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Ovarian cancer targeted medication: PARP inhibitors, anti-angiogenic drugs, immunotherapy, and more, volume II

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Ovarian cancer targeted medication: PARP inhibitors, anti-angiogenic drugs, immunotherapy, and more, volume II

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Editorial: Ovarian cancer targeted medication: PARP inhibitors, anti-angiogenic drugs, immunotherapy, and more, volume II

Zhi-Bin Wang[1,2,3†], De-Hua Liao[3†], Guang Lei[4†], Zhao-Qian Liu[5,6*], Nayiyuan Wu[1,2*] and Jing Wang[1,2*]

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Editorial on the Research Topic
Ovarian cancer targeted medication: PARP inhibitors, anti-angiogenic drugs, immunotherapy, and more, volume II

Ovarian cancer (OC), the deadliest gynecological malignancy, primarily relies on tumor debulking and post-surgical platinum-based chemotherapy. However, platinum resistance often emerges after multiple recurrences. Given OC's heterogeneity and complex molecular landscape, pinpointing specific molecular targets is crucial for understanding its mechanisms and progression. The therapeutic paradigm for OC is evolving from traditional chemotherapy to targeted therapies, with PARP inhibitors and anti-angiogenic agents becoming key maintenance treatments. Despite their promise, these therapies face challenges such as inefficacy, adverse effects, and cost. Next-generation sequencing (NGS) offers a broader spectrum of targeted agents, potentially enhancing personalized treatment strategies. Additionally, immunotherapy and ferroptosis modulation present innovative avenues for OC treatment. Enhancing the efficacy and reducing the side effects of current OC drugs, as well as exploring new targets, are pressing needs. We seek to identify novel therapeutic targets and biomarkers for OC, encouraging both computational and experimental pharmacological studies.

This Research Topic encompasses pharmacological topics, including immune-targeted therapy, prognostic biomarkers, and single-cell sequencing analysis of the immune microenvironment in ovarian cancer (Figure 1). The following section provides a concise summary of the key highlights from the 20 articles featured in this Research Topic.

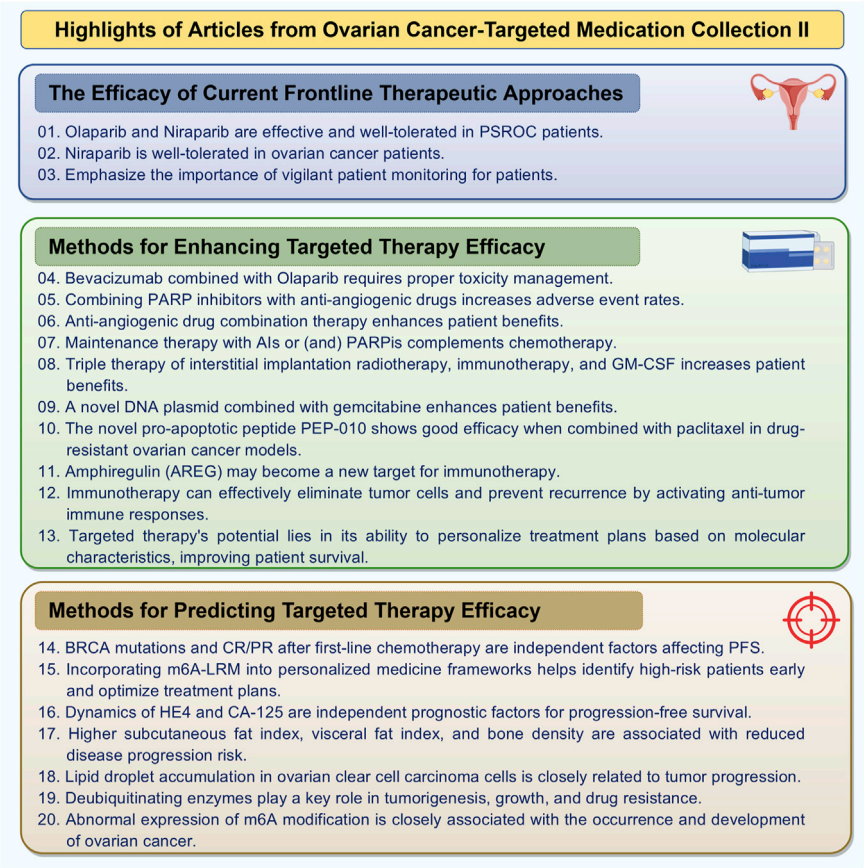

FIGURE 1
Highlights of articles from ovarian cancer-targeted medication collection (By FigDraw).

Data derived from clinical settings have affirmed the efficacy of current frontline therapeutic approaches.

1. In an observational study, Chen et al. scrutinized the efficacy and safety of poly (ADP-ribose) polymerase inhibitors (PARPi) as a maintenance treatment in 75 Chinese patients with platinum-sensitive recurrent ovarian cancer (PSROC). The outcomes demonstrate that both olaparib and niraparib are efficacious and exhibit a favorable tolerability profile in this cohort. They emphasize that their study underscores the value of real-world evidence in elucidating treatment efficacy and bolsters the clinical use of PARPi for PSROC patients.
2. The research spearheaded by Wang et al. delved into treatment-related adverse events (TRAE) in ovarian cancer patients undergoing niraparib treatment subsequent to platinum-based chemotherapy. The study's revelations indicate a markedly reduced incidence of TRAE of any grade and grade ≥3 during niraparib administration compared to chemotherapy, with notable reductions in anemia and neutrophil count decrements. This suggests that niraparib is well-tolerated in this patient cohort, underscoring its clinical potential.
3. Sun and Liu's meta-analysis on the efficacy and safety of PARP inhibitor maintenance therapy for ovarian cancer indicates that PARP inhibitors markedly enhance progression-free survival (PFS) and overall survival (OS) compared to placebo, while concurrently elevating the risk of treatment-related adverse events. This finding accentuates the necessity for vigilant patient monitoring in clinical settings when employing PARP inhibitor maintenance therapy.

However, not all patient populations benefit from existing treatment regimens. The proposition and translational research of novel therapeutic modalities are imperative.

4. The expert synthesis of the safety profile of combining bevacizumab with olaparib as maintenance therapy for patients with newly diagnosed advanced ovarian cancer, based on the PAOLA-1 trial data, indicates that while the combination is deemed safe, there is a notable rate of treatment discontinuations due to adverse events. Romero et al. stress the importance of adept toxicity management to enhance patient quality of life and maximize treatment efficacy.
5. The review and meta-analysis, comparing the efficacy and safety of PARP inhibitors in combination with antiangiogenic agents in ovarian cancer treatment, reveals that combined therapy significantly improves PFS but also increases the incidence of adverse events. Wei et al. note that while combined therapy offers a clear advantage in PFS, its impact on OS remains uncertain.

6. The meta-analysis assesses the efficacy and safety of anti-angiogenic drug monotherapy and combination therapy in ovarian cancer, indicating that combination therapy markedly improves PFS and objective response rate (ORR), while monotherapy does not yield significant survival benefits. Xie and Zhou highlight the critical importance of adverse event monitoring in clinical practice.
7. Hao et al.'s bibliometric analysis, which explores the research progress in the treatment of recurrent ovarian cancer (ROC), shows a consistent increase in ROC treatment literature in recent years, with significant contributions from the United States and Italy. The analysis identifies research hotspots focused on PARP inhibitors and anti-angiogenic agents, indicating the growing importance of these novel therapies in ROC management.
8. A case report investigates a novel triple therapy approach for a patient with oligometastatic platinum-resistant ovarian cancer, combining interstitial implantation radiotherapy, immunotherapy, and granulocyte-macrophage colony-stimulating factor (GM-CSF). The patient showed a partial response to the treatment, with sustained benefits for over 6 months. Qin et al. suggest that this combination therapy may offer additional treatment options for patients with poor prognoses under conventional therapies.
9. The randomized controlled trial evaluates the clinical efficacy and safety of ELENAGEN, a novel DNA plasmid encoding p62/SQSTM1, in combination with gemcitabine for patients with platinum-resistant ovarian cancer. The results indicate that the ELENAGEN group achieved a median PFS of 7.2 months compared to 2.8 months in the gemcitabine-only group. Krasny et al. suggest that ELENAGEN may be effective, particularly in patients with poor prognostic factors, highlighting its potential as a new therapeutic option.
10. Lacroix et al. demonstrate that PEP-010, a first-in-class pro-apoptotic peptide, shows promising efficacy in both monotherapy and in combination with paclitaxel against resistant ovarian adenocarcinoma cell models. The study reveals that PEP-010 effectively induces apoptosis and significantly reduces the IC50 of paclitaxel, suggesting its potential application value in ovarian cancer treatment. This highlights the importance of exploring novel therapeutic strategies in combating drug resistance.
11. One study investigates how increased exposure to amphiregulin (AREG) affects the tumor immune microenvironment in high-grade serous ovarian cancer. The results indicate that increased AREG promotes immune evasion and tumor cell growth. Ebott et al. suggest that AREG may serve as a novel target for immunotherapy, highlighting its potential role in modulating the immune landscape of ovarian tumors.
12. Massariol's study encapsulates recent advancements in immunotherapy for ovarian cancer, with a focus on strategies such as cancer vaccines, CAR-T cell therapy, and immune checkpoint inhibitors (Massariol Pimenta et al.). The researchers emphasize that despite the challenges, the potential of immunotherapy in treating ovarian cancer is substantial. They noted, "By activating anti-tumor immune responses, immunotherapy can effectively eliminate tumor cells and prevent recurrence."
13. The narrative review discusses advancements in targeted treatments for ovarian cancer, including anti-angiogenic agents, PARP inhibitors, and immune checkpoint inhibitors (Satora et al.). The article underscores that while existing therapies delay recurrence, there is an urgent need for new strategies to enhance outcomes. The authors highlight that "the potential of targeted therapies lies in their ability to personalize treatment plans based on molecular characteristics, thereby enhancing patient survival rates."

Regarding the early prediction of the efficacy of targeted therapy for ovarian cancer, several studies have provided commendable attempts and strategies.

14. One real-world study evaluates the use of PARPi as first-line maintenance therapy in newly diagnosed ovarian cancer patients at a major center in China. The study identifies BRCA mutation status and achieving complete or partial response after first-line chemotherapy as independent factors associated with prolonged PFS. As stated by Chen et al., these findings contribute valuable insights into the effectiveness of PARPi in clinical practice for ovarian cancer patients.
15. Ye's study identified six N6-methyladenosine (m6A) effector-related long non-coding RNAs (lncRNAs) through machine learning and constructed a risk prediction model for serous ovarian carcinoma (Ye et al.). The findings indicate that high-risk patients have poorer prognoses and greater sensitivity to immunotherapy. As the researchers noted, "Incorporating the m6A-LRM into personalized medicine frameworks may help identify high-risk patients early and optimize treatment strategies."
16. The META4 clinical trial investigates the prognostic value of HE4 and CA-125 kinetics in patients with recurrent epithelial ovarian carcinoma undergoing chemotherapy. The study finds that baseline concentrations of both biomarkers, as well as their nadir levels and time to nadir, are significant predictors of PFS. As highlighted by Fabbro et al., monitoring HE4 and CA-125 levels could enhance patient management and treatment decision-making in recurrent ovarian cancer.
17. Guo's study examines the prognostic value of body composition and inflammation markers in patients with epithelial ovarian cancer treated with Olaparib. The findings reveal that higher subcutaneous adipose tissue index, visceral adipose tissue index, and bone mineral density are associated with a reduced risk of disease progression. As Guo et al. emphasize, these indicators could provide crucial references for personalized treatment strategies.
18. Koizume et al. propose that lipid droplets may serve as critical factors linking the biological backgrounds of ovarian clear cell carcinoma (OCCC) and clear cell renal cell carcinoma (ccRCC). The research highlights that lipid metabolism in OCCC cells remains underexplored, while lipid droplet accumulation in ccRCC cells is closely associated with tumor progression. This finding opens new avenues for potential therapeutic strategies in OCCC, underscoring the significance of lipid droplets in cancer research.

19. Qiu et al. conducted a bibliometric analysis to systematically review the progress of deubiquitinases (DUBs) in ovarian cancer research. The study finds a steady increase in literature related to DUBs since 1996, with significant contributions from China, the United States, and the United Kingdom. Keyword analysis reveals that DUBs play critical roles in tumor initiation, growth, and resistance, suggesting that future research should focus on their potential as therapeutic targets.
20. Alam's study investigates the role of N6-methyladenosine (m6A) modification in ovarian cancer, emphasizing its significance in cancer progression, drug resistance, and therapeutic prospects. The findings indicate that aberrant expression of m6A modifications is closely associated with the onset and development of ovarian cancer, potentially serving as a novel prognostic marker. As Alam and Giri highlight, the regulatory mechanisms of m6A modifications offer new insights for personalized treatment strategies.

In summary, this Research Topic has provided us with profound insights into the development of targeted therapies for ovarian cancer (OC) and has furnished solid evidence to enhance their efficacy and reduce toxicity. Despite the histological characteristics of the ovarian tissue microenvironment and the "cold tumor" nature of OC, research on targeted therapies, particularly immunotherapy, still faces challenges. However, progress will eventually be made, even though the journey may be long.

Overall, this Research Topic has painted a comprehensive picture of the current state and future perspectives of targeted therapies for ovarian cancer. It has not only highlighted the significant advancements in understanding the complex molecular mechanisms of OC but also underscored the tangible progress in treatment strategies. The evidence accumulated from these studies helps us refine treatment approaches, emphasizing the need for personalized medicine and the potential of combination therapies to improve patient outcomes.

Author contributions

Z-BW: Conceptualization, Visualization, Writing–original draft. D-HL: Writing–original draft. GL: Writing–review and editing. Z-QL: Writing–review and editing. NW: Writing–review and editing. JW: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Case report: Interstitial implantation radiotherapy combined with immunotherapy and GM-CSF in oligometastatic platinum-resistant ovarian cancer

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Background: Treatment for platinum-resistant ovarian cancer is challenging. Currently, platinum-resistant ovarian cancer is typically treated with non-platinum single-agent chemotherapy ± bevacizumab, but the prognosis is often extremely poor. In the treatment of platinum-resistant ovarian cancer patients, reports of triple therapy with interstitial implantation radiotherapy combined with immunotherapy and granulocyte-macrophage colony-stimulating factor (GM-CSF) (PRaG for short) are relatively rare.

Case description: Here, we report a patient with oligometastatic platinum-resistant ovarian cancer. The patient achieved partial response (PR) of the lesion and sustained benefit for more than six months after receiving interstitial implantation radiotherapy combined with immunotherapy along with GM-CSF.

Conclusion: This triple therapy may provide additional options for these patients.

KEYWORDS

platinum-resistant ovarian cancer, interstitial implantation radiotherapy, immunotherapy, GM-CSF, PRaG therapy, case report

Introduction

Ovarian cancer is the second leading cause of death among women from gynecologic malignancies worldwide (1). The majority of ovarian cancer patients are at an advanced stage once confirmed, and the standard of care for advanced ovarian cancer (International Federation of Gynecology and Obstetrics FIGO stage III-IV) is tumor cytoreduction and chemotherapy based on platinum and paclitaxel drugs (2). Platinum-resistant ovarian cancer is a heterogeneous illness with a very bad prognosis and limited survival which commonly advances within 6 months of completing platinum-based therapy. It usually has a survival period of less than 18 months (3). At first recurrence, platinum resistance occurs in about 20% of patients and almost all recurrent patients eventually move toward platinum resistance (4). Currently, platinum-resistant ovarian cancer is typically treated with non-platinum single-agent chemotherapy ± bevacizumab. Non-platinum single-agent chemotherapy has an overall response rate of just 10–15%, a progression-free survival (PFS) of only 4 months, and an overall survival (OS) of roughly 12 months for patients with platinum-resistant ovarian cancer (5). AURELIA clinical trial showed a significant increase in response rates in platinum-resistant patients when combined with bevacizumab, but median survival did not exceed 16 months (6).

Ovarian cancer is immunogenic with immunotherapy promising a role in platinum-resistant ovarian cancer (7, 8). However, programmed cell death protein 1 receptor (PD-1)/PD-1 ligand (PD-L1) antibodies monotherapy has a low response rate in platinum-resistant ovarian cancer, typically no more than 8% (3).Therefore, it is crucial to explore novel approaches to sensitization immunotherapy. Multimodal therapeutic strategies are being investigated to enhance anti-PD1/PD-L1 response rates by the combination of chemotherapy, antiangiogenic agents, radiotherapy, or other immune checkpoint inhibitors (3). Among them, combining stereotactic body radiation therapy (SBRT), hypofractionated radiation therapy (HFRT) or brachytherapy (BT) may be a prospective therapeutic strategy (3, 9, 10).

Here, we present a case of an oligometastatic platinum-resistant ovarian cancer patient. The patient received triple therapy with interstitial implantation radiotherapy combined with immunotherapy and GM-CSF (PRaG for short). At the end of treatment, the patient achieved a PR and sustained benefit for more than 6 months.

Case description

We show the treatment timeline for the patient in Figure 1. In June 2017 (Sichuan, China), a 66-year-old woman was admitted to our hospital with abdominal distension for more than 6 months. The patient had a total of four pregnancies, three abortions and one normal delivery. Ascites cytology result showed malignant cells (poorly differentiated, considered adenocarcinoma). An abdominal computed tomography (CT) scan revealed bilateral adnexal masses and multiple retroperitoneal lymph nodes. The cancer antigen 125 (CA 125) blood test was 698.20 U/ml. Based on the above clinical results, the patient was diagnosed with ovarian adenocarcinoma (FIGO 2017 Stage IIIC). The patient refused surgery for personal reasons and underwent 6 cycles of chemotherapy (paclitaxel 175 mg/m^2 + carboplatin AUC=5, ivgtt, q21d). After completion of 6 cycles of chemotherapy, abdominal CT confirmed a complete response (CR). The physician again recommended surgical resection, which the patient declined. Considering the patient's actual condition, the oncologist implemented the 7th cycle of chemotherapy (paclitaxel 175 mg/m2 + carboplatin AUC=5, ivgtt, q21d). Upon completion of the treatment, follow-up abdominal CT and tumor markers (CA125 and human epitope protein 4 (HE4)) did not show any signs of recurrence for more than 2 years.

In February 2020, the patient presented for vaginal bleeding. A pelvic magnetic resonance imaging (MRI) revealed a cystic solid mass shadow in the pelvis (size 10.3×6.3×8.2 cm). CA125 was greater than 1000 U/ml and HE4 was 114.90 pmol/L. The oncologist considered the patient to be a platinum-sensitive recurrence. The patient still refused surgery and underwent a

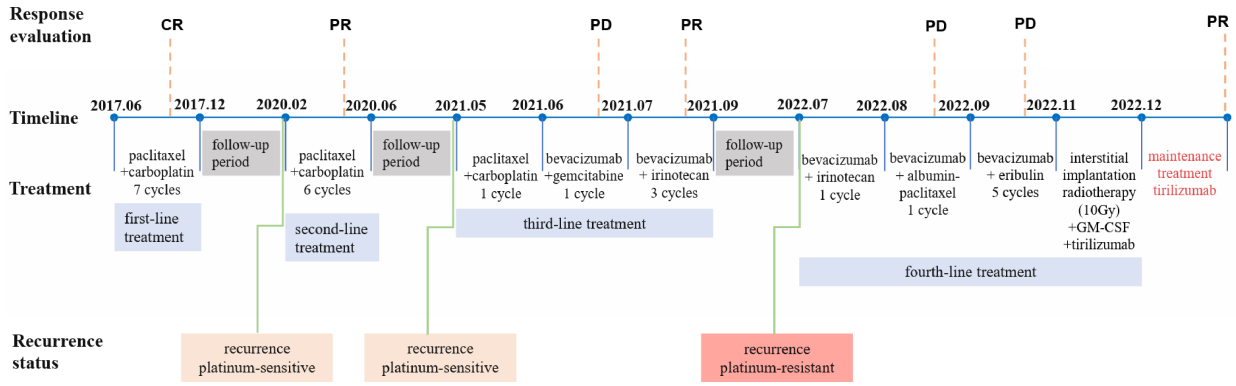

FIGURE 1
Timeline of different treatments and disease status.

second course of 6 cycles of systemic chemotherapy (paclitaxel 175 mg/m^2 + carboplatin AUC=5, ivgtt, q21d). CT confirms that localized lesions achieve PR and CA125 consistently decreases to the normal range (23.30 - >1000 U/ml). After the completion of chemotherapy, the oncologist advised the patient to perform surgical resection or maintenance therapy, but the patient refused all therapeutic recommendations.

Afterward, the patient progressed again in less than a year, still presenting as a localized adnexal mass and same location as the first recurrence. MRI (May 2021) showed a cystic solid mass shadow in the pelvis (size 12.2×7.9×10.0 cm) and CA125 was 560.05 U/ml and HE4 was 186.10 pmol/L. The oncologist considered the patient a platinum-sensitive recurrence again. However, after 1 cycle of chemotherapy (paclitaxel 175 mg/m^2 + carboplatin AUC=5, ivgtt, q21d), the patient's CA125 remained elevated (855.34 U/ml). Positron emission tomography-computed tomography (PET-CT) demonstrated there is a huge mass in the pelvic cavity with increased glucose metabolism, compared with the pelvic MRI in May 2021, the volume of the pelvic lesion has slightly increased. Considered platinum-resistant, it was replaced with bevacizumab combined with gemcitabine (bevacizumab 7.5 mg/kg + gemcitabine 1.0 g/m^2, d1, d8 ivgtt, q21d) in June 2021. As the tumor marker serum CA125 continued to rise, the oncologist implemented 3 cycles of targeted drug combination chemotherapy (bevacizumab 7.5mg/kg d1 + irinotecan 80mg/kg d1, d8, d15 q21d). The last systemic treatment was in September 2021. MRI in October 2021 suggested a significant reduction in the shadow of the cystic solid mass in the pelvis, and efficacy was evaluated as PR of the localized lesion.

In February 2022, the patient experienced abdominal distension again along with a large amount of ascites. In July 2022, the patient was readmitted to our hospital with a worsening condition. Abdominal CT suggested a cystic solid mass in the pelvis (size 12.5×10.6 cm) and CA125 was 1175.16 U/ml and HE4 was 133.80 pmol/L. Tumor recurrence was considered. From July 2022 to November 2022, the patient received 7 cycles of systemic therapy with a targeted agent in combination with a chemotherapeutic agent. On the clinician's recommendation, the patient received 1 cycle of bevacizumab in combination with irinotecan (bevacizumab 7.5 mg/kg + irinotecan 60 mg/kg, ivgtt, q21d), 1 cycle of bevacizumab in combination with albumin-paclitaxel (bevacizumab 7.5 mg/kg + albumin-paclitaxel q21d), and 5 cycles of bevacizumab in combination with eribulin (bevacizumab 7.5mg/kg + eribulin 2mg d1, d8, q21d). During the treatment, the pelvic mass of the patient was still increasing, and the general condition was getting worse. The patient refused to undergo palliative surgery to relieve symptoms and to be enrolled in clinical studies. Considering that the patient has experienced multiple relapses with the same pelvic lesion and the lesion is isolated, local radiotherapy combined with immunotherapy was chosen. Due to financial reasons, the patient refused immune-related genetic tests, including microsatellite instability (MSI) status, programmed cell death-ligand 1 (PD-L1), and tumor mutation burden (TMB). However, considering the MSI-H/dMMR incidence of up to 30% (11), our patient strongly expressed her willingness to do immunotherapy and chose the relatively affordable and cheap the PD-1 inhibitor tirilizumab produced in China. We informed our patient of the treatment purpose and risks, and signed an informed consent form. The patient then received triple therapy from November 30, 2022. The radiation oncologist implemented interstitial implantation radiotherapy at a prescribed dose of 10 Gy, combined with a subcutaneous injection of GM-CSF (200 μg) for one week. The tumor got an actual dose of 926.91 cGy. On December 2, 2022, the patient began immunotherapy with the PD-1 inhibitor tirilizumab (300 mg, ivgtt). Figure 2 shows the three-

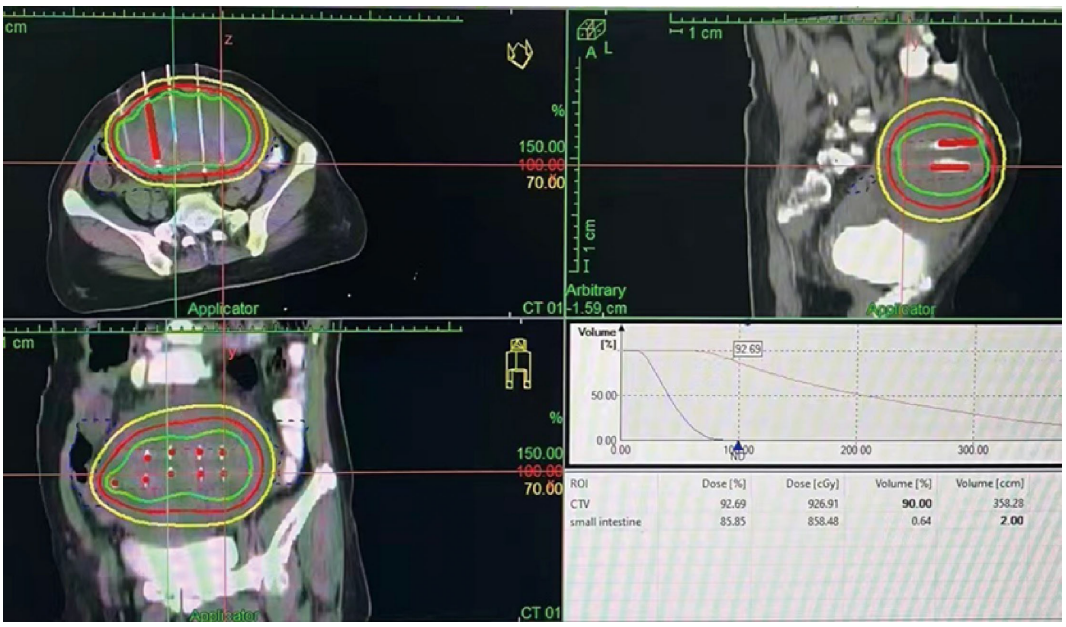

FIGURE 2
Interstitial implantation radiotherapy: Three-dimensional conformal dose assessment.

dimensional conformal dose assessment for interstitial implantation radiation therapy. After radiotherapy, the patient developed mild localized erythema. The patient now has no skin ulcers, no bilateral lower extremity edema or other complications, and only mild localized skin pigmentation. The patient's efficacy evaluation showed a PR. After that, single-agent maintenance therapy with the PD-1 inhibitor tirilizumab was administered every three weeks. During immune maintenance therapy, the patient was temporarily free of treatment-related adverse events (TRAEs), like hemopoietic, thyroid, lung, and heart dysfunction. As of the follow-up in June 2023, abdominal CT suggested a smaller pelvic mass than before (Figure 3) and CA125 was persistently decreasing (most recent CA125 was 18.30 U/ml) (Figure 4). The patient's lesion achieved a PR and continues to benefit for more than six months.

Discussion

Currently, the overall outcome of PD-1/PD-L1 antibody therapy in recurrent ovarian cancer is not good. The use of a single immune checkpoint inhibitor has shown relatively low response rates, usually in the range of 10-15% (12). The response rates to single-agent immunotherapy in platinum-resistant ovarian cancer are even lower (3). Several clinical trials on immune checkpoint inhibitors to platinum-resistant ovarian cancer are ongoing. The JAVELIN phase I clinical study revealed an objective remission rate (ORR) of 9.6% for 125 patients after monotherapy with Avelumab (13). In the phase II clinical study of KEYNOTE-100, the investigators designed two cohorts that had ORRs of 7.4% and 9.9% after treatment with pembrolizumab (14). Several data have shown that combination therapy with immune checkpoint inhibitors shows some advantages over monotherapy. The clinical study of KEYNOTE-162 showed that pembrolizumab and the PARP inhibitor niraparib together had an ORR of 18% for the treatment of platinum-resistant recurrent ovarian cancer (15). Phase II clinical study NCT02853318 evaluated pembrolizumab in combination with bevacizumab and oral cyclophosphamide for recurrent ovarian cancer with an ORR of 47.5% (16). However, the above clinical trials on combination therapy with immune checkpoint inhibitors are small sample studies and their results need to be further validated.

Radiation therapy's clinical success has been linked to the ability of ionizing radiation to cause DNA damage, which can instantly kill tumor cells. However, ionizing radiation can also produce a non-DNA-targeted radiation effect (17). Radiation therapy can initiate the immune system through T-cell mediation. Irradiation-induced immunomodulation can affect both irradiated tumor cells and have an effect on the tumor immune microenvironment (18, 19). Radiation-induced immune stimulation generates a series of molecular reactions through both local and systemic immune mediators, resulting in the creation of a pro-inflammatory environment (20). Irradiation can increase the expression of MHC-I and MHC-II molecules, adhesion molecules, CD80, stress ligands and death receptors on the surface of tumor cells, simultaneously releasing immune-activating danger signals,

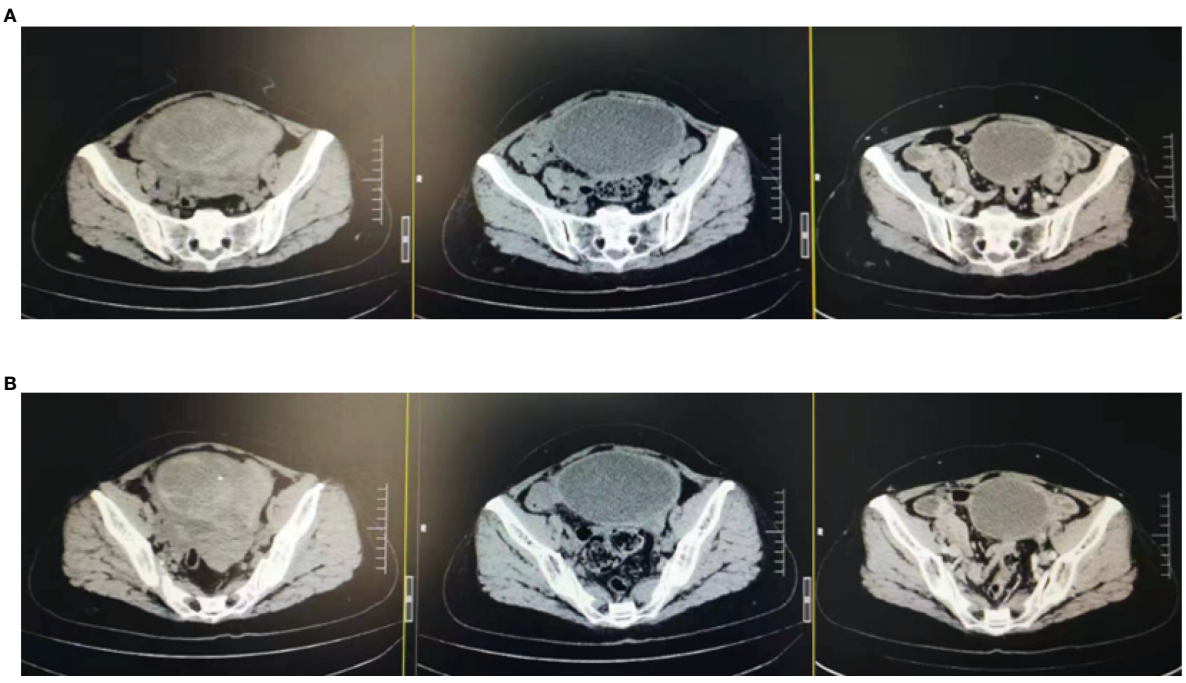

FIGURE 3
Changes in lesions: After the triple therapy, the CT showed a significantly smaller pelvic mass. **(A)** Pelvic mass before the triple therapy. **(B)** Pelvic mass after the triple therapy.

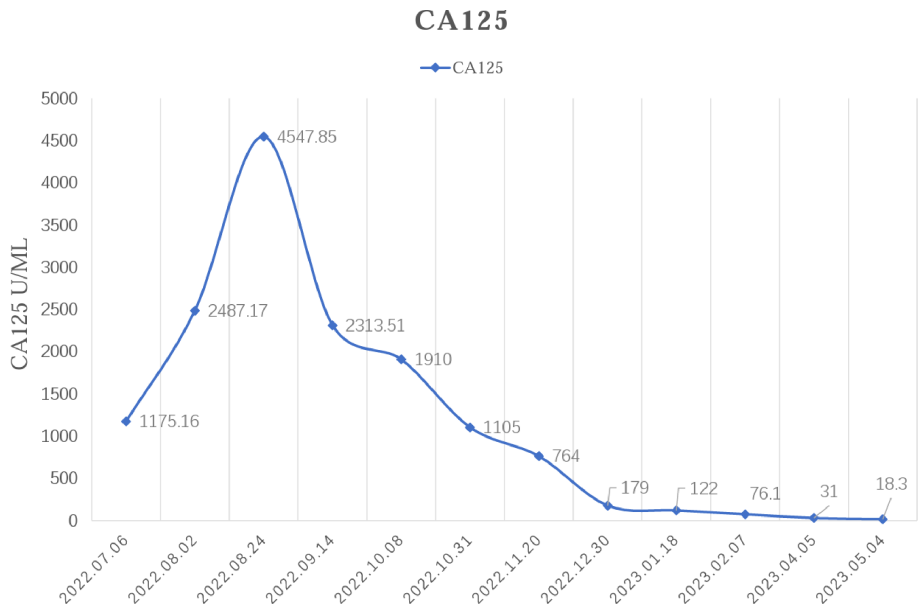

FIGURE 4
Changes in serum CA125 levels before and after the triple therapy.

chemokines, inflammatory cytokines, and possibly even inducing new tumor antigens, which triggers a systemic response (21–23). In this process, mature dendritic cells (DCs) are activated and stimulate the innate immune system, indirectly generating an adaptive immune response (21). It means that tumor cells may be transformed into *in situ* vaccines under irradiation-induced immune stimulation, exerting local tumor control and possibly triggering the so-called "abscopal effect" at distant tumor sites (17, 24). And, immune checkpoint inhibitors act synergistically with radiation therapy to boost local tumor control and systemic response (25). Irradiated tumor cells undergo a specific form of cell death (so-called immune cell death). This cell death exposes tumor cell-associated antigens, allowing for synergy with immunotherapy (26, 27). Thus, radiation therapy combined with immune therapy has been more and more recognized as a possible treatment strategy.

Pelvic irradiation is not included in the National Comprehensive Cancer Network (NCCN) guideline for platinum-resistant ovarian cancer patients (28). However, data have shown that for recurrent ovarian cancer patients, the median survival after HFRT is 17 months, the 1-year survival rate is 66.7% and the 1-year local progression-free survival rate is 45.8% (29). HFRT may be an alternative therapy. In the case of oligometastases, SBRT is a novel high-dose radiation beam treatment. In a study of oligometastatic platinum-resistant ovarian cancer, 156 lesions treated with SBRT were evaluated radiologically. 91 (58%) lesions showed a complete radiologic response, 26 (17%) lesions showed a partial response, 24 (15%) had stable disease, and 11 (7%) showed disease progression (30). Moreover, SBRT has been shown in several clinical trials to have a local control rate of 90-100% in oligometastatic platinum-resistant patients (31, 32). These studies proved the radiosensitivity of platinum-resistant ovarian cancer. Since our patient had a large pelvic lesion, it was difficult to maneuver the SBRT. We chose interstitial implantation radiotherapy for the patient, which used the technique of large fractionation radiotherapy, and only a dose of 10 Gy was given to activate the immune T-cells and synergize with the subsequent immunotherapy. Few reports on interstitial implantation brachytherapy for recurrent ovarian cancer have been reported. In a retrospective study, 47 recurrent ovarian cancer patients were treated with brachytherapy, and the local control rates at 3, 6, 12, 24, and 36 months were 93.3%, 77.7%, 58.9%, 38.7%, and 19.3%, respectively, and the mean OS of 14.6 months (33). Although there are few reports on the local control rate of interstitial implantation radiotherapy for platinum-resistant ovarian cancer, we believe that this treatment is feasible.

GM-CSF is a cytokine which drives the production of myeloid cell subsets including neutrophils, monocytes, macrophages, and dendritic cells (34). Preclinical studies have demonstrated that GM-CSF in combination with immune checkpoint inhibitors enhances innate immune cell activity and indirectly recruits T-cells by promoting antigen cross-presentation, thereby enhancing the immune response (35, 36). PD-1 inhibitors combined with radiotherapy or/and GM-CSF can have a synergistic effect (37–42). Triple-combination therapy of these treatments was called PRaG for short. A clinical trial demonstrated that concurrent radiotherapy with pembrolizumab dramatically enhanced response and prognosis in patients with non-small cell lung carcinoma (median OS: 19.2 months vs. 8.7 months, PFS: 9.0 months vs. 4.4 months) (41). In unresectable advanced melanoma patients, ibritumomab combined with GM-CSF resulted in longer survival and fewer toxic side effects than ibritumomab alone (43). In a phase II clinical trial for refractory metastatic solid tumors, 54

patients were treated with PRaG therapy, resulting in an ORR of 16.7%, a disease control rate of 46.3%, and a median PFS of 4.0 months (42). Consistent with the results of these clinical studies, we have achieved favorable outcomes using interstitial implantation radiotherapy in combination with a PD-1 inhibitor and GM-CSF. By June 2023, the patient's efficacy evaluation was a PR and the general condition has significantly improved and the patient is continuing to benefit.

The most regrettable aspect of this study is that we did not conduct a coarse needle biopsy to obtain tissue pathological diagnosis before undergoing local radiotherapy. We have reason to believe that our patient should be a special case of ovarian cancer, with multiple relapses occurring in the same location. The patient achieved good results in a single fractionated radiotherapy combined with immunotherapy, but the gene expression related to immunotherapy is not very clear. Although our treatment was similar to PRaG therapy, PRaG therapy is usually repeated several times to adequately induce the immune response, which was only done once in this study. Despite these disadvantages, we were pleasantly surprised to find that the triple therapy of single high-dose interstitial implantation radiotherapy for large isolated pelvic masses in combination with immunotherapy and GM-CSF achieved a longer disease progression-free time, which the mechanism of this deserves to be further explored.

Conclusion

In conclusion, oligometastatic platinum-resistant ovarian cancer patients who fail to receive bevacizumab in combination with non-platinum monotherapy tend to have an extremely poor prognosis, and subsequent treatment becomes tricky. In our case, the patient was treated with a triple combination of interstitial implantation radiotherapy, PD-1 inhibitor immunotherapy, and GM-CSF, showing a sustained clinical response. Moreover, the patient had only minor toxic side effects. This might offer a novel treatment option for similar patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YQ: Visualization, Writing – original draft. SH: Writing – original draft. JT: Writing – original draft. YF: Writing – review & editing. XD: Writing – review & editing. PG: Writing – review & editing. ZZ: Supervision, Writing – review & editing. QW: Supervision, Writing – review & editing. DL: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

2. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. *J Clin Oncol* (2002) 20(5):1248–59. doi: 10.1200/JCO.2002.20.5.1248

3. Le Saux O, Ray-Coquard I, Labidi-Galy SI. Challenges for immunotherapy for the treatment of platinum resistant ovarian cancer. *Semin Cancer Biol* (2021) 77:127–43. doi: 10.1016/j.semcancer.2020.08.017

4. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. *Ann Oncol* (2019) 30(5):672–705. doi: 10.1093/annonc/mdz062

5. Naumann RW, Coleman RL. Management strategies for recurrent platinum-resistant ovarian cancer. *Drugs* (2011) 71(11):1397–412. doi: 10.2165/11591720-000000000-00000

6. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian

cancer: The AURELIA open-label randomized phase III trial. *J Clin Oncol* (2014) 32 (13):1302–8. doi: 10.1200/JCO.2013.51.4489

7. Anadon CM, Yu X, Hänggi K, Biswas S, Chaurio RA, Martin A, et al. Ovarian cancer immunogenicity is governed by a narrow subset of progenitor tissue-resident memory T cells. *Cancer Cell* (2022) 40(5):545–57.e13. doi: 10.1016/j.ccell.2022.03.008

8. Jiménez-Sánchez A, Cybulska P, Mager KL, Koplev S, Cast O, Couturier DL, et al. Unraveling tumor-immune heterogeneity in advanced ovarian cancer uncovers immunogenic effect of chemotherapy. *Nat Genet* (2020) 52(6):582–93. doi: 10.1038/s41588-020-0630-5

9. Lee L, Matulonis U. Immunotherapy and radiation combinatorial trials in gynecologic cancer: A potential synergy? *Gynecol Oncol* (2019) 154(1):236–45. doi: 10.1016/j.ygyno.2019.03.255

10. Herrera FG, Irving M, Kandalaft LE, Coukos G. Rational combinations of immunotherapy with radiotherapy in ovarian cancer. *Lancet Oncol* (2019) 20(8): e417–e33. doi: 10.1016/S1470-2045(19)30401-2

11. Hause RJ, Pritchard CC, Shendure J, Salipante SJ. Classification and characterization of microsatellite instability across 18 cancer types. *Nat Med* (2016) 22(11):1342–50. doi: 10.1038/nm.4191

12. Palaia I, Tomao F, Sassu CM, Musacchio L, Benedetti Panici P. Immunotherapy for ovarian cancer: recent advances and combination therapeutic approaches. *Onco Targets Ther* (2020) 13:6109–29. doi: 10.2147/OTT.S205950

13. Disis ML, Taylor MH, Kelly K, Beck JT, Gordon M, Moore KM, et al. Efficacy and safety of avelumab for patients with recurrent or refractory ovarian cancer: phase 1b results from the JAVELIN solid tumor trial. *JAMA Oncol* (2019) 5(3):393–401. doi: 10.1001/jamaoncol.2018.6258

14. Matulonis UA, Shapira-Frommer R, Santin AD, Lisyanskaya AS, Pignata S, Vergote I, et al. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. *Ann Oncol* (2019) 30(7):1080–7. doi: 10.1093/annonc/mdz135

15. Konstantinopoulos PA, Waggoner S, Vidal GA, Mita M, Moroney JW, Holloway R, et al. Single-arm phases 1 and 2 trial of niraparib in combination with pembrolizumab in patients with recurrent platinum-resistant ovarian carcinoma. *JAMA Oncol* (2019) 5(8):1141–9. doi: 10.1001/jamaoncol.2019.1048

16. Zsiros E, Lynam S, Attwood KM, Wang C, Chilakapati S, Gomez EC, et al. Efficacy and safety of pembrolizumab in combination with bevacizumab and oral metronomic cyclophosphamide in the treatment of recurrent ovarian cancer: A phase 2 nonrandomized clinical trial. *JAMA Oncol* (2021) 7(1):78–85. doi: 10.1001/jamaoncol.2020.5945

17. Eckert F, Gaipl US, Niedermann G, Hettich M, Schilbach K, Huber SM, et al. Beyond checkpoint inhibition - Immunotherapeutical strategies in combination with radiation. *Clin Transl Radiat Oncol* (2017) 2:29–35. doi: 10.1016/j.ctro.2016.12.006

18. Wennerberg E, Vanpouille-Box C, Bornstein S, Yamazaki T, Demaria S, Galluzzi L. Immune recognition of irradiated cancer cells. *Immunol Rev* (2017) 280(1):220–30. doi: 10.1111/imr.12568

19. Eckert F, Schilbach K, Klumpp L, Bardoscia L, Sezgin EC, Schwab M, et al. Potential role of CXCR4 targeting in the context of radiotherapy and immunotherapy of cancer. *Front Immunol* (2018) 9:3018. doi: 10.3389/fimmu.2018.03018

20. Sharabi AB, Lim M, DeWeese TL, Drake CG. Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy. *Lancet Oncol* (2015) 16(13):e498–509. doi: 10.1016/S1470-2045(15)00007-8

21. Derer A, Frey B, Fietkau R, Gaipl US. Immune-modulating properties of ionizing radiation: rationale for the treatment of cancer by combination radiotherapy and immune checkpoint inhibitors. *Cancer Immunol Immunother* (2016) 65(7):779–86. doi: 10.1007/s00262-015-1771-8

22. Hodge JW, Ardiani A, Farsaci B, Kwilas AR, Gameiro SR. The tipping point for combination therapy: cancer vaccines with radiation, chemotherapy, or targeted small molecule inhibitors. *Semin Oncol* (2012) 39(3):323–39. doi: 10.1053/j.seminoncol.2012.02.006

23. Frey B, Rubner Y, Kulzer L, Werthmöller N, Weiss EM, Fietkau R, et al. Antitumor immune responses induced by ionizing irradiation and further immune stimulation. *Cancer Immunol Immunother* (2014) 63(1):29–36. doi: 10.1007/s00262-013-1474-y

24. Bardoscia L, Pasinetti N, Triggiani L, Cozzi S, Sardaro A. Biological bases of immune-related adverse events and potential crosslinks with immunogenic effects of radiation. *Front Pharmacol* (2021) 12:746853. doi: 10.3389/fphar.2021.746853

25. Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys* (2004) 58(3):862–70. doi: 10.1016/j.ijrobp.2003.09.012

26. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* (2006) 203(5):1259–71. doi: 10.1084/jem.20052494

27. Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, et al. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J* (2009) 28(5):578–90. doi: 10.1038/emboj.2009.1

28. Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Barroilhet L, Behbakht K, Berchuck A, et al. Ovarian cancer, version 2.2020, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2021) 19(2):191–226. doi: 10.6004/jnccn.2021.0007

29. Komura N, Mabuchi S, Isohashi F, Yokoi E, Shimura K, Matsumoto Y, et al. Radiotherapy for isolated recurrent epithelial ovarian cancer: A single institutional experience. *J Obstet Gynaecol Res* (2019) 45(6):1173–82. doi: 10.1111/jog.13947

30. Lazzari R, Ronchi S, Gandini S, Surgo A, Volpe S, Piperno G, et al. Stereotactic body radiation therapy for oligometastatic ovarian cancer: A step toward a drug holiday. *Int J Radiat Oncol Biol Phys* (2018) 101(3):650–60. doi: 10.1016/j.ijrobp.2018.03.058

31. Kunos CA, Brindle J, Waggoner S, Zanotti K, Resnick K, Fusco N, et al. Phase II clinical trial of robotic stereotactic body radiosurgery for metastatic gynecologic Malignancies. *Front Oncol* (2012) 2:181. doi: 10.3389/fonc.2012.00181

32. Iftode C, D'Agostino GR, Tozzi A, Comito T, Franzese C, De Rose F, et al. Stereotactic body radiation therapy in oligometastatic ovarian cancer: A promising therapeutic approach. *Int J Gynecol Cancer* (2018) 28(8):1507–13. doi: 10.1097/IGC.0000000000001324

33. Liu P, Tong L, Huo B, Dai D, Liu W, Wang K, et al. CT-guided (125)I brachytherapy for recurrent ovarian cancer. *Oncotarget* (2017) 8(35):59766–76. doi: 10.18632/oncotarget.15905

34. Kumar A, Taghi Khani A, Sanchez Ortiz A, Swaminathan S. GM-CSF: A double-edged sword in cancer immunotherapy. *Front Immunol* (2022) 13:901277. doi: 10.3389/fimmu.2022.901277

35. van de Laar L, Coffer PJ, Woltman AM. Regulation of dendritic cell development by GM-CSF: molecular control and implications for immune homeostasis and therapy. *Blood* (2012) 119(15):3383–93. doi: 10.1182/blood-2011-11-370130

36. Gurbatri CR, Lia I, Vincent R, Coker C, Castro S, Treuting PM, et al. Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies. *Sci Transl Med* (2020) 12(530). doi: 10.1126/scitranslmed.aax0876

37. Luke JJ, Lemons JM, Karrison TG, Pitroda SP, Melotek JM, Zha Y, et al. Safety and clinical activity of pembrolizumab and multisite stereotactic body radiotherapy in patients with advanced solid tumors. *J Clin Oncol* (2018) 36(16):1611–8. doi: 10.1200/JCO.2017.76.2229

38. Sundahl N, Seremet T, Van Dorpe J, Neyns B, Ferdinande L, Meireson A, et al. Phase 2 trial of nivolumab combined with stereotactic body radiation therapy in patients with metastatic or locally advanced inoperable melanoma. *Int J Radiat Oncol Biol Phys* (2019) 104(4):828–35. doi: 10.1016/j.ijrobp.2019.03.041

39. Maity A, Mick R, Huang AC, George SM, Farwell MD, Lukens JN, et al. A phase I trial of pembrolizumab with hypofractionated radiotherapy in patients with metastatic solid tumours. *Br J Cancer* (2018) 119(10):1200–7. doi: 10.1038/s41416-018-0281-9

40. Golden EB, Chhabra A, Chachoua A, Adams S, Donach M, Fenton-Kerimian M, et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: a proof-of-principle trial. *Lancet Oncol* (2015) 16(7):795–803. doi: 10.1016/S1470-2045(15)00054-6

41. Theelen W, Chen D, Verma V, Hobbs BP, Peulen HMU, Aerts J, et al. Pembrolizumab with or without radiotherapy for metastatic non-small-cell lung cancer: a pooled analysis of two randomised trials. *Lancet Respir Med* (2021) 9 (5):467–75. doi: 10.1016/S2213-2600(20)30391-X

42. Kong Y, Zhao X, Xu M, Pan J, Ma Y, Zou L, et al. PD-1 inhibitor combined with radiotherapy and GM-CSF (PRaG) in patients with metastatic solid tumors: an open-label phase II study. *Front Immunol* (2022) 13:952066. doi: 10.3389/fimmu.2022.952066

43. Hodi FS, Lee S, McDermott DF, Rao UN, Butterfield LH, Tarhini AA, et al. Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. *Jama* (2014) 312(17):1744–53. doi: 10.1001/jama.2014.13943

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A real-world study of PARP inhibitors in 75 patients with platinum-sensitive recurrent ovarian cancer from China

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Objective: The aim of this study is to assess the efficacy and safety of poly (ADP-ribose) polymerase inhibitor (PARPi) as a maintenance therapy for patients with platinum-sensitive recurrent epithelial ovarian cancer (PSROC) at the largest center of gynecologic oncology in Western China.

Patients and methods: The efficacy of PARPi was evaluated by progression-free survival (PFS) and overall survival (OS) in this real-world single-center retrospective cohort study conducted at West China Second University Hospital. The safety of PARPi was assessed using Common Terminology Criteria for Adverse Events Version 5.0.

Results: In this study, we included a total of 75 eligible patients, of which 54 (72.0%) received olaparib and 21 (28.0%) received niraparib. Among these patients, 24 (32.0%) had breast cancer susceptibility gene (BRCA) mutations, 27 (36.0%) achieved complete response after their last platinum-based therapy, and 22 (29.3%) had previously received ≥3rd-line chemotherapy. The median progression-free survival (mPFS) was 19.1 months (95% CI 8.5-29.7), and the median overall survival (mOS) had not been reached. Log-rank analysis revealed that age (<65 years old V.S. ≥65 years old) and previous lines of chemotherapy (2nd-line V.S. 3rd-line V.S. ≥4th-line) were associated with prolonged PFS (P <0.05). However, multivariate COX regression analysis did not identify any independent factors associated with prognosis (P >0.05). The most common grade≥3 adverse events in the olaparib group were anemia, thrombocytopenia, and leukopenia, while in the niraparib group, they were anemia and thrombocytopenia.

Conclusion: This study confirmed that olaparib and niraparib are effective and tolerate for PSROC in real-world settings. At the follow-up endpoint, no independent prognostic factor associated with prolonged PFS was identified.

KEYWORDS

PARP inhibitor, platinum-sensitive recurrent ovarian cancer, real-world study, progression-free survival, safety

1 Introduction

Ovarian cancer is the third most common female reproductive system malignancy. There were 313,959 new cases of ovarian cancer all around the world in 2020, including 55,342 new cases in China, accounting for 17.62% of the global new cases. A total of 207,252 deaths due to ovarian cancer in the world in 2020, including 37,519 cases in China, accounting for 18.10% of the global total (1). The onset of most patients is insidious, 70% of whom are diagnosed at an advanced stage while 70% relapse within 2-3 years, and the 5-year survival rate is only 30-40%. For patients with newly diagnosed advanced ovarian cancer, initial treatment is particularly crucial in comprehensive management. Maintenance therapy plays a significant role in overall management for ovarian cancer. Poly ADP ribose polymerase inhibitors (PARPi) has astounded the world time and time again with its maturing clinical data (2). Multiple large randomized controlled trials (RCTs) such as SOLO-1 (3), PAOLA-1 (4), PRIMA (5), and PRIME (6) studies have confirmed the curative effect of first-line maintenance therapy for advanced ovarian cancer. The population of SOLO1 trial was limited to BRCA-m patients, while BRCA-wt population was studied in the PRIMA and PRIME trials. They reported that niraparib maintenance therapy provided different degree of benefit in the first-line maintenance treatment of advanced ovarian cancer in the general population (5, 6). The PAOLA-1 study showed that in the HRD-positive population, OS was longer with olaparib plus bevacizumab (HR 0.62, 95% CI 0.45-0.85) (4).

In recent years, PARPi has become a standard treatment for patients with platinum-sensitive recurrent epithelial ovarian cancer (PSROC). The SOLO-2 study (7) revealed a 70% reduction in the risk of disease progression or death (HR=0.30, 95% CI 0.22-0.41) in PSROC patients treated with olaparib. The L-MOCA study (8) demonstrated that after a follow-up of 15.5 months, the median progression-free survival (mPFS) in the overall population, BRCA-mutation (BRCA-m) group, and BRCA wild-type (BRCA-wt) group were 16.1 months, 21.2 months, and 11.0 months, respectively. This study is the first to illustrate the efficacy of olaparib in the PSROC population among Asian individuals, regardless of BRCA mutation status. The NORA study (9) primarily focused on individualized starting doses for Chinese patients with PSROC. In the overall population, the group treated with niraparib demonstrated a 68% reduction in the risk of disease progression or death (HR=0.32, 95% CI 0.23-0.45). Among the gBRCA-m group, the niraparib group showed a 78% reduction in the risk of disease progression or death (HR=0.22, 95% CI 0.12-0.39). In the non-gBRCA-m subgroup, the niraparib group exhibited a 60% reduction in the risk of disease progression or death (HR=0.40, 95% CI 0.26-0.61). These findings highlight the significant impact of niraparib treatment across different patient subgroups. The updated OS data presented at the European Society for Medical Oncology (ESMO) Congress in 2022 revealed that, following the implementation of inverse probability weighting, the niraparib group exhibited a 30.8% reduction in the risk of disease progression or death compared to the placebo group (HR=0.692, 95% CI 0.446-1.074) in the overall population. In the gBRCA-m group, the niraparib group did not reach the mOS (HR=0.882, 95% CI 0.387-2.011). Notably, within the non-gBRCA-m population, the mOS for the niraparib group amounted to 43.1 months, marking a substantial 10.5 months extension compared to the placebo group (HR=0.624, 95%CI 0.368-1.056) (10). These large RCTs have laid a solid foundation for clinical diagnosis and treatment. However, these studies strictly adhere to specified inclusion criteria and treatment protocols, which effectively minimize bias but also result in discrepancies from real-world clinical scenarios (11). Real-world studies have better external validity which are essential to assess the benefit of new drugs in real clinical practice (12). Nevertheless, there is a lack of such real-world studies on PARPi, especially limited data

Abbreviations: PARP, Poly (ADP-ribose), Polymerase; RWS, Real-world Study; RCT, Randomized Controlled Trial; PSROC, Platinum-sensitive Recurrence Ovarian Cancer; PFS, Progression-free Survival; OS, Overall Survival; BRCA, Breast Cancer Susceptibility Gene; BRCA-m, BRCA Mutation Type; BRCA-wt, BRCA Wild Type; HRD, Homologous Recombination Deficiency; FIGO, International Federation of Gynecology and Obstetrics; NCCN, National Comprehensive Cancer Network; ESMO, European Society for Medical Oncology; ASCO, American Society of Clinical Oncology; SGO, Society of Gynecologic Oncology; PDS, Primary Debulking Surgery; IDS, Interval Debulking Surgery; SCR, Secondary Cytoreduction; CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; BMI, Body Mass Index; AE, Adverse Event; CTCAE, Common Terminology Criteria for Adverse Events; RECIST, Response Evaluation Criteria in Solid Tumors; FDA, Food and Drug Administration; NMPA, National Medical Products Administration; ITT, Intention-to-Treat Population.

based on the Chinese population. Consequently, the aim of this study was to evaluate the real-world clinical data from patients with PSROC who were administered PARPi as maintenance therapy and identify factors associated with long-term benefits to accumulate more clinical experience in PARPi maintenance therapy for patients with PSROC.

2 Patients and methods

2.1 Patients and inclusion criteria

The study, conducted in accordance with the principles of the Declaration of Helsinki and the guidelines of the International Conference on Harmonization of Good Clinical Practice, was approved by the Ethics Committee of West China Second University Hospital (approval number: 20220129). As a result of the retrospective design and anonymous data collection of this study, informed consent from the patients was not required.

The clinicopathological data of patients with PSROC treated with PARPi as maintenance therapy after recurrence were collected from August 1, 2018 to September 31, 2022 at the West China Second University Hospital. The inclusion criteria were as follows: (1) age ≥ 18 years old. (2) pathologically confirmed epithelial ovarian cancer, fallopian tube cancer or primary peritoneal cancer with complete clinical and pathological data. (3) patients who achieved complete response (CR) or partial response (PR) after the last platinum-based chemotherapy. (4) patients receiving PARPi for maintenance therapy after platinum-sensitive relapse. Patients who missed important clinical data or declined to follow up were excluded.

2.2 Data collection

Clinical and pathological data collection was conducted to build a real-world database using Microsoft Excel. The basic information of PSROC patients was extracted from the information systems of West China Second University Hospital, Sichuan University (including the Hospital Information System [HIS], laboratory information system, and Picture Archiving and Communication System [PACS]). Patients who met the inclusion and exclusion criteria were selected for the study. The patient-related information collected includes the following: (1) Baseline information: age, body mass index (BMI), comorbidities (hypertension, diabetes, thyroid dysfunction, chronic hepatitis B virus infection, etc.), family history, BRCA gene mutation status, initial treatment, and the number of previous lines of platinum-based chemotherapy. (2) Surgical related data: surgical outcome, postoperative pathological diagnosis, International Federation of Gynecology and Obstetrics (FIGO) 2014 staging. (3) Postoperative treatment status: first-line chemotherapy status (chemotherapy regimen, course of treatment, completion time of chemotherapy, response to chemotherapy), recurrence status (platinum-sensitive recurrence/platinum-resistant recurrence, chemotherapy regimen, course of treatment, completion time of chemotherapy, response to chemotherapy), maintenance treatment (CA125 baseline level before medication, CT/MRI before medication, medication time, starting dose, medication cycle, drug interruption, reduction, discontinuation and reasons, disease progression and time to progression, treatment after progression, and overall survival time). Missing information was supplemented by telephone follow-up or face-to-face inquiries (if alive and accessible).

2.3 Outcomes

The outcome of primary debulking surgery (PDS) or interval debulking surgery (IDS) was assessed based on the postoperative residual lesion size records and imaging data. The classification of the residual disease is defined as R0 for no visible residual lesions after surgical treatment, R1 for postoperative residual lesions ≤ 1 cm, and R2 for postoperative residual lesions > 1 cm. The response to chemotherapy was evaluated with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (13), which categorizes responses as CR, PR, stable disease (SD), or progressive disease (PD) (13). CR is defined as the disappearance of all target lesions, with the short axis of all pathological lymph nodes reduced to <10mm (13). PR indicates a reduction of the sum of target lesion diameters by at least 30% compared with the baseline level (13). SD lies between PR and PD, signifying neither sufficient shrinkage to qualify for PR nor an increase in lesion size to qualify for PD (13). Lastly, PD is marked by a relative increase of at least 20% in the diameter sum of all measured target lesions, and an increase in the absolute value of the diameter sum of at least 5 mm. Additionally, the appearance of one or more new lesions is also considered as part of the classification for PD (13). The efficacy was assessed by PFS and OS. PFS was defined as the period from the initiation of PARPi to radiographic progression according to RECIST version 1.1 (13), death from any cause, or study cutoff. OS was defined as the time from the start of PARPi treatment to death from any cause or study cutoff. The safety of PARPi was evaluated using the Common Terminology Criteria for Adverse Events Version 5.0, (CTCAE5.0) (14), as stipulated by the National Cancer Institute of the United States in 2017. Maintenance therapy after relapse refers to the continuation of treatment after achieving CR or PR following secondary cytoreduction (SCR) or the most recent platinum-based chemotherapy for PSROC patients. It aims to prolong the time to subsequent relapse and lessen associated risk. PSROC is defined as the time between receiving platinum-based chemotherapy and tumor recurrence and progression exceeding 6 months (6, 15, 16). Furthermore, the duration of the platinum-free interval (PFI) ranging from 6 and 12 months is termed as partial platinum-sensitive recurrence, while a PFI of more than 12 months is classified as complete platinum-sensitive recurrence (17).

2.4 Follow-up

This real-world study aimed to gather information about the patient's living status, including the progression of the disease, instances of mortality and the causes of death. Furthermore, the study collected data on adverse events (AEs) experienced after

medication, such as the specific AE terms, the highest CTCAE grade reported, treatment measures employed for AEs, as well as actions taken with regards to PARPi, such as reduction, interruption, and discontinuation. The study utilized various channels for data collection, including telephone, outpatient clinic visits, WeChat groups, and QQ groups. The follow-up endpoint is recurrence, progression, death, or the study cut-off date, which is December 1, 2022.

2.5 Statistical analysis

The statistical analysis was performed with SPSS version 25.0 software. For continuous variables that followed a normal distribution, they were presented as mean ± standard deviation (mean ± SD), and independent sample t-tests were used for group comparisons. If the variables did not follow a normal distribution, they were expressed as median (Q1, Q3), and group comparisons were performed using the Kruskal-Wallis test. Categorical variables were presented as counts (n) and percentages (%), and group comparisons were conducted using the Chi-square test ($\chi 2$). Additionally, survival curves were generated using GraphPad Prism 8.0.1 software. The median follow-up time was calculated using the reverse Kaplan-Meier method. A Log-rank univariate analysis was performed to evaluate factors associated with PFS for patients. Factors with a significance level of $P<0.05$ in the univariate analysis were included in the multivariate Cox regression analysis. A significance level of $P<0.05$ was used to define statistically significant differences.

3 Results

3.1 Baseline characteristics

In this study, a total of 75 eligible patients were enrolled, with 54 (72.0%) receiving olaparib and 21 (28.0%) receiving niraparib as indicated in Figure 1 and Table 1. Among these patients, 24 patients (32.0%) were found to carry BRCA-m, while 5 patients refused genetic testing due to economic reasons. 27 patients (27/75, 36.0%) had received neoadjuvant chemotherapy (NACT) in the past. After the primary surgery, 29 patients (38.7%) had no residual lesions, and 27 patients (36%) achieved R1. Additionally, 11 patients (14.7%) were unaware of any residual lesions after PDS/IDS. Among them, 9 had prior surgeries at different medical facilities, and 2 lacked information concerning residual lesions from their surgical records. Moreover, among 75 patients, 9 cases (12.0%) received SCR after PSR, all achieving R0 status. After the last platinum-based chemotherapy, 27 out of 75 patients (36.0%) achieved CR, while 48 patients (64.0%) achieved PR. Among the cohort, 22 (29.3%) had previously received 3rd-line or more lines of chemotherapy and 29 (38.7%) had experienced partial PSR (PFI 6-12m). It is important to note that the baseline characteristics indicated a balanced and comparable distribution of characteristics between the olaparib and niraparib groups (P>0.05).

3.2 Efficacy

Out of the 75 patients diagnosed with PSROC, the median follow-up time was 20.0 months (95% CI 11.5-28.6). Among these patients, 38 experienced disease progression, and 9 died. The mPFS was 19.1 months (95% CI 8.5-29.7), while the mOS has not been reached yet (Figure 2). In the group receiving olaparib, the mPFS was 19.1 months, while in the group receiving niraparib, the mPFS was 28.2 months.

3.3 Influencing factors for PFS

A Log-rank univariate analysis was conducted to identify factors influencing PFS in patients with PSROC. It was found that age and the number of prior lines of chemotherapy were significantly associated with PFS ($P<0.05$). These factors with a significance level of P<0.05 were included in the multivariate Cox regression analysis. However, the results of the multivariate analysis

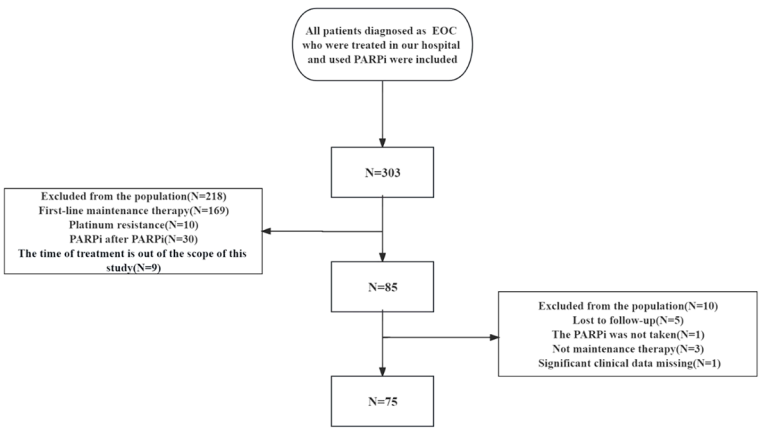

FIGURE 1
Enrollment flow diagram.

10.3389/fonc.2023.1300199

TABLE 1 Clinicopathological characteristic of PSROC patients.

Clinical characteristics	Olaparib (N=54)	Niraparib (N=21)	Statistics	*P*
Age (mean ± SD, year)	52.6 ± 9.2	52.2 ± 6.8	–	0.763
BMI (median (Q1,Q3), kg/m^2)	22.5 (21.4-23.6)	21.8 (20.6-23.5)	t=0.402	0.689
Complication, n (%)			$\chi2$ = 0.151	0.697
Yes	18 (33.3)	8 (38.1)		
No	36 (66.7)	13 (61.9)		
Family history, n (%)			$\chi2$ = 0.158	0.691
Yes	18 (33.3)	6 (28.6)		
No	36 (66.7)	15 (71.4)		
BRCA gene, n (%)			$\chi2$ = 0.413	0.814
Wild type	32 (59.3)	14 (66.7)		
Mutation type	18 (33.3)	6 (28.6)		
Unknown	4 (7.4)	1 (4.8)		
NACT, n (%)			$\chi2$ = 0.090	0.764
Yes	20 (37.0)	7 (33.3)		
No	34 (63.0)	14 (66.7)		
The residual disease			$\chi2$ = 4.175	0.243
R0	18 (33.3)	11 (52.4)		
R1	21 (38.9)	6 (28.7)		
R2	5 (9.3)	3 (14.3)		
Unknown	10 (18.5)	1 (4.8)		
Histology, n (%)			$\chi2$ = 0.089	0.765
Serous	50 (92.6)	19 (92.0)		
Others	4 (7.4)	2 (9.5)		
Previous lines of chemotherapy, n (%)			$\chi2$ = 0.009	0.924
2	38 (70.4)	15 (71.4)		
3	13 (24.1)	4 (19.0)		
≥4	3 (5.6)	2 (9.5)		
PFI, n (%)			$\chi2$ = 0.216	0.642
6-12 months	20 (37.0)	9 (42.9)		
>12 months	34 (63.0)	12 (57.1)		
Response to the last platinum-based therapy, n (%)			$\chi2$ = 1.881	0.170
CR	22 (40.7)	5 (23.8)		
PR	32 (59.3)	16 (76.2)		
SCR, n (%)			$\chi2$ = 0.000	0.987
Yes	7 (13.0)	2 (9.5)		
No	47 (87.0)	19 (90.5)		
The interval between the last chemotherapy and maintenance therapy, n (%)			$\chi2$ = 1.498	0.221
4-8 weeks	34 (63.0)	17 (81.0)		
>8 weeks	20 (37.0)	4 (19.0)		
Combined with bevacizumab in maintenance therapy, n (%)			$\chi2$ = 0.029	0.865
Yes	5 (9.3)	1 (4.8)		
No	49 (90.7)	20 (95.2)		
CA125 before PARPi, n (%)			$\chi2$ = 0.750	0.386
<35U/ml	53 (98.1)	19 (90.5)		
≥35U/ml	1 (1.9)	2 (9.5)		
Time of PARPi treatment, median (Q1,Q3)	14 (8-21)	6 (4.5-15)	–	0.056
PARPi, n (%)				
Dose reduction	21 (38.9)	4 (19.0)	$\chi2$ = 2.679	0.102
Dose interruption	13 (24.1)	9 (42.9)	$\chi2$ = 2.573	0.109
Dose discontinuation	0 (0)	2 (9.5)	–	0.157

indicated that neither age nor the number of prior lines of chemotherapy were independent factors influencing PFS in patients with PSROC (P>0.05). More detailed information can be found in Tables 2, 3.

3.4 Safety for PARPi in the real world

The safety of PARPi in real-world clinical practice was evaluated in two groups, olaparib and niraparib (refer to Table 4). In the olaparib group (N=54), the most ccommon AEs included leukopenia (30/54, 40.0%), anemia (26/54, 34.7%), vomiting (24/54, 32.0%), and thrombocytopenia (21/54, 28.0%). The most common grade ≥3 AEs were anemia (8/54, 10.7%), thrombocytopenia (4/54, 5.3%), and leukopenia (1/54, 1.3%). In niraparib group (N=21), anemia (10/21, 47.6%), vomiting (10/21, 47.6%), leukopenia (9/21, 42.9%), and nausea (9/21, 42.9%) were the most common AEs. Moreover, grade ≥3 AEs included anemia (4/21, 19.0%) and thrombocytopenia (1/21, 4.8%). Notably, no MDS/AML events or new primary malignant tumors were reported by the end of the study. Furthermore, no additional safety signals were identified.

In this single-center real-world study, approximately 29.3% of patients (22/75) interrupted treatment, 13.3% (10/75) of whom interrupted the medication due to grade ≥3 AEs, and all 10 cases

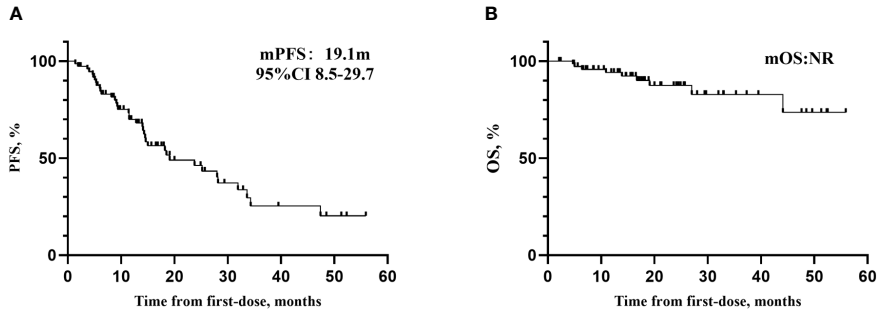

FIGURE 2
(A) Kaplan–Meier curves for PFS. **(B)** Kaplan–Meier curves for OS.

were anemia (see Table 5). Moreover, 33.3% of patients (25/75) experienced dose reduction, 20.0% (15/75) of which were associated with hematological AEs. Specifically, 5.3% (4/75) underwent dose reduction due to grade≥3 AEs, including anemia (3/75, 4.0%) and thrombocytopenia (1/75, 1.3%). Notably, no patients discontinued PARPi treatment due to AEs.

4 Discussion

PARPi has become the standard treatment for maintenance therapy in patients with PSROC, as it has successfully broken two "70%" barriers for ovarian cancer patients (4, 5). This validation was achieved through multiple large-scale Phase III RCTs. However, the complexity of the relationship between patients and doctors in the clinical practice exceeds that observed in RCTs. The relationship between healthcare providers and patients has evolved from the traditional model of passive-active and guidance-cooperation to a new model of shared participation. This model involves joint consultation to make individualized diagnosis and treatment decisions tailored to the patient's condition, while also considering the patient's preferences and economic status. Thus, the evaluation of the efficacy and safety of PARPi in real-world clinical settings, alongside the findings of large-scale RCT studies, offers enhanced external validity. This study, conducted using real-world clinical data at the largest gynecologic oncology center in Western China, reaffirmed the effectiveness and favorable tolerability of PARPi in patients with PSROC. Moreover, it contributed significantly to the growing body of knowledge on maintenance therapy for ovarian cancer and offered valuable insights for the clinical implementation of PARPi.

The initial strategy for managing PSROC focuses on prolonging the time to recurrence and diminishing the likelihood of recurrence (18). According to the 2023 version of the National Comprehensive Cancer Network(NCCN) Ovarian Cancer Guidelines, patients with PSROC who have achieved CR or PR after last platinum-based chemotherapy, and have not received PARPi before, were advised to undergo maintenance therapy with PARP inhibitors. This guideline recommends Olaparib for all PSROC patients, irrespective of their BRCA status, while limiting the use of niraparib to gBRCAm patients and rucaparib to BRCAm patients (19). The 2022 American Society of Clinical Oncology (ASCO) guidelines recommend PARPi monotherapy as a maintenance therapy after platinum-sensitive recurrence, regardless of the BRCA mutation status (20). Many studies have demonstrated the efficacy of PARPi monotherapy in PSROC patients who previously received ≥2nd-line of platinum-based chemotherapy. The mPFS was 8.4 months in Study19 (N=136, olaparib) (21), 16.1 months in L-MOCA study (N=224, olaparib) (8), 15 months in NORA study (N=177, niraparib) (9), and 12.9 months in FZOCUS-2 study (N =167, fluzoparib) (22). In this real-world study, the mPFS of overall population was 19.1 months (95%CI 8.5-29.7). Specifically, the mPFS for the olaparib and niraparib groups were 19.1 months and 28.2 months, respectively. It is noteworthy that in our center, both in the overall population and in the olaparib or niraparib group, the mPFS was longer than that in the previously mentioned large clinical trials. The follow-up time may be the reason for this difference. From the published data so far, the median follow-up time is 6.9 months (206.5 days) in Study19 (21), 15.5 months in L-MOCA study (8), 15.8 months in NORA study (9), and 8.5 months in FZOCUS-2 study (22). Notably, the follow-up time in our center was longer at 20.0 months (95%CI 11.5-28.6), which might be a reason for this difference. According to a domestic study on 106 PSROC patients, with a median follow-up of 17.5 months (95% CI 13-22), 49 patients had received PARPi for at least 12 months at the time of analysis. The mPFS from the initiation of PARPi was 21 months (95% CI 13–24.5) (23). In another study conducted in China, 48 PSROC patients who achieved CR or PR after last platinum-based chemotherapy were included. This study reported a median follow-up time of 17.8 months and a mPFS of 26.1 months (95% CI 20.2-32.1) (24). Hence, the patients with PSROC in the Chinese population experienced significant PFS benefits from PARPi. However, as the studies were non-head-to-head and the RWE was limited, the results can only be considered as a reference.

SCR in patients with PSROC is controversial (25). Whether SCR will affect the efficacy of PARPi is worth exploring. A non-randomized case-control study (26) included 46 patients with PSROC carrying BRCA-m. The case group received SCR + chemotherapy + olaparib (N=23), and the control group received chemotherapy + olaparib (N=23), the baseline data of the two groups were well balanced and comparable. The case group exhibited a significantly longer median duration of subsequent

TABLE 2 Log-rank analysis of factors associated with PFS.

Clinical characteristics		Log-Rank analysis		
		mPFS (95%CI)	χ^2	*P*
Age	<65	25.2 (13.0-37.4)	4.701	**0.030***
	≥ 65	8.9 (2.3-15.4)		
Complication	Yes	18.5 (12.4-24.6)	0.399	0.527
	No	23.8 (9.2-38.3)		
Family history	Yes	28.0 (8.3-47.7)	1.540	0.215
	No	18.2 (12.1-24.3)		
BRCA gene	Wild type	23.8 (6.7-40.8)	0.052	0.974
	Mutation type	18.2 (12.2-24.2)		
	Unknown	NE		
NACT	Yes	14.0 (3.9-24.1)	2.992	0.084
	No	28.0 (10.3-45.7)		
The residual disease	≤R1	28.2 (13.2-43.2)	4.161	0.125
	R2	8.9 (0.1-17.7)		
	unknown	18.2 (11.9-24.5)		
Previous lines of chemotherapy	2	28.0 (14.7-41.3)	7.241	**0.027***
	3	23.8 (9.9-37.6)		
	≥4	5.3 (.0-12.7)		
Response to the last platinum-based therapy	CR	25.2 (10.7-39.8)	1.172	0.279
	PR	14.6 (9.7-19.6)		
PFI	6-12months	18.5 (3.8-33.1)	0.291	0.589
	>12months	19.1 (1.7-36.6)		
SCR	Yes	31.9 (NE)	0.482	0.487
	No	19.1 (7.3-30.9)		
The interval between the last chemotherapy and maintenance therapy	4-8 weeks	23.8 (11.1-36.4)	0.067	0.795
	>8weeks	19.1 (1.5-36.8)		
The type of PARPi	Olaparib	19.1 (8.7-29.6)	0.000	0.986
	Niraparib	28.2 (0.3-56.1)		
Combined with bevacizumab in maintenance therapy	Yes	NE	0.020	0.887
	No	19.1 (8.3-30.0)		
PARPi interruption	Yes	15.0 (11.5-18.5)	0.109	0.741
	No	25.2 (12.0-38.4)		
PARPi reduction	Yes	47.4 (5.8-89.0)	3.618	0.057
	No	18.5 (12.2-24.8)		

* The factor with a significance level of P<0.05 was included in the multivariate analysis.

treatment compared to the control group (42 months versus 16 months, *P* =0.05). Furthermore, the 3-year survival rate after recurrence was significantly higher in the case group than in the control group (79% V.S. 42%, *P* =0.02). A RWS in China included 106 PSROC patients with 19 patients (17.9%) receiving SCR after relapse. COX regression analysis indicated that receiving SCR was not significantly associated with prolonged PFS (HR=0.88, 95% CI 0.38-2.00, *P*=0.761). A phase II RCT (27) (NCT03983226) is currently underway to investigate the potential benefits of niraparib for PSR ovarian cancer patients undergoing SCR. In this study, among the patients with PSROC and achieving CR or PR after the last chemotherapy, 9 cases underwent SCR after recurrence. The SCR + chemotherapy group (N=9) demonstrated a prolongation of mPFS by 12.8 months (31.9 months V.S. 19.1 months) compared to the chemotherapy-only group (N=57). This outcome suggested a potential benefit of adding SCR to chemotherapy for PSR ovarian cancer patients. However, further evidence from high-quality clinical trials is needed to determine whether the use of PARPi would weaken the effect of SCR.

PFI is used to measure the sensitivity of platinum-based drugs. In the L-MOCA study conducted on the Asian-Pacific Chinese population (8), the mPFS in the complete platinum-sensitive group (N=70) was 20.9 months (95% CI 16.2-24.1), while that in the partial platinum-sensitive group (N=67) was 9.3 months (95% CI 8.3-14.1). Patients with a PFI >12 months showed a potential trend towards benefit. However, in the NORA study (9), the risk of disease progression or death who taking niraparib was reduced by 69% (95%CI 0.17-0.55) and 67% (95% CI 0.22-0.51) in the partial platinum-sensitive group and the complete platinum-sensitive group. Interestingly, the study revealed that PFI>12 months did not have a significant impact on PFS in patients. Additionally, the forest plot presented in Study 19 (21) did not show a significant difference in PFS between partial platinum-sensitive and complete platinum-sensitive patients. A European multi-center retrospective study (28) included 114 patients with recurrent ovarian cancer carrying BRCA mutation. In patients with a PFI<12 months(N=40), the mPFS was 10.4 months (95% CI 6.3-17.1), while in patients with a PFI≥12 months (N=74), the mPFS was 18.0 months (95% CI 10.1-26.8). Compared to patients with a PFI<12 months, patients with a PFI≥12 months showed a significant extension in PFS (HR=0.5, 95% CI 0.6-0.8, *P*<0.01). In this study, the mPFS was 18.5m for patients with PFI of 6-12 months, and 19.1 months for patients with PFI>12 months, and the difference was not statistically significant (Log-rank, $\chi 2$ = 0.291, *P*=0.589). Further exploration is needed to determine whether the degree of platinum sensitivity affects the efficacy of PARPi in PSROC patients.

In this RWS, the mPFS of BRCA-m group (N=24) and BRCA-wt group (N=46) were 18.2 months and 23.8 months, respectively. There was no statistically significant difference in PFS between patients (Log-rank, $\chi 2$ = 0.052, *P*=0.974). The reasons for the analysis are as follows: (1) The proportion of partially platinum-sensitive patients in the BRCA-m group was found to be higher than that in the BRCA-wt group (45.8% V.S. 34.8%). (2) This observation may be attributed to factors such as the relatively small sample size of the subgroup and data immaturity. In addition, it is pertinent to note that 5 patients in our center declined genetic

TABLE 3 COX analysis of factors associated with PFS.

Clinical characteristics	COX analysis							
	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI	
							Lower	Upper
Age(<65y V.S.≥65y)	0.678	0.540	1.572	1	0.210	1.969	0.683	5.680
Previous lines of chemotherapy			3.126	2	0.209			
2 V.S. 3	0.284	0.387	0.538	1	0.463	1.328	0.622	2.834
2 V.S. ≥4	0.966	0.573	2.842	1	0.092	2.628	0.855	8.085

testing due to economic constraints. Nevertheless, it is noteworthy that the completion rate of BRCA gene testing reached 93.3%. This high completion rate underscored our center's strict adherence to diagnostic and treatment guidelines, as well as our commitment to patient education. The Society of Gynecologic Oncology (SGO) in 2021 released the latest data from the NOVA study (29, 30), indicating that non-BRCAm patients in the niraparib group exhibited a 5.4-month reduction in mOS compared to the control group (31.1 months V.S. 36.5 months).This suggests that niraparib maintenance therapy in patients without BRCA-m may be associated with a detrimental effect on OS (HR=1.10, 95% CI 0.831-1.459). Hence, it is evident that while patients without BRCA-m may potentially benefit from niraparib in terms of PFS, there is a decreasing trend in OS. Consequently, the first version of the NCCN guidelines (19) in 2023 was revised to specify that niraparib is restricted to gBRCAm patients. Furthermore, the 2022 ASCO meeting highlighted the need to carefully consider the balance between potential PFS benefits and OS decline when utilizing niraparib maintenance therapy for patients with non-BRCA mutations (20). The NORA study, which focused on the Chinese population and utilized individualized starting doses, demonstrated OS benefits for the entire population receiving niraparib as maintenance therapy, regardless of BRCA gene status after applying inverse probability weighting (10). The survival differences between the NOVA and NORA studies are summarized in Table 6. This suggested that the individualized starting doses used in the NORA study may have contributed to the observed OS benefits, which is an important consideration when evaluating the efficacy of niraparib as a maintenance therapy. In conclusion, it is currently uncertain whether the NCCN guidelines will impose more stringent restrictions on the utilization of PARP inhibitors in the PSROC population. Nevertheless, irrespective of the guidelines, healthcare professionals should prioritize patient education, emphasize the importance of genetic test in genetic assessment, efficacy, and prognosis, and promote patients' willingness to undergo HRD testing. Additionally, after examining studies including NOVA study (30), SOLO-2 study (7), and Study 19 (21), it is evident that there is a limited representation of Chinese patients in these global clinical trials. The clinical trial evidence for PARP inhibitors approved by the U.S. Food and Drug Administration (FDA) may differ from that approved by the China National Medical Products Administration (NMPA). We look forward to the development of more multi-center clinical studies conducted on Chinese and Asian populations.

The majority of advanced ovarian cancer patients experience recurrent or progressive disease, leading to a gradual shortening of the PFI after multiple lines of chemotherapy, and ultimately developing drug resistance. Therefore, in our study, we conducted a subgroup analysis based on the number of prior lines of chemotherapy. An analysis of the data showed that 70.4% of patients had received 2nd-line chemotherapy, 24.1% had received 3rd-line chemotherapy, and 5.6% had received more than 4th-line chemotherapy. Furthermore, a trend was revealed wherein patients receiving 2nd-line chemotherapy exhibited a potentially enhanced PFS compared to those who had undergone 3rd-line or more than 4th-line chemotherapies. (2nd-line V.S. 3rd-line V.S. ≥ 4th-line: 28.0 months V.S. 23.2 months V.S. 5.3 months). In the L-MOCA study (23), patients who previously received 2nd-line chemotherapy demonstrated a mPFS of 9.2 months longer than patients who previously received 3rd-line chemotherapy (18.0 months V.S. 8.8 months). In a RWS involving 234 PSROC patients with BRCA-m treated with olaparib (31), the median follow-up time was 15.5 months (95% CI 13.0-18.2). Patients who had received 2nd-line chemotherapy had a longer PFS than those who had received 3rd-line or more chemotherapy, with mPFS of 16.6 months, 15.5 months, and 8.2 months, respectively (2nd-line V.S. 3rd-line: HR=1.9, 95% CI 1.1-3.6, P=0.03; 2nd-line V.S. 3rd-line: HR=2.5, 95% CI 1.34-4.8, P=0.004). It is expected to increase the sample size, lengthen the follow-up time, and further explore the impact of the number of prior lines of chemotherapy on the prognosis of patients.

In this study, the maturity of PFS data in the PSR population was 50.7% (38/75). Among patients who achieved CR and PR after the last chemotherapy, the mPFS was 25.2 months (95%CI 10.7-39.8) and 14.6 months (95%CI 9.7-39.8). Notably, the mPFS of the CR group was 10.6 months longer than that of the PR group. However, a statistical analysis revealed no significant difference in PFS between the CR and PR groups (Log-rank, $\chi 2$ = 1.172, P=0.279), which could be attributed to the sample size and follow-up time. In the subgroup analysis of the SOLO2 study (32), the mPFS of patients who achieved CR at the last chemotherapy (N=91) has not yet reached, while the mPFS of the PR group (N=105) was 13.8 months. The risk of disease progression or death was reduced by 74% (HR=0.26, 95%CI 0.16-0.42) and 63% (HR=0.37, 95%CI 0.25-0.54), respectively. At the same time, the CR group showed a significant benefit compared with the PR group.

TABLE 4 Common AEs for olaparib and niraparib in the real world.

Terms	Olaparib(N=54)		Niraparib(N=21)	
	N(%)	≥G3(%)	N(%)	≥G3(%)
Hematological system				
Anemia	26(34.7)	8(10.7)	10(47.6)	4(19.0)
Leukopenia	30(40.0)	1(1.3)	9(42.9)	0
Thrombocytopenia	21(28.0)	4(5.3)	5(23.8)	1(4.8)
Gastrointestinal system				
Nausea	22(29.3)	0	9(42.9)	0
Vomiting	24(32.0)	0	10(47.6)	0
Diarrhea	4(5.3)	0	2(9.5)	0
Constipation	10(13.3)	0	3(14.3)	0
Abdominal pain	0	0	0	0
Loss of appetite	20(26.7)	0	6(28.6)	0
Fatigue	10(13.3)	0	6(28.6)	0
Infection and invasive disease				
Upper respiratory tract infection	6(8.0)	0	1(4.8)	0
Urinary tract infection	2(2.7)	0	4(19.0)	0
Pneumonia	1(1.3)	0	0	0
Neurological System				
Dizziness/Headache	0	0	0	0
Sleeping disorders	13(17.3)	0	4(19.0)	0
Cardiovascular System				
Tachycardia	5(6.7)	0	4(19.0)	0
Hypertension	0	0	2(9.5)	0
Abdominal liver and kidney function				
Elevated transaminases	6(8.0)	0	5(23.8)	0
Elevated creatinine	14(18.7)	0	5(23.8)	0
Kidney failure	0	0	0	0
Others				
Muscle, skeletal and joint pain	10(13.3)	0	1(4.8)	0
Dermatitis, rash, photosensitivity	3(4.0)	0	3(14.3)	0
Oral ulcers, oral mucositis	8(10.7)	0	1(4.8)	0

This finding aligned with the results of the NORA study (9), wherein similar trends were observed. In the NORA study (9), patients in the CR and PR groups experienced a reduction in the risk of disease progression or death by 74% (95%CI 0.15-0.45) and 67% (95%CI 0.21-0.52), respectively. In the L-MOCA study (8), the mPFS for the CR group (N=43) was 19.7 months (95%CI 15.8-22.2), while that of the PR group was 13.9 months (95%CI 11.0-16.6). The mPFS of the CR group was 5.8 months longer than that of the PR group. According to Study19 (21), the risk of disease progression or death in CR patients decreased by 54% (HR=0.46, P<0.001). Additionally, several real-world studies based on the Chinese population also found that achieving CR after the last chemotherapy was associated with improved PFS (31, 33). After 15.5 months (95%CI 13.0-18.2) follow-up, a study of 234 PSROC patients with BRCA-m treated with olaparib showed that the mPFS for patients who achieved CR, PR, SD and PD after last chemotherapy was 33.4 months, 10.4 months, and 9.2 months, respectively (CR V.S. PR: HR=3.1, 95% CI 1.6-5.8, P=0.001; CR V.S. SD+PD: HR=2.7, 95% CI 1.2-6.1, P=0.017). This indicates that patients who achieved CR had significant PFS benefit compared to

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TABLE 5 Common AEs for PARPi Interruption, Reduction, and Discontinuation.

Terms	Dose interruption		Dose reduction		Dose discontinuation	
	N(%)	≥G3(%)	N(%)	≥G3(%)	N(%)	≥G3(%)
	22(29.3)	10(13.3)	25(33.3)	4(5.3)	0	0
Hematological system						
Anemia	10(13.3)	10(13.3)	5(6.7)	3(4.0)	0	0
Leukopenia	2(2.7)	0	4(5.3)	0	0	0
Thrombocytopenia	5(6.7)	0	2(2.7)	0	0	0
Bone marrow suppression	1(1.3)	0	4(5.3)	1(1.3)	0	0
Gastrointestinal system						
Nausea	0	0	3(4.0)	0	0	0
Vomiting	1(1.3)	0	0	0	0	0
Diarrhea	0	0	0	0	0	0
Constipation	0	0	0	0	0	0
Abdominal pain	0	0	0	0	0	0
Loss of appetite	1(1.3)	0	2(2.7)	0	0	0
Fatigue	0	0	0	0	0	0
Infection and invasive disease						
Upper respiratory tract infection	0	0	0	0	0	0
Urinary tract infection	1(1.3)	0	0	0	0	0
Neurological System						
Dizziness/Headache	0	0	0	0	0	0
Sleeping disorders	0	0	0	0	0	0
Cardiovascular System						
Tachycardia	0	0	0	0	0	0
Hypertension	0	0	0	0	0	0
Abdominal liver and kidney function						
Elevated transaminases	0	0	0	0	0	0
Elevated creatinine	1(1.3)	0	5(6.7)	0	0	0
Kidney failure	0	0	0	0	0	0

those with PR or SD+PD. (CR V.S. PR: HR=3.1, 95%CI 1.6-5.8, *P*=0.001; CR V.S. SD+PD: HR=2.7, 95%CI 1.2-6.1, *P*=0.017) (31). That is, patients who achieved CR with the last chemotherapy had a prolonged PFS compared with patients with PR or SD+PD. In a RWS with a total of 106 patients, 47 patients achieved CR and 59 patients achieved PR, with a median follow-up time of 17.5 months (95% CI: 13-22) (23). The study revealed that achieving CR after the last chemotherapy was an independent factor influencing PFS in patients with PSROC (HR=0.42, 95% CI 0.21-0.85, *P*=0.016). This finding was supported by another RWS of olaparib in 97 patients with PSROC, which found that after 13 months of follow-up, the risk of disease progression or death in patients who achieved CR decreased by 58.6% (HR=0.414, 95% CI 0.205 -0.836, *P*=0.014) (33). Therefore, it is evident that the achievement of CR following the last chemotherapy has a substantial impact on PFS in patients with PSROC.

Maintenance therapy aims to achieve long-term disease control, and the timely identification, continuous monitoring, and effective management of AEs directly influence patient compliance with the medication, thereby impacting treatment efficacy. PARP inhibitor-related AEs commonly occur within the first three months of treatment and are primary reasons for patients to adjust the drug dosage, interrupt treatment, or even discontinue the medication (34, 35). Based on the real-world safety data of patients in our hospital receiving PARP inhibitors (PARPi), this study found that the most common adverse events (AEs) were hematologic toxicity and gastrointestinal reactions. Hematologic toxicity was the most common grade ≥3 AE. This may be attributed to the fact that PARPi

TABLE 6 The survival comparison between NOVA and NORA.

Study	NOVA (30)				NORA (10)			
Groups	Niraparib	Placebo	HR	95%CI	Niraparib	Placebo	HR	95%CI
BRCA-m	43.5	41.6	0.93	0.63-1.34	NR	42.1	0.88	0.39-2.01
Non-BRCAm	31.1	36.5	1.10	0.83-1.46	43.1	32.6	0.62	0.37-1.51
ITT*	38.5	39.1	N/A	N/A	46.3	34.3	0.69	0.45-1.07

*ITT, intention-to-treat population. N/A,not available.

can target PARP enzymes involved in various physiological processes, including the regulation of cell differentiation in the bone marrow or hematopoietic system by PARP1, and the involvement of PARP2 in the regulation of red blood cell production (36, 37). Different PARP inhibitors can cause varying hematologic toxicities (34, 35). In our center, the population receiving olaparib, the incidence of grade ≥3 anemia was 10.7% (8/54). In the SOLO-1 study (5), the incidence of grade ≥3 anemia was 22%, while in the SOLO-2 study (7), it was 19%. The incidence of grade ≥3 leukopenia was 1.3% (1/54) in olaparib group in this RWS, compared to 2% in the SOLO-2 study (7). The incidence of grade≥3 thrombocytopenia in olaparib group was 5.3% (4/54), while in the SOLO-1 study (5) and SOLO-2 study (7) the incidence of grade≥3 thrombocytopenia was 1%. The incidence of hematological AEs of olaparib is basically consistent with the data of large clinical trials. In this study, 4 patients taking niraparib had grade ≥ 3 anemia (4/21, 19.0%), and 1 patient had grade≥3 thrombocytopenia (1/21, 4.8%). In the PRIMA study (38) and NOVA study (30), the incidence rates of grade ≥3 anemia were 31% and 25%, and the incidence rates of grade ≥3 thrombocytopenia were 29% and 34%, respectively. Compared with the data of large clinical trials, the incidence of hematological in niraparib group in our center is relatively low. This may be related to the individualized starting dose administration, and the rigorous monitoring and management of complete blood counts. Notably, none of the patients taking niraparib discontinued treatment due to thrombocytopenia at the end of the follow-up period, in contrast to 4% reported in the NORA study (9, 10) and 14.7% in the NOVA study (30).

MDS/AML is a delayed adverse event associated with PARPi therapy. Morice et al. (39) conducted a meta-analysis of 28 randomized controlled trials (RCTs) of PARP inhibitors published between March 2012 and April 2020 to assess the occurrence of MDS/AML associated with PARPi and found that all cases of MDS/AML were observed in ovarian cancer patients. The World Health Organization's VigiBase database from 2015 to 2020 revealed a total of 178 cases of MDS/AML associated with the use of PARPi. Among these cases, 58 patients developed MDS/AML after their initial use of PARPi, with a median latency period of 17.8 months (39, 40). In the SOLO2 study (7), there were 4 cases of MDS/AML in the olaparib group. In the NOVA study (29, 30), the niraparib group had 5 cases of MDS/AML. In the PAOLA-1 study (41), the olaparib + bevacizumab group had 5 cases of MDS/AML. In the ARIEL-3 study (42), the rucaparib group had 4 cases of MDS/AML. As of the follow-up endpoint, no cases of MDS/AML have been observed in patients receiving olaparib or niraparib in our center. However, as a delayed AE, it is necessary to be vigilant against MDS/AML in the follow-up time. Once it occurs, the treatment should be stopped immediately and go to the hematology department (35). Currently, there is a lack of real-world clinical studies on the safety monitoring for various PARP inhibitors in China. We look forward to more research in this area.

5 Limitation

Due to the small sample size, this single-center real-world clinical study has limitations in performing more specific subgroup analyses. Additionally, the insufficient data maturity, particularly with regards to the OS data, demands long-term follow-up to analyze the factors influencing the clinical benefit of PARPi. Additionally, this study primarily relied on data collection from the West China Second University Hospital's Hospital Information System (HIS), and it was retrospective in nature, which led to some limitations in the collection of safety data, such as the exact time when the AEs started and stopped during the PARPi period, the investigator's assessment of the causal relationship between the AEs and PARPi, the assessment of the causal relationship with other medications, the treatment measures taken for the AEs, and the outcome. Further standardization is needed in the collection and administration of safety data. The 2023 NCCN guidelines recommend explicitly determining the HRD status. For BRCA-wt patients, testing for HRD can improve patient prognosis. However, our center is located in the western China with relatively less-developed economy. Most patients were unable to complete HRD testing due to the high cost of HRD testing and the limited availability of HRD testing kits in the domestic market, especially for those who have completed BRCA testing. Therefore, this study did not analyze HRD-related data.

6 Conclusion

This real-world study confirmed the efficacy and safety of olaparib and niraparib in the treatment of PSROC. No MDS/AML was observed in this study. However, it remains necessary to exercise close follow-up to remain vigilant for the occurrence of secondary tumors. The importance of genetic testing is emphasized, and it is encouraged to improve HRD testing for non-BRCAm patients to guide treatment and improve patient prognosis.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

This study followed the Declaration of Helsinki and was approved by the Ethics Committee of West China Second Hospital of Sichuan University (approval number: 20220129). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because Informed consent was waived by competent authorities due to the anonymized nature of patient data and the retrospective design of the study.

Author contributions

RY: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. JC: Data curation, Formal analysis, Methodology, Software, Writing – original draft. MZ: Data curation, Investigation, Writing – original draft. KL: Formal analysis, Methodology, Software, Writing – original draft. YD: Data curation, Writing – original draft. XL: Validation, Writing – original draft. LZ: Validation, Writing – original draft. QL: Supervision, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Giannini A, Di Dio C, Di Donato V, D'oria O, Salerno MG, Capalbo G, et al. PARP inhibitors in newly diagnosed and recurrent ovarian cancer. *Am J Clin Oncol* (2023) 46(9):414–9. doi: 10.1097/COC.0000000000001024

3. DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim BG, Oaknin A, et al. SOLO1 investigators. Overall survival with maintenance olaparib at a 7-year follow-up in patients with newly diagnosed advanced ovarian cancer and a BRCA mutation: the SOLO1/GOG 3004 trial. *J Clin Oncol* (2023) 41(3):609–17. doi: 10.1200/JCO.22.01549

4. Ray-Coquard I, Leary A, Pignata S, Cropet C, González-Martín A, Marth C, et al. PAOLA-1/ENGOT-ov25 investigators. Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. *Ann Oncol* (2023) 34(8):681–92. doi: 10.1016/j.annonc.2023.05.005

5. O'Cearbhaill RE, Pérez-Fidalgo JA, Monk BJ, Tusquets I, McCormick C, Fuentes J, et al. Efficacy of niraparib by time of surgery and postoperative residual disease status: A *post hoc* analysis of patients in the PRIMA/ENGOT-OV26/GOG-3012 study. *Gynecol Oncol* (2022) 166(1):36–43. doi: 10.1016/j.ygyno.2022.04.012

6. Li N, Zhu J, Yin R, Wang J, Pan L, Kong B, et al. Treatment with niraparib maintenance therapy in patients with newly diagnosed advanced ovarian cancer: A phase 3 randomized clinical trial. *JAMA Oncol* (2023) 9(9):1230–7. doi: 10.1001/jamaoncol.2023.2283

7. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. SOLO2/ENGOT-Ov21 investigators. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol* (2017) 18(9):1274–84. doi: 10.1016/S1470-2045(17)30469-2

8. Gao Q, Zhu J, Zhao W, Huang Y, An R, Zheng H, et al. Olaparib maintenance monotherapy in asian patients with platinum-sensitive relapsed ovarian cancer: phase III trial (L-MOCA). *Clin Cancer Res* (2022) 28(11):2278–85. doi: 10.1158/1078-0432.CCR-21-3023

9. Wu XH, Zhu JQ, Yin RT, Yang JX, Liu JH, Wang J, et al. Niraparib maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer using an individualized starting dose (NORA): a randomized, double-blind, placebo-controlled phase III trial☆. *Ann Oncol* (2021) 32(4):512–21. doi: 10.1016/j.annonc.2020.12.018

10. Wu XH, Zhu JQ, Yin RT, Yang JX, Liu JH, Wang J, et al. Overall survival of niraparib with individualized starting dose as maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer adjusted for subsequent PARPi use in placebo group: Results from an *ad hoc* interim analysis for the phase III NORA study. *2023 ESMO Gynecological Cancers Congress abstract 35O.* (2023) 8(1S1):4. doi: 10.1016/j.annonc.2020.12.018

11. Yang S, Ma LT, Zhang QQ. Chinese practice algorithm on real world study of clinical medicine [J]. *PLA Med J* (2018) 43(01):1–6. doi: 10.3969/j.issn.1674-7380.2017.04.001

12. Wang RP. Critical points of real world clinical study classification and design [J]. *Shanghai Med Pharm J* (2022) 43(23):63–7.

13. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026

14. US Department of Health and Human Services. *Common terminology criteria for adverse events. Version 5.0. Published November 27, 2017.* Available at: https://ctep.

cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.

15. Tapia G, Diaz-Padilla I. Molecular mechanisms of platinum resistance in ovarian cancer. In: Diaz-Padilla I, editor. *Ovarian cancer: A clinical and translational update. Rijeka, Croatia, inTechOpen* (2013). (Rijeka, Croatia: InTechOpen) p. 205–23. doi: 10.5772/55562

16. Del Campo JM, Matulonis UA, Malander S, Provencher D, Mahner S, Follana P, et al. Niraparib maintenance therapy in patients with recurrent ovarian cancer after a partial response to the last platinum-based chemotherapy in the ENGOT-OV16/NOVA trial. *J Clin Oncol* (2019) 37(32):2968–73. doi: 10.1200/JCO.18.02238

17. Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C, et al. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2013) 6:vi24–32. doi: 10.1093/annonc/mdt333

18. Kong BH, Liu JH, Huang H, Wang XY, Lu X, Li N, et al. Clinical practice guidelines for PARP inhibitors in ovarian cancer (2022 edition) [J]. *Prog Obstet. Gynecol* (2022) 31(08):561–72. doi: 10.13283/j.cnki.xdfckjz.2022.08.001

19. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). *Ovarian cancer, including fallopian tube cancer and primary peritoneal cancer*. Fort Washington: National Comprehansive Cancer Network (2023).

20. Tew WP, Lacchetti C, Kohn EC. PARP inhibitors in the management of ovarian cancer guideline expert panel. Poly(ADP-ribose) polymerase inhibitors in the management of ovarian cancer: ASCO guideline rapid recommendation update. *J Clin Oncol* (2022) 40(33):3878–81. doi: 10.1200/JCO.22.01934

21. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med* (2012) 366(15):1382–92. doi: 10.1056/NEJMoa1105535

22. Li N, Zhang Y, Wang J, Zhu J, Wang L, Wu X, et al. Fuzuloparib maintenance therapy in patients with platinum-sensitive, recurrent ovarian carcinoma (FZOCUS-2): A multicenter, randomized, double-blind, placebo-controlled, phase III trial. *J Clin Oncol* (2022) 40(22):2436–46. doi: 10.1200/JCO.21.01511

23. Zhang D, Li S, Zhang X, Peng J, Zhang S. What predicts the clinical benefits of PARP inhibitors in platinum-sensitive recurrent ovarian cancer: A real-world single-center retrospective cohort study from China. *Front Oncol* (2022) 12:955124. doi: 10.3389/fonc.2022.955124

24. Wang DF, Shi XW, Zhang C, Zhang J, Liu H, Huang JM, et al. Real-world applications of PARPi maintenance therapy for recurrent ovarian cancer: A single-center study in China. *Gynecol Oncol* (2023) 170:25–31. doi: 10.1016/j.ygyno.2022.12.014

25. Chen YL, Shi TY, Zang RY, et al. Problems related to surgery for platinum-sensitive relapsed ovarian cancer and standardized diagnosis and treatment. *Chin J Pract Gynecol. Obstet.* (2021) 37(06):616–9. doi: 10.19538/j.fk2021060105

26. Marchetti C, Rosati A, Scaletta G, Pietragalla A, Arcieri M, Ergasti R, et al. Secondary cytoreductive surgery in platinum-sensitive recurrent ovarian cancer before olaparib maintenance: Still getting any benefit? A case-control study. *Gynecol Oncol* (2019) 155(3):400–5. doi: 10.1016/j.ygyno.2019.09.020

27. Shi T, Yin S, Zhu J, Zhang P, Liu J, Zhu Y, et al. A phase II trial of cytoreductive surgery combined with niraparib maintenance in platinum-sensitive, secondary recurrent ovarian cancer: SGOG SOC-3 study. *J Gynecol Oncol* (2020) 31(3):e61. doi: 10.3802/jgo.2020.31.e61

28. Labidi-Galy SI, de la Motte Rouge T, Derbel O, Wolfer A, Kalbacher E, Olivier T, et al. Clinical factors associated with prolonged response and survival under olaparib as maintenance therapy in BRCA mutated ovarian cancers. *Gynecol Oncol* (2019) 155 (2):262–9. doi: 10.1016/j.ygyno.2019.09.008

29. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. ENGOT-OV16/NOVA investigators. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N Engl J Med* (2016) 375(22):2154–64. doi: 10.1056/NEJMoa1611310

30. Matulonis UA, Herrstedt J, Oza A, Mahner S, Redondo A, Berton D, et al. Long-term safety and secondary efficacy endpoints in the ENGOT-OV16/NOVA phase 3 trial of niraparib in recurrent ovarian cancer. *Gynecol. Oncol* (2021) 162, S24–S25. doi: 10.1016/S0090-8258(21)00693-4

31. Cecere SC, Giannone G, Salutari V, Arenare L, Lorusso D, Ronzino G, et al. Olaparib as maintenance therapy in patients with BRCA 1-2 mutated recurrent platinum sensitive ovarian cancer: Real world data and post progression outcome. *Gynecol Oncol* (2020) 156(1):38–44. doi: 10.1016/j.ygyno.2019.10.023

32. Oza AM, Combe P, Ledermann J, Marschner S, Amit A. T.Huzarski,et al., 965Pevaluation of tumour responses and olaparib efficacy in platinum-sensitive recurrenced ovarian cancer (PSROC) patients (pts) with or without measurable disease in the SOLO2 trial (ENGOT Ov-21), Ann. *Oncol* (2017) 28:v344. doi: 10.1093/annonc/mdx372.036

33. Zheng H, Gao Y, Guo H, Li L, Li Q, Cui H, et al. Real-world experience of olaparib treatment in patients with ovarian cancer: A chinese multicenter study. *Mol Cancer Ther* (2021) 20(9):1735–42. doi: 10.1158/1535-7163.MCT-20-1064

34. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). *Ovarian cancer, including fallopian tube cancer and primary peritoneal cancer. Version 2.2022*. Fort Washington: National Comprehansive Cancer Network (2022).

35. Wen H. Chinese expert consensus on the management of adverse reactions to PARP inhibitors (2021 edition). *Chin J Pract Gynecol. Obstet* (2021) 37(11):1119–30. doi: 10.19538/j.fk2021110111

36. Hopkins TA, Ainsworth WB, Ellis PA, Donawho CK, DiGiammarino EL, Panchal SC, et al. PARP1 trapping by PARP inhibitors drives cytotoxicity in both cancer cells and healthy bone marrow. *Mol Cancer Res* (2019) 17(2):409–19. doi: 10.1158/1541-7786.MCR-18-0138

37. Farrés J, Martín-Caballero J, Martínez C, Lozano JJ, Llacuna L, Ampurdanés C, et al. Parp-2 is required to maintain hematopoiesis following sublethal γ-irradiation in mice. *Blood.* (2013) 122(1):44–54. doi: 10.1182/blood-2012-12-472845

38. González-Martín A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. PRIMA/ENGOT-OV26/GOG-3012 investigators. *Niraparib Patients Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med* (2019) 381(25):2391–402. doi: 10.1056/NEJMoa1910962

39. Morice PM, Leary A, Dolladille C, Chrétien B, Poulain L, González-Martín A, et al. Myelodysplastic syndrome and acute myeloid leukaemia in patients treated with PARP inhibitors: a safety meta-analysis of randomised controlled trials and a retrospective study of the WHO pharmacovigilance database. *Lancet Haematol* (2021) 8(2):e122–34. doi: 10.1016/S2352-3026(20)30360-4

40. Zeng J, Yin RT. Interpretation of Myelodysplastic syndrome and acute myeloidleukaemia in patients treated with PARP inhibitors: a safety metaanalysis of randomised controlled trials and a retrospective study of the WHO pharmacovigilance database [J]. *Chin J Med Front* (2021) 13(07):128–32. doi: 10.12037/YXQY.2021.07-21

41. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. PAOLA-1 investigators. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N Engl J Med* (2019) 381(25):2416–28. doi: 10.1056/NEJMoa1911361

42. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. ARIEL3 investigators. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* (2017) 390(10106):1949–61. doi: 10.1016/S0140-6736(17)32440-6

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HE4 and CA-125 kinetics to predict outcome in patients with recurrent epithelial ovarian carcinoma: the META4 clinical trial

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HE4 and CA-125 are used for epithelial ovarian cancer (EOC) screening, diagnosis, and follow-up. Our objective was to study HE4 and CA-125 kinetics in patients treated for recurrent EOC. Serum samples were prospectively collected before the first chemotherapy cycle and every 3 months until disease progression. Data from 89/101 patients could be analyzed. At baseline, the median CA-125 and HE4 concentrations were 210 IU/L (7–10,310) and 184 pM (31–4,836). Among the 12 patients (13%) with normal CA-125 (<35 IU/L) concentration, eight had HE4 concentration ≥75 pM, and among the 16 patients with normal HE4 concentration (18%), 12 had increased CA-125 concentration. The median nadir concentrations were 31 IU/L (3–8,744) for CA-125 and 75 pM (20–4,836) for HE4. The median times to nadir were 14 (0–130) weeks for CA-125 and 12 (0–52) weeks for HE4. In multivariate analysis, CA-125 and HE4 nadir concentrations (<35 IU/L, HR 0.35, 95% CI: 0.17–0.72 and<75 pM, HR 0.40, 95% CI: 0.20–0.79) and time to CA-125 and HE4 nadir (>14 weeks, HR 0.37, 95% CI: 0.20–0.70 and >12 weeks, HR 0.43, 95% CI: 0.23–0.83) were prognostic factors of progression-free survival. More investigations on HE4 kinetics could help to better monitor patients with CA-125 concentration within normal values.

KEYWORDS

epithelial ovarian carcinoma, biomarkers kinetic, CA-125, HE4 epithelial ovarian carcinoma, HE4

1 Introduction

Epithelial ovarian cancer (EOC) is diagnosed late (advanced disease) in 75% of patients, and therefore its prognosis is poor and the 5-year overall survival rate is approximately 20–25% (1). Peritoneal invasion is a very frequent recurrence site. Besides clinical status, tumor markers are used for EOC detection, diagnosis, disease monitoring, and prognosis prediction (2). Clinical imaging (CT, PET, and MRI) has a limited value for EOC screening, diagnosis, peritoneal invasion quantification, and treatment efficacy assessment (3, 4).

Cancer antigen 125 (CA-125) is a dynamic marker of ovarian cancer. Its decrease predicts ovarian cancer cell death and response to therapy, whereas its increase is often the first indication of disease recurrence. CA-125 is the only tumor marker currently used for EOC diagnosis and follow-up. Many guidelines on CA-125 use in EOC management have been published to help with treatment decision-making (5–7). Moreover, serial CA-125 testing is commonly used to detect EOC recurrence after surgery and adjuvant therapy (8). In a meta-analysis, elevated CA-125 values correlated with disease progression in 89% of patients (9). Therefore, after treatment end, recurrence monitoring includes CA-125 measurement (7, 10). According to the Gynecologic Cancer Inter-group criteria, during serial CA-125 measurements, disease progression is suspected when CA-125 concentration doubles the upper limit of the reference range in two occasions separated by at least 1 week (11, 12). As CA-125 concentration is increased in 90% of patients with advanced EOC at diagnosis, treatment response monitoring with this serum marker is generally part of the follow-up. CA-125 half-life represents a prognostic factor for recurrence after chemotherapy. Specifically, a half-life<20 days has been associated with better disease-free survival compared with a half-life >20 days (28 months vs. 19 months) (13, 14). In patients with EOC who receive chemotherapy but not primary debulking surgery at diagnosis, CA-125 concentration normalization after three chemotherapy cycles has been correlated with better survival (15), although the number of cycles of chemotherapy remains a point of debate (16, 17). These results were confirmed by Riedinger et al. who showed that CA-125 nadir and half-life during induction chemotherapy were independent predictors of recurrence (18). Recently, it has been shown that the CA-125 ELIMination rate constant K value, defined as the CA-125 clearance during the first 100 days of chemotherapy in retrospective studies, represents a good prognostic factor of subsequent platinum-resistant disease relapse, progression-free survival (PFS), and also overall survival (15, 19).

Human epididymis protein 4 (HE4) belongs to the family of whey acidic four-disulfide core proteins (20) that are expressed in the epididymis epithelium and play a role in sperm maturation. HE4 is also strongly secreted by EOC cells (21). This marker is not increased in benign ovarian pathologies, unlike CA-125 (22). Moreover, HE4 is elevated in 50% of EOC in which the CA-125 concentration is within the normal range. Therefore, it is a more specific and sensitive EOC marker than CA-125 (23). Previous studies showed HE4's usefulness in combination with CA-125 for EOC diagnosis in women with a pelvic mass, and it is included in the Risk of Ovarian Malignancy Algorithm (24, 25). The HE4 and CA-125 combination displays increased sensitivity and specificity compared with CA-125 alone. A meta-analysis performed using data from more than 6,000 patients confirmed HE4's sensitivity and specificity for EOC diagnosis (26). An elevated pre-operative HE4 concentration in patients with known EOC has been associated with shorter overall survival (27–29), and the HE4 levels correlate with chemoresistance (30). HE4's role in EOC detection and diagnosis is well known, but it should be better studied in patients with ovarian cancer recurrence during chemotherapy (31, 32). It could be useful particularly in patients with tumors that do not express CA-125. Moreover, HE4 prognosis and predictive value should be compared with the information provided by CA-125. HE4 concentration should be analyzed also during the post-treatment follow-up to determine whether HE4 could be useful for recurrence detection.

Besides their concentration at diagnosis, CA-125 and HE4 kinetics, half-life, and nadir are relevant to predict the prognostic outcomes in primary EOC (22).

The aim of this study was to determine in patients treated for recurrent EOC the value of HE4 and/or CA-125 baseline concentrations and kinetics to predict the response to chemotherapy and the post-treatment prognosis. Our analysis showed that elevated baseline CA-125 and HE4 concentrations predicted a shorter PFS in patients with recurrent EOC. Moreover, CA-125 and HE4 nadir concentrations and the time to nadir were prognostic factors when included in the same model.

2 Materials and methods

2.1 Study design

META4 was a multicenter observational study carried out at three French Comprehensive Cancer Centers between September 2010 and September 2014 to evaluate HE4 and CA-125 kinetics in patients with recurrent EOC. The main objective of the study was to assess the prognostic value of HE4, compared to CA-125, for PFS. The secondary objective was to assess the kinetic parameters of both markers and their prognostic values.

This study (EudraCT 2010-A00152-37) was approved by the local ethics committee (CPP Sud Méditerranée). All patients provided a written informed consent before inclusion in the study. The study was performed in accordance with the Good Clinical Practice Requirements and the Helsinki Declaration.

2.2 Patients

The inclusion criteria were as follows: ≥18-year-old patients with fallopian tube, ovarian, or peritoneum EOC recurrence and programmed to receive at least three cycles of chemotherapy (first, second, or third recurrence). The main exclusion criteria were as follows: another cancer treated in the previous 5 years and number of chemotherapy lines >3.

Serum samples were prospectively collected before the first chemotherapy cycle, during treatment, and every 3 months until disease progression.

2.3 CA-125 and HE4 quantification

CA-125 and HE4 were quantified using immunoassays. HE4 in serum was measured with the commercial EIA method (Fujirebio Diagnostics, Malvern, PA, USA; www.fujirebio.com). This test is a solid-phase, non-competitive immunoassay based on the direct sandwich technique using two mouse monoclonal antibodies, 2H5 and 3D8, against two epitopes in the C-terminal WAP-type four-disulfide core (WFDC) domain of HE4. The CA-125 concentration in serum was measured by electrochemiluminescence using the CA-125 II cobas kit (Roche). The following standard cutoffs were considered: 35 IU/L for CA-125 and 75 pmol/L for HE4, as previously defined (16). The results of CA-125 and HE4 quantification were blinded and did not contribute to the therapeutic decision-making.

2.4 Endpoints and assessment

The primary endpoint was to study the prognostic value of the baseline HE4 and CA-125 serum concentrations.

The secondary endpoints were to study the HE4 and CA-125 kinetics in patients with a recurrent disease and who are receiving chemotherapy, namely: plasma concentration at baseline (C_0), half-life ($t_{1/2}$), time to normalization (t_{norm}), plasma concentration at nadir (C_{nadir}), time to nadir (t_{nadir}), doubling time (t_{d}), and time to exceed the clinical threshold (t_{ex}). The kinetic parameters (definition and calculation method) are precisely defined in Table 1. Mono-compartmental models were performed, first, with k_1, the slope associated with the decrease of the logarithm of the marker between baseline and nadir, and then with k_2, the slope associated with the increase of the logarithm of the marker after nadir. Linear regression was used to estimate k_1 and k_2 (in semi-logarithmic scale).

PFS was defined from inclusion to the date of the first documented progression or the date of death from any cause. Treatment efficacy was assessed every three cycles of chemotherapy by clinical examination and CT according to the RECIST 1.1 criteria (33). Patients with partial or complete response to chemotherapy were considered responders, whereas patients with progressive disease were considered non-responders. In responders, a follow-up visit was performed every 3 months.

2.5 Statistical analyses

Descriptive analyses were performed on the per-protocol population defined as all eligible patients with CA-125 and/or HE4 data to allow calculating the kinetic parameters before nadir (at least two assessments before nadir—including nadir—and with a decreasing slope: $\widehat{k_1} < 0$) and/or after nadir (at least two assessments after nadir—including nadir—and with an increasing

TABLE 1 Definition and calculation of kinetic parameters.

Notation	Definition	Calculation method
C_0	Plasma concentration at baseline	
$t_{1/2}$	Half-life Note: If the calculated half-life was higher than the time to nadir, it was not considered in the analysis (replaced by missing data).	Time required to observe a 50% decrease in the plasma concentration from baseline: $t_{1/2} = \frac{-\ln(2)}{k_1}$ (mono-compartmental model) with k_1 the slope associated with the decrease of the neperian logarithm of the marker between baseline and nadir. A linear regression (in semi-logarithmic scale) between baseline and nadir is used to estimate k_1 (and thus $t_{1/2}$).
t_{norm}	Time to normalization Notes: (i) It cannot be calculated if the baseline concentration is lower than the threshold; (ii) if the calculated time to normalization was higher than the time to nadir, it was not considered in the analysis (replaced by missing data).	Time required (from baseline to nadir) to observe a value below the clinical threshold C_s (i.e. 35 IU/L for CA-125 and 75 pM for HE4): $t_{norm} = \frac{\ln(C_s) - \ln(C_0)}{k_1}$ (mono-compartmental model) with k_1, the slope associated with the decrease of the neperian logarithm of the marker between baseline and nadir.
C_{nadir}	Plasma concentration at nadir	Lowest plasma concentration observed during treatment until progression (if progression occurs during treatment) or until 1 month ± 7 days (maximum 38 days) after the treatment end date (otherwise).
t_{nadir}	Time to nadir	Time from baseline to nadir.
t_d	Doubling time Note: If the calculated doubling time was higher than the time to progression or time to follow-up, it was not considered in the analysis (replaced by missing data).	Time required to observe a 100% increase in the plasma concentration at nadir (from nadir): $t_d = \frac{\ln(2)}{k_2}$ (mono-compartmental model) with k_2, the slope associated with the increase of the neperian logarithm of the marker after nadir. An estimate of k_2 (and thus t_d) is obtained using a linear regression (in semi-logarithmic scale) between nadir and progression (if progression occurs) or nadir and the last value assessed (otherwise).
t_{ex}	Time to exceed the clinical threshold (from nadir) Notes: (i) It cannot be calculated if the concentration at nadir is higher than the threshold concentration; (ii) if the calculated time to exceed the clinical threshold was higher than the time to progression or time to	Time required (from nadir) to observe a value above the clinical threshold C_s (i.e. 35 IU/L for CA-125 and 75 pM for HE4): $t_{elev} = \frac{\ln(C_s) - \ln(nadir)}{k_2}$ with k_2, the slope associated with the increase of the neperian logarithm of the marker after nadir.

(Continued)

TABLE 1 Continued

Notation	Definition	Calculation method
	follow-up, it was not considered in the analysis (replaced by missing data).	

slope: $\widehat{k_2} > 0$). Survival analyses were performed only on patients with high-grade serous carcinoma.

Categorical variables were reported as the number of observations (*N*) and the frequency (%) of each modality. Continuous variables were reported as median, minimum, and maximum.

The median follow-up was calculated using the Schemper and Smith method. PFS was estimated using the Kaplan–Meier method. Multivariate analyses were performed using Cox proportional hazards models. Variables with (univariate) *p*-values<0.05 were selected for multivariate analysis, and a backward covariate selection was performed. Hazard ratios (HR) were reported with 95% confidence intervals (CI). The two parameters "time to normalization" and "time to exceed the clinical threshold" were not included in the multivariate model due to the large number of missing values and because the analyses would have been performed on a specific subpopulation of patients. Three multivariate analyses were performed: with only the CA-125 kinetic parameters, with only the HE4 kinetic parameters, and with CA-125 and HE4 kinetic parameters and the patients' clinical characteristics. The validity of the proportional hazard assumptions was verified using Schoenfeld residuals in the final models. The Harrell's C-index (which corresponds to the percentage of concordance between prediction and outcome) was calculated to evaluate the predictive accuracy of the different models. All tests were two-sided, and *p*-values<0.05 were considered significant. Statistical analyses were performed with STATA 16.0 (StatCorp, College Station, TX, USA).

3 Results

3.1 Patients' characteristics

From September 2010 to September 2014, 101 patients were included at the three centers (intention-to-treat population). Finally, 89 patients were included in the final analysis (per-protocol population). Figure 1 summarizes the CONSORT flow chart.

The patient's characteristics and treatment data are listed in Tables 2, 3. At inclusion, the median age was 65 (34–83) years. The World Health Organization (WHO) performance status scores were 0, 1, and 2 in 40.5%, 48.3%, and 11.2% of patients, respectively; 88.5% of patients had high-grade carcinoma. The main histological sub-type was serous (87.5%).

At diagnosis, most patients (93.2%) had FIGO stage III or IV tumor, and 96.6% had undergone a surgery previously. Macroscopic residual disease was not detected in 35.3% of patients. This was the first, second, and third recurrence in 70%, 23%, and 7% of patients, respectively. Chemotherapy choice was left to the investigating physician according to the current recommendations on platinum-free interval before recurrence (shorter vs. longer than 6 months). Briefly, 64% of patients were treated with platinum-based chemotherapy [alone (4.4%) or associated with pegylated liposomal doxorubicin, paclitaxel, or gemcitabine (59.6%)], and 57% were platinum-sensitive. The other patients received mainly weekly paclitaxel (29.2%).

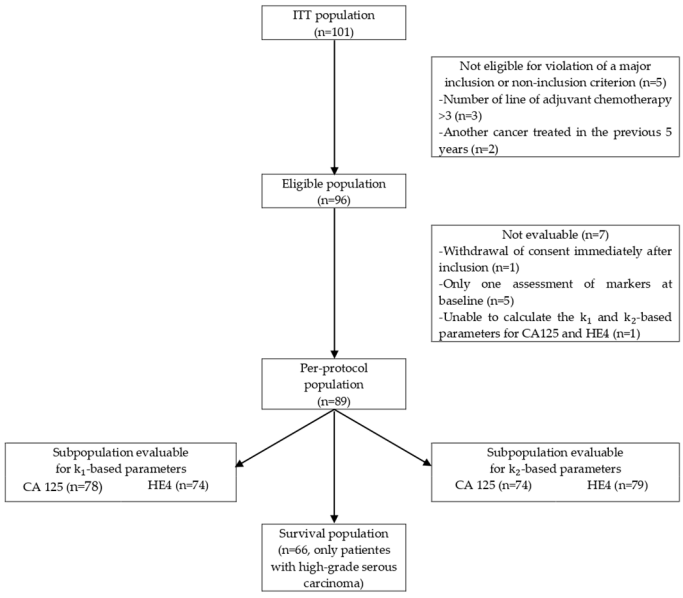

FIGURE 1
CONSORT flow chart.

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TABLE 2 Patients' demographic, clinical and histological characteristics.

	n=89	%
Center		
1 - ICM, Montpellier	72	80.9
2 - Institut Bergonié, Bordeaux	15	16.9
3 - Centre Léon Bérard, Lyon	2	2.3
Age		
Median (range)	65	(34-83)
WHO Performance status		
0	36	40.5
1	43	48.3
2/3	10	11.2
Residual disease		
No residual disease	35	35.3
≤ 1cm	37	19.6
> 1 cm	14	45.1
Missing	3	
FIGO stage		
I/II	6	6.8
III/IV	82	93.2
Missing	1	
Grade		
Low grade (1)	10	11.5
High grade (2 + 3)	77	88.5
Missing	2	
Histological type		
Serous	77	87.5
Endometrioïd	7	7.9
Undifferenciated	4	4.5
Clear Cell Carcinoma	1	0.1
CA-125 concentration (IU/L)		
Median (range)	210	7-10309
HE4 concentration (pM)		
Median (range)	184	31-4836
Creatinine clearance		
Median (range)	75	25-163
Missing	2	
Number of chemotherapy lines		
1	61	70.1
2	20	23.0
3	6	6.9
Missing	2	

(Continued)

TABLE 2 Continued

3.2 Biomarker kinetic parameters

At baseline (recurrence detection), the median CA-125 concentration was 210 IU/L (range, 7–10,310) and was ≥35 IU/L in 86.5% of patients (Table 4; Supplementary Table S1). The baseline HE4 median level was 184 pM (31–4,836), and was ≥75 pM in 82.0% of patients. The HE4 concentration was ≥75 pM in eight of the 12 patients with a normal CA-125 concentration (<35 IU/L) (Supplementary Table S2). The CA-125 concentration was increased in 12/16 patients with a normal HE4 concentration (<75 pM).

The median CA-125 concentration at nadir was 31 IU/L (3–8,744) and was ≥35 IU/L in 48.3% of patients. The median HE4 concentration at nadir was 75 pM (21–4,836), and was ≥75 pM in 50.6% of patients. At nadir time, the HE4 concentration was ≥75 pM in 14 of the 46 patients with CA-125<35 IU/L (Supplementary Table S2).

The other kinetic parameters (half-time, time to normalization, time to nadir, doubling time, and time to exceed the clinical threshold) are described in Table 4. Two examples of CA-125 and HE4 kinetics (in semi-logarithmic scale) are shown in Figure 2.

Treatment response could be assessed in all patients, and 55% were considered as responders. The baseline CA-125 and HE4 concentrations were not significantly different in responders and non-responders: 197 IU/L (7–7,341) and 217 IU/L (25–10,309) for CA-125 (p = 0.21) and 176 pM (31–2,911) and 205 pM (46–4,836) for HE4 (p = 0.38), respectively. The half-life of both markers was not different in the responders and non-responders. Conversely, the CA-125 nadir concentration was significantly lower in the responders (16 IU/L, range: 3–796) than the non-responders (115 IU/L, range: 12–8,744, p< 0.001), and the time to nadir was longer in the responders (20 weeks, range: 4–130) than in the non-responders (8 weeks, range: 0–30, p< 0.001). Similar results were observed for HE4 (Supplementary Table S1).

3.3 Pronostic factors (univariate analysis)

The analysis was performed using data from 66 patients with high-grade carcinoma. Four patients were alive without progression at the study end, and the median follow-up was 12.1 months (95% CI: 9.3–12.6). The median PFS was 8.6 months (95% CI: 6.7–10.8).

The univariate analysis (Table 5) revealed that, among the clinical variables, only WHO performance status was a significant prognostic factor of PFS (0–1 vs. 2–3: HR 2.93, 95% CI: 1.34–6.39).

High baseline CA-125 and HE4 concentrations were associated with shorter PFS (HR 2.07, 95% CI: 0.89–4.84 and HR 2.96, 95% CI:

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TABLE 3 Treatment and efficacy.

	n=89	p%
Chemotherapy regimen		
Platinum-based	57	64
Carboplatin alone	4	4.4
Carboplatin in association (PLD, paclitaxel, gemcitabin)	53	59.6
Not Platinum-based	32	36
Paclitaxel weekly	26	29.3
PLD +/- trabectedin	5	5.6
Cyclophosphamide/bevacizumab	1	0.1
Best response		
CR	10	11.6
PR	39	45.7
SD	26	30.2
PD	11	12.8
Missing	3	
Reasons for stopping treatment		
Progression	34	38.2
Toxicity	3	3.4
Patient's decision	2	2.3
Physician's decision	37	41.6
Other	13	14.6

PLD, pegylated liposomal doxorubicin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

1.24–7.06, respectively). Conversely, low CA-125 and HE4 nadir concentrations were associated with longer PFS (HR 0.23, 95% CI: 0.13–0.39 and HR 0.27, 95% CI: 0.15–0.48, respectively). For CA-125, time to nadir ≥14 weeks and time to exceed the clinical threshold ≥34.4 weeks were strong prognostic factors of longer PFS (HR 0.32, 95% CI: 0.19–0.54 and HR 0.21, 95% CI: 0.08–0.57, respectively). Half-life and doubling time were not associated with PFS.

HE4 doubling time ≥14.7 weeks, time to nadir ≥12 weeks, and time to exceed the clinical threshold ≥21.7 weeks were strong prognostic factors of longer PFS (HR 0.44, 95% CI: 0.22–0.86, HR 0.27, 95% CI: 0.16–0.45; and HR 0.33, 95% CI: 0.15-0.73, respectively). As observed for CA-125, HE4 half-life was not a prognostic factor.

The most significant prognostic parameters were baseline CA-125 and HE4 concentrations. Figure 3 shows PFS in function of the baseline and nadir CA-125 and HE4 concentrations. PFS was always worse in patients with baseline CA-125 ≥ 35 IU/L and HE4 ≥ 75 pM (52/66; HR 3.65, 95% CI: 1.74–7.68 after grouping the other modalities) and nadir CA-125 ≥ 35 IU/L and HE4 ≥ 75 pM (27/66, HR 4.62, 95% CI: 2.62–8.13 after grouping the other modalities) (Figure 4).

TABLE 4 Kinetic parameters.

	n=89	%
CA-125		
Concentration at baseline (IU/L)		
Median (min-max)	210 (7-10310)	
< 35	12	13.5
≥ 35	77	86.5
Half-life (weeks)		
Median (min-max)	6.5 (1.3-48.9)	
Missing	34	
Time to normalization (weeks)		
Median (min-max)	11.2 (2.8-20)	
Missing	57	
Nadir (IU/L)		
Median (min-max)	31 (3-8744)	
< 35	46	51.7
≥ 35	43	48.3
Time to nadir (weeks)		
Median (min-max)	14 (0-130)	
Doubling time (weeks)		
Median (min-max)	10.7 (1.1-39.9)	
Missing	34	
Time to exceed the clinical threshold (>35, weeks)		
Median (min-max)	34.4 (0.3-147)	
Missing	52	
HE4		
Concentration at baseline (pM)		
Median (min-max)	184 (31-4836)	
< 75	16	18.0
≥ 75	73	82.0
Half-life (weeks)		
Median (min-max)	8.5 (1.6-41.7)	
Missing	47	
Time to normalization (weeks)		
Median (min-max)	8 (1.8-23)	
Missing	60	
Nadir (pM)		
Median (min-max)	75 (21-4836)	
< 75	44	49.4
≥ 75	45	50.6

(Continued)

TABLE 4 Continued

	n=89	%
Time to nadir (weeks)		
Med (min-max)	12 (0-52)	
Doubling time (weeks)		
Median (min-max)	14.7 (2.1-67.3)	
Missing	36	
Time to exceed the clinical threshold (>75, weeks)		
Median (min-max)	21.7 (0.1-85.8)	
Missing	47	

3.4 Prognostic factors (multivariate analysis)

Three models were used from the multivariate analysis to identify the prognostic factors for PFS. The results are summarized in Table 5.

The multivariate analysis that included only CA-125 kinetic parameters led to a final model (model 1) with two significant factors: nadir concentration ($p< 0.001$) and time to nadir (weeks) ($p< 0.001$). Low CA-125 nadir concentration (<35 IU/L; HR 0.19, 95% CI: 0.10–0.35) and time to nadir ≥14 weeks (HR 0.27, 95% CI: 0.15–0.48) were independent favorable prognostic factors of PFS.

The multivariate analysis that included only HE4 kinetic parameters led to a final model (model 2) with three significant factors, namely: nadir concentration ($p = 0.024$), time to nadir ($p< 0.001$), and doubling time ($p = 0.004$). Low HE4 nadir concentration (≤75 pM; HR 0.44, 95% CI: 0.21–0.92), time to nadir ≥12 weeks (HR 0.20, 95% CI: 0.10–0.43), and doubling time ≥14.7 weeks (HR 0.35, 95% CI: 0.17–0.74) were independent favorable prognostic factors of PFS.

The multivariate analysis that included CA-125 and HE4 kinetic parameters and the patients' clinical characteristics led to a final model (model 3) with four significant factors, namely: CA-125 nadir concentration ($p = 0.004$), time to CA-125 nadir ($p = 0.002$), HE4 nadir concentration ($p = 0.008$), and time to HE4 nadir ($p = 0.013$). Conversely, low CA-125 nadir concentration (<35 IU/L; HR 0.35, 95% CI: 0.17–0.72), time to CA-125 nadir ≥14 weeks (HR 0.37, 95% CI: 0.20–0.70), low HE4 nadir concentration (<75 pM; HR 0.40, 95% CI: 0.20–0.79), and time to HE4 nadir ≥12 weeks (HR 0.43; 95% CI: 0.23–0.83) were favorable prognostic factors.

For the three models, the proportional hazards assumption was not violated. According to the Harrell's C-index, model 3 that included the kinetic parameters of both markers and the patients' clinical characteristics was the best model, although the index values were similar for all models (0.75, 0.77, and 0.78 for model 1, 2, and 3, respectively).

4 Discussion

Most studies on the EOC biomarkers CA-125 and HE4 focused mainly on only one of them, although the main issue should be to

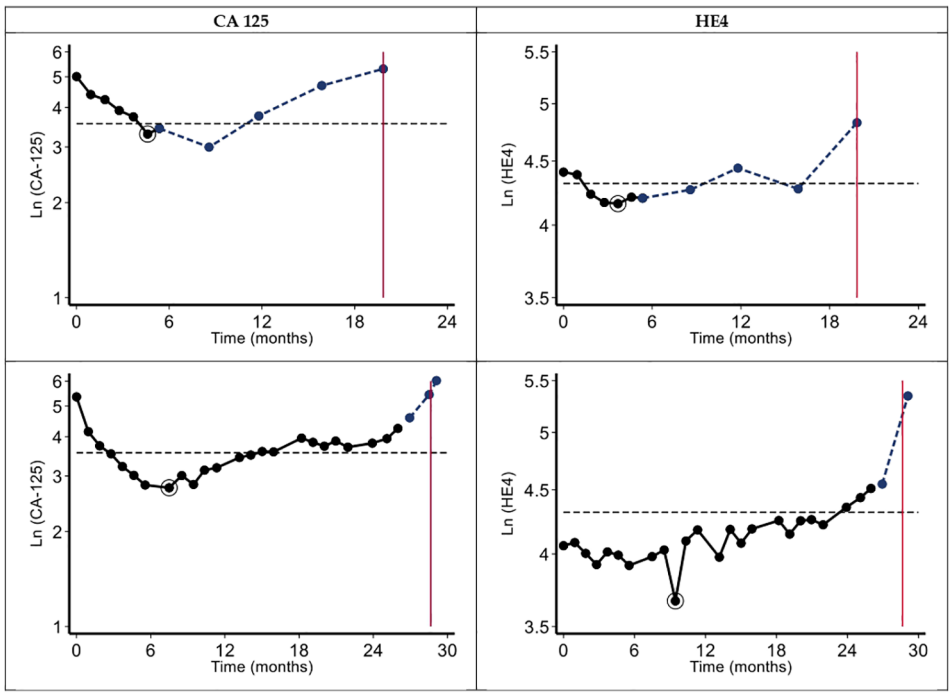

FIGURE 2
Trajectory of CA-125 and HE4. Black lines and dots represent the values of the markers observed under treatment, circled black dots show the value at nadir, blue lines and dots represent the values of the markers after the end of treatment, vertical red lines indicate the occurrence of progression, and horizontal dash lines indicate the threshold value.

TABLE 5 Results of the univariate and multivariate analyses (n = 66).

	Univariate analysis				Multivariate analysis (model 1)				Multivariate analysis (model 2)				Multivariate analysis (model 3)			
	n	HR	95% CI	*P*					n	HR	95% CI	*P*	n	HR	95% CI	*P*
Clinical characteristics																
Age (years)	66			0.386												
< 65		1														
≥ 65		1.25 [0.75, 2.08]														
WHO Performance status	66			0.016												
0-1		1														
2-3		2.93 [1.34; 6.39]														
FIGO stage	65			0.209												
I/II		1														
III/IV		2.90 [0.40; 21.13]														
Residual disease	64			0.224												
No residual disease		1														
≤ 1 cm or > 1 cm		1.39 [0.81, 2.40]														
CA-125																
Concentration at baseline (IU/l)	66			0.065												
< 35		1														
≥ 35		2.07 [0.89, 4.84]														
Time to normalization (weeks)	25			0.306												
< 11.2		1														
≥ 11.2		0.64 [0.27, 1.53]														
Half-life (weeks)	42			0.744												
< 6.5		1														
≥ 6.5		0.90 [0.47, 1.71]														
Nadir (IU/l)	66			<0.001	66			<0.001					66			0.004
< 35		1				1								1		
≥ 35		0.23 [0.13, 0.39]				0.19 [0.10, 0.35]								0.35 [0.17, 0.72]		

(Continued)

TABLE 5 Continued

	Univariate analysis				Multivariate analysis (model 1)				Multivariate analysis (model 2)				Multivariate analysis (model 3)			
	n	HR	95% CI	*P*					n	HR	95% CI	*P*	n	HR	95% CI	*P*
Time to nadir (weeks)	66			<0.001	66			<0.001					66			0.002
< 14		1				1								1		
≥ 14		0.32 [0.19, 0.54]				0.27 [0.15, 0.48]								0.37 [0.20, 0.70]		
Doubling time (weeks)	42			0.599												
< 10.7		1														
≥ 10.7		0.85 [0.46, 1.57]														
Time to exceed the clinical threshold (>35, weeks)	27			0.010												
< 34.4		1														
≥ 34.4		0.21 [0.08, 0.57]														
HE4																
Concentration at baseline (pM)	66			0.006												
< 75		1														
≥ 75		2.96 [1.24, 7.06]														
Time to normalization (weeks)	21			0.980												
< 8		1														
≥ 8		1.01 [0.39, 2.58]														
Half-life (weeks)	34			0.664												
< 8.5		1														
≥ 8.5		0.86 [0.43, 1.72]														
Nadir (pM)	66			<0.001					42			0.024	66			0.008
< 75		1								1				1		
≥ 75		0.27 [0.15, 0.48]								0.44 [0.21,0.92]				0.40 [0.20, 0.79]		
Time to nadir (weeks)	66			<0.001					42			<0.001	66			0.013
< 12		1								1				1		
≥ 12		0.27 [0.16, 0.45]								0.20 [0.10, 0.43]				0.43 [0.23, 0.83]		

(Continued)

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TABLE 5 Continued

	Univariate analysis				Multivariate analysis (model 1)				Multivariate analysis (model 2)				Multivariate analysis (model 3)			
	n	HR	95% CI	*P*					n	HR	95% CI	*P*	n	HR	95% CI	*P*
Doubling time (weeks)	42			0.0141					42			0.004				
< 14.7		1								1						
≥ 14.7		0.44 [0.22, 0.86]								0.35 [0.17, 0.74]						
Time to exceed the clinical threshold (>75, weeks)	30			0.008												
< 21.7		1														
≥ 21.7		0.33 [0.15, 0.73]														

determine what HE4 brings in addition to the well-known and universally used CA-125 marker. Moreover, many studies were carried out in neo-adjuvant settings where chemotherapy efficacy is tested in treatment-naive patients (34). On the other hand, the META 4 study assessed the prognostic values of both CA-125 and HE4 (baseline concentrations and kinetics) in patients with disease recurrence after previous chemotherapy cycles (i.e., not in adjuvant or neo-adjuvant settings). As described previously (REF), low (below the thresholds) CA-125 and HE4 nadir concentrations and long time to nadir were the main prognostic kinetic factors in addition to low grade histology.

At EOC recurrence time, prognostic factors are needed, for instance, to help with treatment decision-making, to monitor the treatment response, and to obtain information on survival. In our sample, the median baseline CA-125 concentration was 210 IU/L (7–10,310), similar to the 263 IU/L (5–52,000) concentration reported in a French multicenter study on 631 patients with EOC (18). Our study showed that both baseline CA-125 and HE4 concentrations have a high prognostic value, which is in agreement with previous studies. Elevated baseline CA-125 and HE4 concentrations predicted shorter PFS in patients with recurrent EOC: HR 2.07, 95% CI 0.89–4.84 and HR 2.96, 95% CI: 1.24–7.06, respectively (35). The same results are observed in neo-

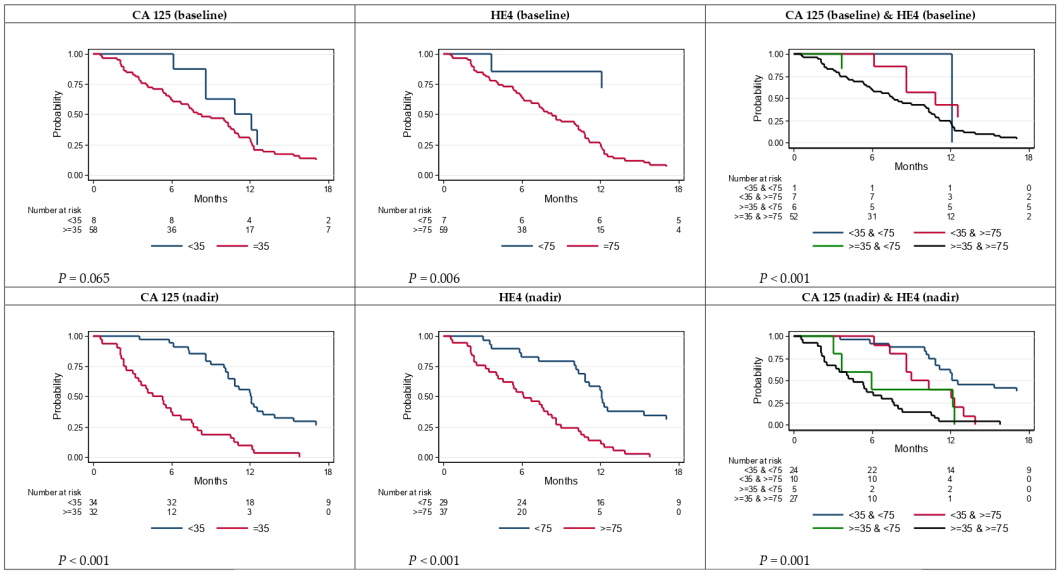

FIGURE 3
Progression-free survival according to CA-125 (baseline, nadir) and HE4 (baseline, nadir).

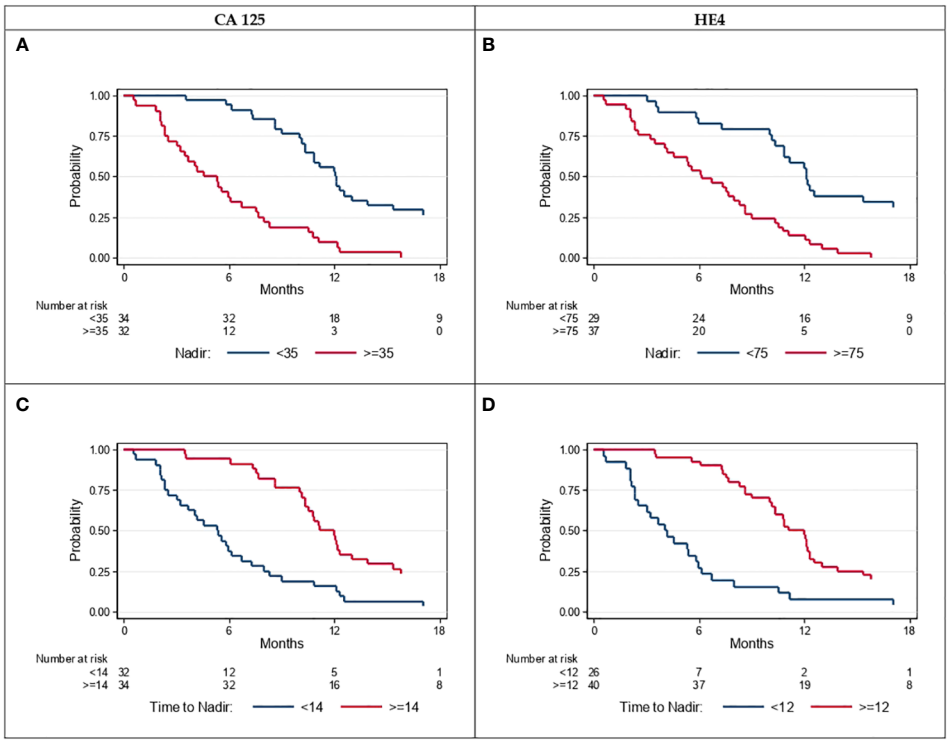

FIGURE 4
Progression-free survival according to CA-125 **(A)** threshold<35 vs. ≥35, **(C)** time to nadir<14 vs. ≥14 weeks and according to HE4 **(B)** threshold<75 vs. ≥75, **(D)** time to nadir<12 vs. ≥12 weeks.

adjuvant treatment. Sensitivity to chemotherapy was predicted by both CA-125 and HE4, as described in previous studies (36–38).

The main result of our study was provided by the multi-variate analysis showing that both CA-125 and HE4 were independent prognostic factors for PFS, as indicated by the robust hazard ratios (0.35 and 0.40 for CA-125 and HE4 nadir concentrations, respectively). The nadir concentration and the time to nadir of CA-125 and HE4 were prognostic factors when included in the same model. This means that HE4 brings additional information to CA-125 nadir and time to nadir. This novel result could justify the use of both biomarkers. The role of HE4 in patients where CA-125 kinetic data do not correlate with disease progression warrants more investigation. In some cases (e.g., oligometastatic disease), early detection of progression could allow reductive surgery.

In EOC, surgical reduction of the tumor mass followed by platinum-based chemotherapy leads to complete remission in approximately 60% of patients and to CA-125 concentration normalization in 86% of patients receiving first-line chemotherapy (39–41). The relationship between chemotherapy efficacy, CA-125 concentration decrease, and survival has been strongly validated by several studies (9, 11, 42). In a recurrent disease, complete response and/or CA-125 normalization translates into a PFS improvement (43, 44). Our study confirmed the very strong prognostic value of CA-125 nadir concentration below the threshold (<35 IU/L) [HR = 0.23 and 0.35 in the uni- and multi-variate analyses, respectively, versus HR = 0.46 in previous studies (44, 45)]. Another study found that CA-125 nadir concentration after first-line treatment was associated with PFS, but not with overall survival (46).

Conversely, our study did not find any correlation of CA-125 or HE4 baseline concentration, half-time, and time to nadir with sensitivity to platinum-based chemotherapy, unlike what we observed for first-line chemotherapy (38). This highlights the fact that kinetic parameters represent more valuable information than a single quantification (even when abnormal) (42).

Surprisingly, long time to nadir (i.e., the slope between chemotherapy onset and the nadir) correlated with longer PFS. This suggests that the time won before reaching the nadir is time added to the date of recurrence. These results are not in accordance with what we previously observed during first-line chemotherapy, particularly in neo-adjuvant settings: faster CA-125 concentration decrease was associated with better treatment efficacy and significant PFS and overall survival improvement (15, 47). In recurrent EOC, reaching disease control, even partial, is more important than reaching rapidly the biomarker nadir. This time is currently prolonged by maintenance treatment, such as bevacizumab (43) and, more recently, PARP inhibitors (48–50).

This study presents some limitations, particularly the sample heterogeneity in terms of histology, although most patients (88.5%) had serous high-grade carcinoma. The second main limitation was the heterogeneity in platinum-free interval. Indeed 64% of patients received platinum-based chemotherapy, and 57% of them were platinum-sensitive. The third limitation was the treatment heterogeneity: platinum-based chemotherapy (alone or in association) and platinum-free treatment (36%). Lastly, the power of our study was limited by the small sample size.

5 Conclusions

Our study showed that HE4 kinetic information, in addition to CA-125 kinetic data, contributes to predict the prognosis (PFS) of patients with recurrent EOC treated by chemotherapy. More studies are needed especially in patients in whom the CA-125 concentration does not correlate with the disease course.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by EudraCT 2010-A00152-37 Local ethics committee (CPP Sud Méditerranée). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MF: Conceptualization, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. P-JL: Conceptualization, Resources, Validation, Writing – original draft, Writing – review & editing. CT: Methodology, Writing – review & editing. AF: Investigation, Writing – review & editing. IR-C: Investigation, Writing – review & editing. CM: Conceptualization, Formal analysis, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1308630/full#supplementary-material

References

1. Kotsopoulos J, Rosen B, Fan I, Moody J, McLaughlin JR, Risch H, et al. Ten-year survival after epithelial ovarian cancer is not associated with BRCA mutation status. *Gynecologic Oncol* (2016) 140(1):42–47. doi: 10.1016/j.ygyno.2015.11.009

2. Sölétormos G, Duffy MJ, Hassan SOA, Verheijen RHM, Tholander B, Bast RC Jr, et al. Clinical use of cancer biomarkers in epithelial ovarian cancer: updated guidelines from the European group on tumor markers. *Int J Gynecological Cancer* (2016) 26 (1):43. doi: 10.1097/IGC.0000000000000586

3. Forstner R, Meissnitzer M, Cunha TM. Update on imaging of ovarian cancer. *Curr Radiol Rep* (2016) 4(6). doi: 10.1007/s40134-016-0157-9

4. Delvallée J, Rossard L, Bendifallah S, Touboul C, Collinet P, Bricou A, et al. Accuracy of peritoneal carcinomatosis extent diagnosis by initial FDG PET CT in epithelial ovarian cancer: A multicentre study of the FRANCOGYN research group. *J Gynecology Obstetrics Hum Reprod* (2020), 101867. doi: 10.1016/j.jogoh.2020.101867

5. Duffy MJ, Bonfrer JM, Kulpa J, Rustin GJS, Soletormos G, Torre GC, et al. CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. *Int J Gynecologic Cancer* (2005) 15(5):679–91. doi: 10.1136/ijgc-00009577-200509000-00001

6. Vasey PA, Herrstedt J, Jelic. ESMO Minimum Clinical Recommendations for diagnosis S. treatment and follow-up of epithelial ovarian carcinoma. *Ann Oncol* (2005) 16:i13–i15. doi: 10.1093/annonc/mdi823

7. Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Barroilhet L, Behbakht K, Berchuck A, et al. Ovarian cancer, version 2.2020, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Network* (2021) 19(2):191–226. doi: 10.6004/jnccn.2021.0007

8. Santillan A. Risk of epithelial ovarian cancer recurrence in patients with rising serum CA-125 levels within the normal range. *J Clin Oncol* (2005) 23(36):9338–9343. doi: 10.1200/JCO.2005.02.2582

9. Meyer T, Rustin GJS. Rustin. Role of tumour markers in monitoring epithelial ovarian cancer. *Br J Cancer* (2000) 82(9):1535. doi: 10.1054/bjoc.2000.1174

10. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. *Ann Oncol* (2019) 30(5):672–705. doi: 10.1093/annonc/mdz062

11. Rustin GJ, Marples M, Nelstrop AE, Mahmoudi M, Meyer T. Use of CA-125 to define progression of ovarian cancer in patients with persistently elevated levels. *J Clin Oncol* (2001) 19(20):4054–4057. doi: 10.1200/JCO.2001.19.20.4054

12. Rustin GJ, van der Burg ME, Griffin CL, Guthrie D, Lamont A, Jayson GC, et al. Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. *Lancet* (2010) 376(9747):1155–1163. doi: 10.1016/S0140-6736(10)61268-8

13. Bast RC Jr, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. *Int J Biol Markers* (1998) 13(4):179–87. doi: 10.1177/172460089801300402

14. Verheijen RHM, von Mensdorff-Pouilly S, van Kamp GJ, Kenemans P. CA 125: fundamental and clinical aspects. *Semin Cancer Biol* (1999) 9(2):117–124. doi: 10.1006/scbi.1998.0114

15. You B, Robelin P, Tod M, Louvet C, Lotz J-P, Abadie-Lacourtoisie S, et al. CA-125 ELIMination rate constant K (KELIM) is a marker of chemosensitivity in patients with ovarian cancer: results from the phase II CHIVA trial. *Clin Cancer Res* (2020) 26 (17):4625–4632. doi: 10.1158/1078-0432.CCR-20-0054

16. Marchetti C, Rosati A, Felice FD, Boccia SM, Vertechy L, Pavone M, et al. Optimizing the number of cycles of neoadjuvant chemotherapy in advanced epithelial ovarian carcinoma: A propensity-score matching analysis. *Gynecologic Oncol* (2021) 163(1):29–35. doi: 10.1016/j.ygyno.2021.07.025

17. Colombo PE, Labaki M, Fabbro M, Bertrand M, Mourregot A, Gutowski M, et al. Impact of neoadjuvant chemotherapy cycles prior to interval surgery in patients with advanced epithelial ovarian cancer. *Gynecologic Oncol* (2014) 135(2):223–230. doi: 10.1016/j.ygyno.2014.09.002

18. Riedinger JM, Wafflart J, Ricolleau G, Eche N, Larbre H, Basuyau JP, et al. CA 125 half-life and CA 125 nadir during induction chemotherapy are independent predictors of epithelial ovarian cancer outcome: results of a French multicentric study. *Ann Oncol* (2006) 17(8):1234–1238. doi: 10.1093/annonc/mdl120

19. You B, Colomban O, Heywood M, Lee C, Davy M, Reed N, et al. The strong prognostic value of KELIM, a model-based parameter from CA 125 kinetics in ovarian cancer: Data from CALYPSO trial (a GINECO-GCIG study). *Gynecologic Oncol* (2013) 130(2):289–294. doi: 10.1016/j.ygyno.2013.05.013

20. Bouchard D, Morisset D, Bourbonnais Y, Tremblay. Proteins with whey-acidic-protein motifs GM. and cancer. *Lancet Oncol* (2006) 7(2):167–174. doi: 10.1016/S1470-2045(06)70579-4

21. Emanuela A. HE4: a new potential early biomarker for the recurrence of ovarian cancer. *Tumor Biol* (2010) 31(2):113–9. doi: 10.1007/s13277-009-0015-y

22. Hellström I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, MacIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res* (2003) 63(13):3695–700.

23. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecologic Oncol* (2008) 108(2):402–408. doi: 10.1016/j.ygyno.2007.10.017

24. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecologic Oncol* (2009) 112(1):40–46. doi: 10.1016/j.ygyno.2008.08.031

25. Nolen B, Velikokhatnaya L, Marrangoni A, De Geest K, Lomakin A, Bast RC, et al. Serum biomarker panels for the discrimination of benign from Malignant cases in patients with an adnexal mass. *Gynecologic Oncology* (2010) 117(3):440–5. doi: 10.1016/j.ygyno.2010.02.005

26. Yu S, Yang H, Xie S, Bao Y-X. Diagnostic value of HE4 for ovarian cancer: a meta-analysis. *Clin Chem Lab Med* (2012) 50(8):1439–1446. doi: 10.1515/cclm-2011-0477

27. Trudel D, Têtu B, Grégoire J, Plante M, Renaud M-C, Bachvarov D, et al. Human epididymis protein 4 (HE4) and ovarian cancer prognosis. *Gynecologic Oncol* (2012) 127(3):511–515. doi: 10.1016/j.ygyno.2012.09.003

28. Braicu EI, Chekerov R, Richter R, Pop C, Nassir M, Loefgren H, et al. HE4 expression in plasma correlates with surgical outcome and overall survival in patients with first ovarian cancer relapse. *Ann Surg Oncol* (2014) 21(3):955–962. doi: 10.1245/s10434-013-3347-1

29. Chudecka-Głaz A, Cymbaluk-Płoska A, Wężowska M, Menkiszak J. Could HE4 level measurements during first-line chemotherapy predict response to treatment among ovarian cancer patients? *PloS One* (2018) 13(3):e0194270. doi: 10.1371/journal.pone.0194270

30. Lee S, Choi S, Lee Y, Chung D, Hong S, Park N. Role of human epididymis protein 4 in chemoresistance and prognosis of epithelial ovarian cancer. *J Obstetrics Gynaecology Res* (2017) 43(1):220–227. doi: 10.1111/jog.13181

31. Havrilesky LJ, Whitehead CM, Rubatt JM, Cheek RL, Groelke J, He Q, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. *Gynecologic Oncol* (2008) 110(3):374–382. doi: 10.1016/j.ygyno.2008.04.041

32. Potenza E, Parpinel G, Laudani ME, Macchi C, Fuso L, Zola P. Prognostic and predictive value of combined HE-4 and CA-125 biomarkers during chemotherapy in patients with epithelial ovarian cancer. *Int J Biol Markers* (2020) 35(4):20–27. doi: 10.1177/1724600820955195

33. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* (2009) 45(2):228–247. doi: 10.1016/j.ejca.2008.10.026

34. Vallius T, Hynninen J, Auranen A, Carpén O, Matomäki J, Oksa S, et al. Serum HE4 and CA125 as predictors of response and outcome during neoadjuvant chemotherapy of advanced high-grade serous ovarian cancer. *Tumor Biol* (2014) 35 (12):12389–12395. doi: 10.1007/s13277-014-2553-1

35. Zorn KK, Tian C, McGuire WP, Hoskins WJ, Markman M, Muggia FM, et al. The prognostic value of pretreatment CA 125 in patients with advanced ovarian carcinoma. *Cancer* (2009) 115(5):1028–1035. doi: 10.1002/cncr.24084

36. Pelissier A, Roulot A, Guéry B, Bonneau C, Bellet D, Rouzier R. Serum CA125 and HE4 levels as predictors for optimal interval surgery and platinum sensitivity after neoadjuvant platinum-based chemotherapy in patients with advanced epithelial ovarian cancer. *J Ovarian Res* (2016) 9:61. doi: 10.1186/s13048-016-0270-7

37. Alegría-Baños JA, Jiménez-López JC, Vergara-Castañeda A, de León DFC, Mohar-Betancourt A, Pérez-Montiel D, et al. Kinetics of HE4 and CA125 as prognosis biomarkers during neoadjuvant chemotherapy in advanced epithelial ovarian cancer. *J Ovarian Res* (2021) 14(1):96. doi: 10.1186/s13048-021-00845-6

38. Han Y, Jiang L, Liu K, Ouyang L, Li Y. Predictive value of HE4 in platinum-based chemotherapy for ovarian cancer: A systematic review. *Front Oncol* (2021) 11:703949. doi: 10.3389/fonc.2021.703949

39. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *New Engl J Med* (2019) 381(25):2416–2428. doi: 10.1056/NEJMoa1911361

40. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. *New Engl J Med* (2011) 365 (26):2484–2496. doi: 10.1056/NEJMoa1103799

41. Burger RA, Fleming GF, Mannel RS, Greer BE, Liang SX. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *New Engl J Med* (2011) 11:2473–83. doi: 10.1056/NEJMoa1104390

42. Almufti R, Wilbaux M, Oza A, Henin E, Freyer G, Tod M, et al. A critical review of the analytical approaches for circulating tumor biomarker kinetics during treatment. *Ann Oncol* (2014) 25(1):41–56. doi: 10.1093/annonc/mdt382

43. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: A randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *JCO* (2012) 30(17):2039–2045. doi: 10.1200/JCO.2012.42.0505

44. Crawford SM, Peace J. Does the nadir CA125 concentration predict a long-term outcome after chemotherapy for carcinoma of the ovary? *Ann Oncol* (2005) 16(1):47–50. doi: 10.1093/annonc/mdi012

45. Prat A, Parera M, Peralta S, Perez-Benavente MA, Garcia A, Gil-Moreno A, et al. Nadir CA-125 concentration in the normal range as an independent prognostic factor for optimally treated advanced epithelial ovarian cancer. *Ann Oncol* (2008) 19(2):327–331. doi: 10.1093/annonc/mdm495

46. Piatek S, Panek G, Lewandowski Z, Piatek D, Kosinski P, Bidzinski M. Nadir CA-125 has prognostic value for recurrence, but not for survival in patients with ovarian cancer. *Sci Rep* (2021) 11:18190. doi: 10.1038/s41598-021-97564-1

47. Colomban O, Tod M, Leary A, Ray-Coquard I, Lortholary A, Hardy-Bessard AC, et al. Early modeled longitudinal CA-125 kinetics and survival of ovarian cancer patients: A GINECO AGO MRC CTU study. *Clin Cancer Res* (2019) 25(17):5342–5350. doi: 10.1158/1078-0432.CCR-18-3335

48. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *New Engl J Med* (2016) 375(22):2154–2164. doi: 10.1056/NEJMoa1611310

49. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* (2017) 90(10106):1949–61. doi: 10.1016/S0140-6736(17)32440-6.

50. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol* (2014) 15(8):852–861. doi: 10.1016/S1470-2045(14)70228-1

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Safety of bevacizumab and olaparib as frontline maintenance therapy in advanced ovarian cancer: expert review for clinical practice

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Olaparib, a poly(ADP-ribose) polymerase inhibitor, in combination with the antiangiogenic agent bevacizumab, is approved as maintenance therapy for patients with newly diagnosed stage III or IV epithelial ovarian cancer who have homologous recombination deficient tumors with a deleterious or suspected deleterious *BRCA* mutation and/or genomic instability based on the long-lasting survival benefit observed in the PAOLA-1 trial. Despite treatment with olaparib and bevacizumab showing an acceptable safety profile, the rate of discontinuations due to adverse events was relatively high, and toxicity related to this regimen may restrict its clinical use. Proper management of olaparib/bevacizumab-related adverse events is important for the improvement of quality of life and maximization of the efficacy of maintenance therapy. Here, we summarize the safety results of the PAOLA-1 study, focusing on treatment discontinuation reasons and adverse event profiles. We sought to shed light on toxicity monitoring and prevention, providing concise recommendations for the clinical management of the most relevant side effects.

KEYWORDS

ovarian cancer, olaparib, bevacizumab, maintenance therapy, first-line, toxicity management

1 Introduction

Epithelial ovarian cancer (EOC) continues to be the most lethal gynecological tumor. Diagnosis is usually made at advanced stages, and cytoreductive surgery and first-line platinum-based chemotherapy have been the standard of care for decades. However, the majority of patients relapse within 3 years, with no reliable biomarkers to timely detect disease recurrence (1, 2). Two pivotal trials, GOG-0218 (3) and ICON7 (4), confirmed in

2011 the benefit of continuation maintenance therapy with bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), leading to its approval in the USA, the European Union, and other countries worldwide to treat patients with FIGO (International Federation of Gynecology and Obstetrics) stage III or IV EOC following debulking surgery (5).

Genome instability is a hallmark of EOC. In 50% of high-grade serous epithelial carcinoma, the most common histological subtype, there is a homologous recombination deficiency (HRD) due to different mechanisms, with mutations in *BRCA1/2* (14.5% germline and 7% somatic mutations) the most prevalent (6). Based on the molecular mechanisms of actions that target key DNA repair pathways in cancer cells, poly(ADP-ribose) polymerase inhibitors (PARPis) have emerged as a new therapeutic option in the management of EOC, particularly for tumors presenting HRD (7, 8). Currently, there are three PARPis in EOC approved by U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA)—olaparib, niraparib, and rucaparib—with different clinical indications and toxicity profiles (Table 1).

In the frontline maintenance setting, olaparib was the first PARPi to be approved for the treatment of *BRCA* mutated FIGO stage III or IV EOC, as a switch maintenance strategy, based on the SOLO-1 (NCT01844986) study results (9). Niraparib was approved with the same indication regardless of the biomarker status, following PRIMA (NCT02655016) study results (10). Both are approved for patients who have complete or partial response upon completion of first-line platinum-based chemotherapy. In this setting, although not yet granted regulatory approval, rucaparib maintenance was also associated with longer progression-free survival (PFS) (11). An additional PARPi, veliparib, not yet approved but has been added to first-line chemotherapy and continued as maintenance monotherapy, has been shown to improve the PFS of EOC patients, particularly those with HRD (12). With a different approach, olaparib is the only PARPi approved in combination with bevacizumab for newly diagnosed stage III or IV EOC with HRD tumors harboring deleterious or suspected deleterious *BRCA* mutations and/or genomic instability based on the benefit observed in the PAOLA-1 study (13).

Preclinical studies suggest that PARPis and antiangiogenic combinations may provide enhanced benefits in EOC (14, 15). Exploratory analyses point out that the combination of olaparib and bevacizumab may derive higher improvement in PFS compared to monotherapy (16, 17). Despite olaparib and bevacizumab showing acceptable safety profiles, up to 20% of participants in the PAOLA-1 trial discontinued due to adverse events (AEs) (13). Thus, toxicity related to this regimen and its management may raise concerns about the use of the combination in clinical practice. The aim of this review was to examine safety data from the PAOLA-1 study, focusing on treatment discontinuation reasons and the AE profile. In addition, we sought to shed light on toxicity monitoring and management to optimize the integration of this maintenance regimen in clinical practice. Management recommendations for treatment-emergent AEs (TEAEs) are proposed based on trial protocol, prescribing information, published supportive cancer care guidelines, and the authors' clinical experience.

TABLE 1 Poly(ADP-ribose) polymerase inhibitor (PARPi) approved indications in ovarian cancer (updated to June 2023).

Drug	FDA-approved indications	EMA-approved indications
Olaparib	• Maintenance treatment of adult patients with deleterious or suspected deleterious germline or somatic *BRCA*-mutated advanced EOC, FTC, or PPC who are in CR or PR to first-line platinum-based chemotherapy • In combination with bevacizumab, for the maintenance treatment of adult patients with advanced EOC, FTC, or PPC who are in CR or PR to first-line platinum-based chemotherapy and whose cancer is associated with HRD-positive status defined by either a deleterious or suspected deleterious *BRCA* mutation and/or genomic instability • Maintenance of recurrent EOC, FTC, or PPC in patients with CR or PR to platinum-based chemotherapy, regardless of *BRCA* status	• Maintenance treatment of adult patients with advanced (FIGO stages III and IV) *BRCA1/2*-mutated (germline and/or somatic) high-grade EOC, FTC, or PPC who are in response (CR or PR) following completion of first-line platinum-based chemotherapy • Maintenance treatment of adult patients with platinum-sensitive relapsed high-grade EOC, FTC, or PPC who are in response (CR or PR) to platinum-based chemotherapy • In combination with bevacizumab, for the maintenance treatment of adult patients with advanced high-grade EOC, FTC, or PPC who are in response (CR or PR) following completion of first-line platinum-based chemotherapy in combination with bevacizumab and whose cancer is associated with HRD positive status defined by either a *BRCA1/2* mutation or genomic instability
Niraparib	• Maintenance treatment of adult patients with advanced EOC, FTC, or PPC who are in a CR or PR to first-line platinum-based chemotherapy • Maintenance treatment of adult patients with deleterious or suspected deleterious gBRCAm recurrent EOC, FTC, or PPC who are in a CR or PR to platinum-based chemotherapy	• Maintenance treatment of adult patients with advanced EOC, FTC, or PPC who are in response (CR or PR) following completion of first-line platinum-based chemotherapy • Maintenance treatment of adult patients with platinum-sensitive relapsed high-grade serous EOC, FTC, or PPC who are in response (CR or PR) to platinum-based chemotherapy
Rucaparib	• Treatment of adult patients with deleterious *BRCA* mutation (germline and/or somatic)-associated EOC, FTC, or PPC who are in a CR or PR to platinum-based chemotherapy	• Maintenance treatment of adult patients with platinum-sensitive relapsed high-grade serous EOC, FTC, or PPC who are in response (CR or PR) to platinum-based chemotherapy

CR, complete response; EOC, epithelial ovarian cancer; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; FIGO, International Federation of Gynecology and Obstetrics; FTC, fallopian tube cancer; gBRCAm, germline BRCA-mutated; HRD, homologous recombination deficiency; PPC, primary peritoneal cancer; PR, partial response.

2 Delving into the PAOLA-1 study

PAOLA-1 was a randomized, double-blind study that compared olaparib (300 mg, twice daily for up to 24 months) with bevacizumab (15 mg/kg every 3 weeks for up to 15 months) as maintenance therapy after first-line chemotherapy in patients with

newly diagnosed, advanced FIGO stage EOC, regardless of *BRCA* mutation status and surgical outcomes, who were in complete or partial response to standard first-line platinum–taxane-based chemotherapy and bevacizumab (13). As the primary endpoint, time from randomization to investigator-assessed disease progression or death was chosen. After a median follow-up of 22.9 months, significant increases in PFS were observed for olaparib plus bevacizumab compared with placebo plus bevacizumab in the overall population (median PFS 22.1 *vs.* 16.6 months; hazard ratio, 0.59; 95% confidence interval [CI], 0.49 to 0.72; $p < 0.001$), with the greatest PFS benefit seen in patients with *BRCA* mutations (37.2 *vs.* 21.7 months) and those with HRD-positive status, regardless of *BRCA* mutation status (37.2 *vs.* 17.7 months). In patients with HRD-positive tumors without *BRCA* mutations, the median PFS increased to 28.1 months for olaparib compared to 16.6 months in the placebo group (13). Significant increases were also observed for time to second objective disease progression (PFS2) (36.5 months for olaparib plus bevacizumab *vs.* 32.6 months for placebo plus bevacizumab; hazard ratio, 0.78; 95% CI, 0.64 to 0.95; $p = 0.0125$). Median time to second subsequent therapy and death (TSST) was 38.2 months and 31.5 months, respectively (hazard ratio, 0.78; 95% CI, 0.64 to 0.95; $p = 0.0115$). In the placebo plus bevacizumab group, 72 (27%) patients received a PARPi as the first subsequent therapy (18). The final analysis of the overall survival (OS) at 5 years has shown that, in HRD-positive patients, olaparib plus bevacizumab reduced the mortality risk by 38% versus bevacizumab, and 65.5% of patients treated with the combination were still alive at 5 years compared to 48.4% of those treated with bevacizumab alone (19). These numbers of long survivors are similar to those reflected by the OS rates at 7 years of follow-up in the SOLO-1 trial (67.0% of olaparib patients *vs.* 46.5% of placebo patients) (20), which support the potential for cure of maintenance olaparib.

In the PAOLA-1 trial, more patients in the olaparib plus bevacizumab group (148/535 [27.7%] patients) completed the per protocol maximum 2-year treatment period than those in the placebo plus bevacizumab group (53/267 [19.9%] patients). The main toxicities reported in the PAOLA-1 study are summarized in Figure 1. Overall, the most common TEAEs (≥ 20%) associated with olaparib and bevacizumab versus bevacizumab and placebo were fatigue/asthenia (53% *vs.* 32%, respectively), nausea (53% *vs.* 22%), hypertension (46% *vs.* 60%), anemia (41% *vs.* 10%), lymphopenia (24% *vs.* 9%), vomiting (22% *vs.* 11%), and arthralgia (22% *vs.* 24%). The most relevant grade 3–4 toxicities (≥ 5%) with olaparib and bevacizumab compared to bevacizumab and placebo were hypertension (19% *vs.* 30%), anemia (17% *vs.* < 1%), lymphopenia (7% *vs.* 1%), fatigue/asthenia (5% *vs.* 1%), and neutropenia (6% *vs.* 3%).

The combined olaparib and bevacizumab regimen led to dose interruptions in 54% of patients, reductions in 41%, and discontinuations in 20%, compared to bevacizumab and placebo, which caused 24%, 7%, and 6% interruption, reduction, and discontinuation, respectively. Of note, patients who discontinued study treatment in PAOLA-1 were proactively questioned if this was due to an AE, whereas this approach was not taken in other trials (9–11). This difference in managing discontinuations may have contributed to the high rate of discontinuation due to AEs observed in this study. In fact, the overall discontinuation rates due to TEAEs, patient decision, and other reasons were comparable in the PAOLA-1 (25% of patients in the olaparib plus bevacizumab arm) (13), SOLO-1 (24% of patients in the olaparib arm) (9), PRIMA (18% of patients in the niraparib arm) (10), and ATHENA-

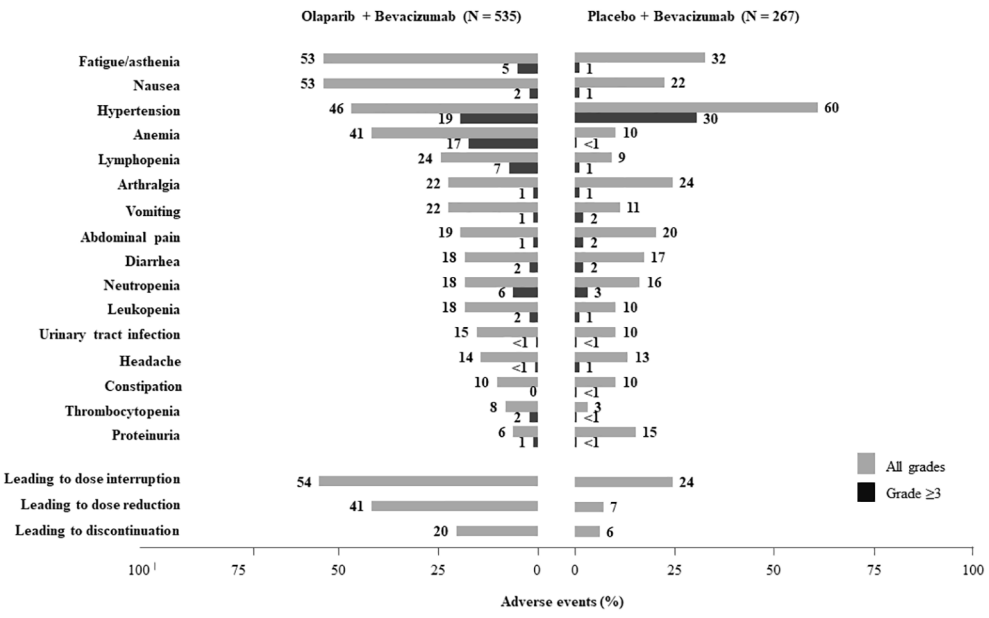

FIGURE 1
Summary of adverse events in the PAOLA-1 trial (13).

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MONO (18% of patients in the rucaparib arm) (11) studies (Table 2). The tolerability profile of olaparib plus bevacizumab versus placebo plus bevacizumab was consistent across the higher and lower risk of progression subgroups of patients and similar regardless of biomarker status (21). There was no clinically significant change in health-related quality of life in either group and no relevant difference between the treatment groups (13).

Toxicities observed during treatment with olaparib and bevacizumab were mostly managed by dose reductions and interruptions. Olaparib dose reductions were scarcely required, and AEs were managed by dose interruptions in most patients. Dose reductions occurred mainly during the first 3 months of therapy, in parallel with the temporal onset of the most common AEs (22). Prompt identification of any toxicity is mandatory, aiming to continue the treatment to optimize clinical benefit. If dose reductions are needed despite preventive/supportive measures, they should be implemented decreasing to 250 mg twice/day as a first step and further decreasing to 200 mg twice/day as a second step. Preliminary data indicate that there is a significant relationship between plasma olaparib exposure and the occurrence of serious AE (SAE). A trough plasma concentration threshold >2,500 ng/mL may be associated with a higher risk of SAE, which could guide dose adjustments in certain patients (23). Anticipatory and effective supportive care is critical to avoid dose changes. In the following sections, we propose practical guidance for the management of the most relevant AEs.

3 Management of adverse events in clinical practice (beyond drug label information)

3.1 Hematologic toxicity

3.1.1 Anemia

In the PAOLA-1 study, more than half of anemia events were grade 1 or 2 (Figure 1), and anemia rarely leads to permanent discontinuation of treatment. According to the EMA's Variation Assessment Report of Lynparza (22), olaparib dose was reduced in 99 (18.5%) patients, interrupted in 110 (20.6%), and permanently discontinued in 19 (3.6%). Anemia started early, generally within the first 3 months of olaparib initiation (median time to first event was 1.54 months), with no evidence of cumulative effect, as the risk of developing anemia remained quite constant over the entire exposure period. The majority (209/219 patients) of the first events of anemia with olaparib/bevacizumab were controlled satisfactorily (median time to resolution 1.41 months). Blood transfusions were required by 94 (17.6%) patients, and 26 (4.9%) of them needed more than one transfusion after starting study treatment. Most of the transfusions took place during the first 4 months of treatment. Thirty (5.6%) patients treated in the olaparib/bevacizumab arm received an erythropoiesis-stimulating agent (22).

Thus, frequent monitoring for hematologic toxicity is recommended at the beginning of olaparib/bevacizumab maintenance therapy. A hemogram should be performed monthly

TABLE 2 Discontinuation rates in poly(ADP-ribose) polymerase inhibitor (PARPi) clinical trials.

Reasons for discontinuation other than disease progression, n (%)	PAOLA-1 (13)		SOLO-1 (9)		PRIMA (10)			ATHENA-MONO (11)	
	Olaparib (n = 535)	Placebo (n = 269)	Olaparib (n = 260)	Placebo (n = 130)	Niraparib, fixed dose (n = 317)	Niraparib, individualized dose (n = 170)	Placebo (n = 244)	Rucaparib (n = 425)	Placebo (n = 110)
TEAE	109 (20)	13 (5)	30 (12)	3 (2)	35 (11)	23 (14)	5 (2)	50 (12)	6 (5)
Patient decision	4 (1)	4 (2)	22 (8)	2 (2)	10 (3)	2 (1)	1 (0)	21 (5)	3 (3)
Other	19 (4)	6 (2)	11 (4)	9 (7)	14 (4)	4 (2)	7 (3)	7 (1)	2 (2)
Total	**132 (25)**	**23 (9)**	**63 (24)**	**14 (11)**	**59 (19)**	**29 (17)**	**13 (5)**	**78 (18)**	**11 (10)**

TEAE, treatment-emergent adverse event.

for the first 12 months and periodically after this time to monitor for clinically significant changes in any parameter during treatment. If anemia appears, it is also necessary to study other possible causes, and monitoring of iron, folic acid, and vitamin B_{12} levels is recommended. Folate deficiency has been observed in some patients receiving olaparib, so administering folate supplements and/or other hematinics could ameliorate the risk of severe anemia in these cases (24). When hemoglobin values fall below 8 g/dL, olaparib dose reduction and/or blood transfusion should be considered (Table 3). If anemia persists below 8 g/dL for more than 4 weeks, refer the patient to hematology before continuing treatment and recommend bone marrow and/or blood cytogenetic analysis. Given that bevacizumab may increase the risk of bleeding, the presence of active bleeding should be checked. If the hemoglobin falls back to less than 8 g/dL after dose reductions or there is a need for periodic transfusions, then definitive discontinuation of olaparib treatment is recommended.

TABLE 3 Proposal for anemia management.

Severity	Recommended action
CTCAE grade 2 (Hb < 10.0 to ≥ 8.0 g/dL)	First event - Provide supportive treatment and investigate causality - According to physician's judgment, continue olaparib or interrupt dose for a maximum of 4 weeks
	Recurrent event - Interrupt olaparib for a maximum of 4 weeks until Hb ≥ 10 g/dL. If more than 4 weeks is required, refer the patient to hematology service - When Hb ≥ 10 g/dL, resume olaparib with a reduced dose: • 250 mg twice daily as a first step and • 200 mg twice daily as a second step if it recurs
CTCAE grade 3 (Hb < 8.0 g/dL)	First event - Interrupt olaparib for a maximum of 4 weeks until Hb ≥ 10 g/dL. If more than 4 weeks is required, refer the patient to hematology service - When Hb ≥ 10 g/dL, resume olaparib with the first step of reduced dose (250 mg twice daily)
	Recurrent event - Interrupt olaparib for a maximum of 4 weeks until Hb ≥ 10 g/dL with supportive treatment. If more than 4 weeks is required, refer the patient to hematology service - When Hb ≥ 10 g/dL, resume olaparib with the second step of reduced dose (200 mg twice daily)
CTCAE grade 3 (Hb < 8.0 g/dL) with concurrent neutropenia and/or thrombocytopenia	- Interrupt olaparib and bevacizumab for a maximum of 4 weeks until recovery with supportive treatment. If more than 4 weeks is required, refer the patient to hematology service - When Hb ≥ 10 g/dL, resume bevacizumab according to clinical practice and resume olaparib with the second step of reduced dose (200 mg twice daily)
CTCAE grade 3 (Hb < 8.0 g/dL) despite dose reduction or more than one transfusion is needed for anemia recovery	Definitely discontinue olaparib treatment

CTCAE, Common Terminology Criteria for Adverse Events; Hb, hemoglobin.

3.1.2 Thrombocytopenia

In the PAOLA-1 study, thrombocytopenia or decreased platelet count of any grade was reported in 8% of patients in the olaparib plus bevacizumab arm (Figure 1), but no event led to treatment discontinuation. The development of thrombocytopenia was not associated with the duration of olaparib/bevacizumab treatment. Thrombocytopenia events appeared initially during the first 12 months of the study period in the olaparib/bevacizumab arm (median time to first onset, 1.41 months); most of the patients (42/43 patients) resolved satisfactorily (median time to resolution of the first event was 0.82 month) (22). Regarding hemorrhage events, 52/535 (9.7%) patients treated with olaparib plus bevacizumab had a total of 65 AEs, whereas in the placebo/bevacizumab arm, only 36 AEs were observed in 28/267 (10.5%) patients. Bleeding events were predominantly grade 1 or 2. Similar proportions of patients in each group (5/43 [11.6%] olaparib/bevacizumab and 1/9 [11.1%] placebo/bevacizumab) received treatment for thrombocytopenia AEs. Five (0.9%) versus one (0.4%) patients received platelet transfusions (22).

Although it seems that olaparib-associated thrombocytopenia rarely translates into bleeding risk, grade 1 thrombocytopenia (platelets <75,000/mm^3) requires close monitoring and possible dose reduction (Table 4). If grade 2 or higher thrombocytopenia occurs, olaparib should be interrupted, and weekly monitoring should be carried out until recovery of platelets to the level of 100,000/mm^3. Platelet transfusions are recommended when platelet counts are below 20,000/mm^3, or higher with active bleeding or planned invasive procedure, or in patients with ulcerative tumors. In addition, patients who receive anticoagulants or antiplatelet therapy should also be considered for transfusion at higher platelet counts or when anticoagulation is interrupted (25).

3.1.3 Myelodysplastic syndrome/acute myeloid leukemia

Nine (1.7%) versus six (2.2%) cases of myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) were reported in the olaparib versus placebo groups in the PAOLA-1 study, respectively (17). MDS/AML is considered an important identified risk of olaparib treatment. Warnings regarding blood count monitoring and discontinuation of treatment in the event of MDS/AML are included in the product information (26).

3.2 Non-hematologic toxicity

3.2.1 Nausea and vomiting

In the PAOLA-1 study, most nausea and vomiting events were grade 1–2 (Figure 1), and most did not require treatment discontinuation (22). These gastrointestinal toxicities generally occurred early during treatment (median time to onset of nausea and vomiting were 0.16 and 1.38 months, respectively), and most events with olaparib/bevacizumab subsequently improved or

TABLE 4 Recommendations for managing the main adverse events associated with the combination of olaparib and bevacizumab.

Adverse event		Proposed management
Hematologic toxicity	• Hemogram should be performed twice weekly for the first 2 months and monthly thereafter	
	Anemia	• Investigate other possible causes and monitor iron, folic acid, and vitamin B_{12} levels • Provide folate supplements and/or other hematinics, if required • Investigate the presence of active bleeding • If hemoglobin falls < 8 g/dL, follow the actions described in Table 3
	Thrombocytopenia	• Grade 1: Close monitoring and potential dose reduction • Grade ≥ 2: Interrupt olaparib and monitor weekly until recovery (100,000 platelets/mm^3) • Consider platelet transfusion when platelets fall below 20,000/mm^3, or higher with active bleeding, planned surgery, or ulcerative tumors • Be aware of concomitant anticoagulant or antiplatelet therapy
Non-hematologic toxicity	Nausea and vomiting	• Advise patients regarding these side effects • Dietary modifications: protein-rich foods, taking olaparib after meals • Consider antiemetic prophylaxis
	Fatigue/asthenia	• Inform patients of the expected patterns of fatigue • Recommend regular exercise, massage therapy, and/or psychosocial interventions • Consider vitamin B supplementation • If grade ≥ 3 fatigue, consider dose reductions or interruption
	Arthralgia	• Inform patients regarding this side effect • Consider referral to rheumatology • For mild pain, treat with common analgesics
	Hypertension	• Check and control pre-existing hypertension before initiating bevacizumab therapy • Measure blood pressure before and after the first few bevacizumab infusions and then every 3 weeks • In patients with a blood pressure of 150/100 mmHg or more, interrupt bevacizumab until normal pressure is restored using antihypertensive medication • When hypertension persists, refer the patient to specialized hypertension units or the general practitioner for adequate monitoring and follow-up
	Proteinuria	• Regular urine assessment • If abnormal urine results, perform 24-hour urine analysis • If proteinuria ≥ 2 g/24 hours, interrupt bevacizumab until recovery to < 2 g/24 hours

resolved (median time to resolution of the first event of 1.28 and 0.10 months, respectively). Nausea prevalence decreased from a range of 0.3%–0.2% in the first 6 months of treatment to 0.2%–0.1% thereafter. Vomiting prevalence was approximately 0.05% throughout the study. A total of 104 (19.4%) patients in the olaparib plus bevacizumab arm reported both nausea and vomiting. In the olaparib plus bevacizumab treatment arm, approximately half of the patients with nausea (158/285 [55.4%] patients) required treatment, and 58/117 (49.6%) patients who experienced vomiting received treatment. Fewer patients received treatment for nausea and vomiting in the placebo plus bevacizumab group (24/58 [41.4%] and 10/29 [34.5%] patients, respectively). The incidence of nausea and vomiting was higher (≥5 percentage points difference) in patients aged <65 years when compared with older patients (22).

Patient advice regarding these side effects can help them be prepared and thereby continue with the treatment (Table 4). Eating bland foods and liquids that are easy on the stomach and consuming protein-rich foods are common dietary changes recommended by nutritionists (27). Taking olaparib tablets after breakfast and dinner can also be helpful. In patients with recurrent emesis, a prophylactic approach with antiemetic therapy (e.g., metoclopramide) may avoid dose adjustments or discontinuation (28).

3.2.2 Fatigue/asthenia

More than half of patients on olaparib/bevacizumab treatment experienced fatigue or asthenia, most of the time graded 1 or 2 (Figure 1). Up to 28/535 (5.2%) patients had grade 3 fatigue (i.e., interfering with activities of daily living and self-care). These AEs were reported early, as most of the first events in the olaparib/bevacizumab arm were reported within the first 3 months of treatment. The median time to onset was 0.72 months, and the incidence plateaued at approximately 1 month (20). The majority (220/283 [77.7%] patients) of fatigue and asthenia events with olaparib/bevacizumab resolved in a median time of 2.10 months. Few patients in the olaparib/bevacizumab arm experiencing fatigue and asthenia (6/283 [2.1%] patients) required treatment compared to 3/86 (3.5%) in the placebo/bevacizumab group (22).

Although treatment-related fatigue represents a common class effect of PARPis, some subjective and objective underlying causes not directly related to these drugs could also be contributing to fatigue, such as prior chemotherapy, anemia, poor nutrition, emotional distress, or insomnia (Table 4) (29). Fatigue is a distressing symptom that negatively impacts patients' quality of life and can provoke a lack of adherence (30). Patients should be informed of the expected patterns of fatigue, aiming to facilitate patients' adaptation to the ongoing treatment. Regular exercise (for example, Pilates) may be recommended, along with massage therapy and/or psychosocial interventions (31). Vitamin B supplementation seems to improve anemia and fatigue and may be considered in selected cases (32). For patients with grade ≥ 3 fatigue, intolerable or long-lasting low-grade fatigue, dose reductions, or interruption may be necessary.

3.2.3 Arthralgia

All grade arthralgia afflicted 116 (22%) patients of the olaparib plus bevacizumab group and 64 (24%) patients of the placebo plus bevacizumab group (Figure 1). In other studies, the incidence of arthralgia in bevacizumab-treated patients was higher, reaching up to 50% (33–35), with evidence of cumulative toxic effect (36). Health professionals should be aware of this side effect to inform patients and if needed refer them to a rheumatologist to properly manage joint pain. Treatment for mild pain may include common analgesics, but severe pain could require corticosteroids or methotrexate (Table 4) (35).

3.2.4 Hypertension

Hypertension events were reported in a lower percentage of patients in the olaparib/bevacizumab arm (45.8%) when compared to the placebo/bevacizumab arm (59.9%). Fewer patients presented with grade 3 hypertension in the olaparib/bevacizumab group, compared with the placebo/bevacizumab group of the PAOLA-1 study (Figure 1). The majority of first hypertension events in both treatment arms occurred during the first 12 months of treatment. It was suggested that hypertension AEs were associated with bevacizumab treatment, as these events occurred at the same time of bevacizumab exposure (median duration of bevacizumab treatment, 11.0 months with olaparib/bevacizumab and 10.4 months with placebo/bevacizumab). In the first month of treatment, similar numbers of patients in each arm experienced a first hypertension AE, with rates of 17.8% for olaparib/bevacizumab and 20.2% for placebo/bevacizumab. Hypertension in the study rarely resulted in dose changes for olaparib or placebo, and none resulted in discontinuation (22).

Patients with pre-existing hypertension should have adequate blood pressure control prior to initiation of bevacizumab therapy. All patients should have blood pressure monitoring before and after the first few bevacizumab infusions and then every 3 weeks. In patients with a blood pressure of 150/100 mm Hg or higher, bevacizumab should be interrupted until normal blood pressure is restored with antihypertensive medications. If hypertension persists, the patient should be referred to specialized hypertension units or the general practitioner for adequate monitoring and follow-up (Table 4) (37, 38).

3.2.5 Proteinuria

Proteinuria is one of the commonly reported side effects caused by bevacizumab, especially in patients with a history of hypertension (39). In the PAOLA-1 study, proteinuria was reported in more patients in the placebo/bevacizumab group (15.4%) than in the olaparib/bevacizumab one (5.8%) (Figure 1). Most proteinuria events were grade 1 or 2, and none led to treatment withdrawal. The majority of proteinuria AEs were observed during the combined treatment period in both groups (26/31 [83.9%] patients in the olaparib/bevacizumab arm and 37/41 [90.2%] patients in the placebo/bevacizumab arm). Similar onset times of proteinuria were observed in both arms. Most first-time proteinuria events occurred in the first 450 days of the study, which is consistent with an association with bevacizumab treatment (22).

Patients being treated with bevacizumab require regular systematic urine assessment. If any alteration is detected (two consecutive positive dipstick tests), a 24-hour urine analysis should be performed. In the event of urine proteinuria being greater than 2 g/24 hours, bevacizumab should be interrupted until recovery to <2 g/24 hours (Table 4) (40).

4 Concluding remarks and summary of recommendations

In summary, the PAOLA-1 study confirmed that adding olaparib to bevacizumab as maintenance therapy is beneficial in the group of patients who have HRD-positive tumors harboring a deleterious or suspected deleterious *BRCA* mutation and/or genomic instability, with manageable toxicity profile and no deterioration of health-related quality of life. Although the majority of olaparib-related AEs were mild and there was no evidence of an increase in the known toxic effects associated with bevacizumab, as with the administration of any treatment, a full discussion of the benefits and risks of the combination should take place with the patient as part of the informed-consent process. In addition, clinicians should detect and adequately manage the side effects of olaparib treatment as early as possible to maximize the efficacy of maintenance therapy. Toxic effects, many of which appear to be class effects of PARPis, are mostly self-limiting and can be managed with the use of preventive and supportive measures and, sometimes, with treatment interruptions and/or dose reductions. We provide the summary of recommendations in Table 4, hoping to contribute to better clinical management of the potential toxicity. Although we provide a comprehensive and practical synthesis of the safety results of the PAOLA-1 study, the main limitation in making recommendations is the lack of real-world evidence on the long-term use of olaparib plus bevacizumab. However, on the basis of the critical review of the (scant) literature and the experience of the authors, we consider that the benefits of olaparib plus bevacizumab therapy currently outweigh potential risks, and therefore, patients with *BRCA* mutation or genomic instability in whom bevacizumab is added to the combination with first-line chemotherapy should not be deprived of the benefit of continuing bevacizumab in maintenance with olaparib.

Author contributions

IR: Writing – original draft, Writing – review & editing. EG: Writing – original draft, Writing – review & editing. AM: Writing – original draft, Writing – review & editing. LM: Writing – original draft, Writing – review & editing.

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Conflict of interest

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References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71:7–33. doi: 10.3322/caac.21654

2. Giampaolino P, Foreste V, Della Corte L, Di Filippo C, Iorio G, Bifulco G. Role of biomarkers for early detection of ovarian cancer recurrence. *Gland Surg* (2020) 9:1102–11. doi: 10.21037/gs-20-544

3. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med* (2011) 365:2473–83. doi: 10.1056/NEJMoa1104390

4. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* (2011) 365:2484–96. doi: 10.1056/NEJMoa1103799

5. Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2013) 24:vi24–32. doi: 10.1093/annonc/mdt333

6. The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* (2011) 474:609–15. doi: 10.1038/nature10166

7. Franzese E, Centonze S, Diana A, Carlino F, Guerrera LP, Di Napoli M, et al. PARP inhibitors in ovarian cancer. *Cancer Treat Rev* (2019) 73:1–9. doi: 10.1016/j.ctrv.2018.12.002

8. Della Corte L, Foreste V, Di Filippo C, Giampaolino P, Bifulco G. Poly (ADP-ribose) polymerase (PARP) as target for the treatment of epithelial ovarian cancer: what to know. *Expert Opin Investig Drugs* (2021) 30:543–54. doi: 10.1080/13543784.2021.1901882

9. Moore K, Colombo N, Scambia G, Kim B-G, Oaknin A, Friedlander M, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* (2018) 379:2495–505. doi: 10.1056/NEJMoa1810858

10. González-Martín A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* (2019) 381:2391–402. doi: 10.1056/NEJMoa1910962

11. Monk BJ, Parkinson C, Lim MC, O'Malley DM, Oaknin A, Wilson MK, et al. A randomized, phase III trial to evaluate rucaparib monotherapy as maintenance treatment in patients with newly diagnosed ovarian cancer (ATHENA-MONO/GOG-3020/ENGOT-ov45). *J Clin Oncol* (2022) 40:3952–64. doi: 10.1200/JCO.22.01003

12. Coleman RL, Fleming GF, Brady MF, Swisher EM, Steffensen KD, Friedlander M, et al. Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. *N Engl J Med* (2019) 381:2403–15. doi: 10.1056/NEJMoa1909707

13. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N Engl J Med* (2019) 381:2416–28. doi: 10.1056/NEJMoa1911361

14. Lim JJ, Yang K, Taylor-Harding B, Wiedemeyer WR, Buckanovich RJ. VEGFR3 inhibition chemosensitizes ovarian cancer stemlike cells through down-regulation of BRCA1 and BRCA2. *Neoplas* (2014) 16:342–3. doi: 10.1016/j.neo.2014.04.003

15. Ivy SP, Liu JF, Lee J-M, Matulonis UA, Kohn EC. Cediranib, a pan-VEGFR inhibitor, and olaparib, a PARP inhibitor, in combination therapy for high grade serous ovarian cancer. *Expert Opin Investig Drugs* (2016) 25:597–611. doi: 10.1517/13543784.2016.1156857

16. Hettle R, McCrea C, Lee CK, Davidson R. Population-adjusted indirect treatment comparison of maintenance PARP inhibitor with or without bevacizumab versus bevacizumab alone in women with newly diagnosed advanced ovarian cancer. *Ther Adv Med Oncol* (2021) 13:17588359211049640. doi: 10.1177/17588359211049639

17. Vergote I, Ray-Coquard I, Anderson DM, Cantuaria G, Colombo N, Garnier-Tixidre C, et al. Population-adjusted indirect treatment comparison of the SOLO1 and PAOLA-1/ENGOT-ov25 trials evaluating maintenance olaparib or bevacizumab or the combination of both in newly diagnosed, advanced BRCA-mutated ovarian cancer. *Eur J Cancer* (2021) 157:415–23. doi: 10.1016/j.ejca.2021.08.023

18. González-Martín A, Desauw C, Heitz F, Cropet C, Gargiulo P, Berger R, et al. Maintenance olaparib plus bevacizumab in patients with newly diagnosed advanced high-grade ovarian cancer: Main analysis of second progression-free survival in the phase III PAOLA-1/ENGOT-ov25 trial. *Eur J Cancer* (2022) 174:221–31. doi: 10.1016/j.ejca.2022.07.022

19. Ray-Coquard IL, Leary A, Pignata S, Cropet C, Martin AJG, Bogner G, et al. LBA29 Final overall survival (OS) results from the phase III PAOLA-1/ENGOT-ov25 trial evaluating maintenance olaparib (ola) plus bevacizumab (bev) in patients (pts) with newly diagnosed advanced ovarian cancer (AOC). *Ann Oncol* (2022) 33:S1396–7. doi: 10.1016/j.annonc.2022.08.025

20. DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim B-G, Oaknin A, et al. Overall survival with maintenance olaparib at a 7-year follow-up in patients with newly diagnosed advanced ovarian cancer and a BRCA mutation: the SOLO1/GOG 3004 trial. *J Clin Oncol* (2023) 41:609–17. doi: 10.1200/JCO.22.01549

21. Harter P, Mouret-Reynier MA, Pignata S, Cropet C, González-Martín A, Bogner G, et al. Efficacy of maintenance olaparib plus bevacizumab according to clinical risk in patients with newly diagnosed, advanced ovarian cancer in the phase III PAOLA-1/ENGOT-ov25 trial. *Gynecol Oncol* (2022) 164:254–64. doi: 10.1016/j.ygyno.2021.12.016

22. European Medicines Agency (EMA). *Lynparza assessment report* (2020). Available at: https://www.ema.europa.eu/en/documents/variation-report/lynparza-h-c-3726-ii-0035-epar-assessment-report-variation_en.pdf (Accessed August 23, 2022).

23. Velev M, Puszkiel A, Blanchet B, de Percin S, Delanoy N, Medioni J, et al. Association between olaparib exposure and early toxicity in BRCA-mutated ovarian cancer patients: results from a retrospective multicenter study. *Pharmaceuticals* (2021) 14:804. doi: 10.3390/ph14080804

24. Colombo N, Moore K, Scambia G, Oaknin A, Friedlander M, Lisyanskaya A, et al. Tolerability of maintenance olaparib in newly diagnosed patients with advanced ovarian cancer and a BRCA mutation in the randomized phase III SOLO1 trial. *Gynecol Oncol* (2021) 163:41–9. doi: 10.1016/j.ygyno.2021.07.016

25. Schiffer CA, Bohlke K, Delaney M, Hume H, Magdalinski AJ, McCullough JJ, et al. Platelet transfusion for patients with cancer: american society of clinical oncology clinical practice guideline update. *J Clin Oncol* (2018) 36:283–99. doi: 10.1200/JCO.2017.76.1734

26. European Medicines Agency (EMA). *Lynparza : EPAR - product information* (2023). Available at: https://www.ema.europa.eu/en/documents/product-information/lynparza-epar-product-information_en.pdf (Accessed October 10, 2023).

27. Crichton M, Marx W, Marshall S, Isenring E. What are the optimal dietary strategies for the management of cancer-related nausea and vomiting in adults: A PEN guideline update. *Nutr Diet* (2019) 76:77. doi: 10.1111/1747-0080.12567

28. Friedlander M, Banerjee S, Mileshkin L, Scott C, Shannon C, Goh J. Practical guidance on the use of olaparib capsules as maintenance therapy for women with BRCA mutations and platinum-sensitive recurrent ovarian cancer. *Asia Pac J Clin Oncol* (2016) 12:323–31. doi: 10.1111/ajco.12636

29. Bower JE. Cancer-related fatigue: mechanisms, risk factors, and treatments. *Nat Rev Clin Oncol* (2014) 11:597–609. doi: 10.1038/nrclinonc.2014.127

30. Poort H, Fenton ATHR, Thompson E, Dinardo MM, Liu JF, Arch JJ, et al. Lived experiences of women reporting fatigue during PARP inhibitor maintenance treatment for advanced ovarian cancer: A qualitative study. *Gynecol Oncol* (2021) 160:227–33. doi: 10.1016/j.ygyno.2020.10.034

31. Fabi A, Bhargava R, Fatigoni S, Guglielmo M, Horneber M, Roila F, et al. Cancer-related fatigue: ESMO Clinical Practice Guidelines for diagnosis and treatment. *Ann Oncol* (2020) 31:713–23. doi: 10.1016/j.annonc.2020.02.016

32. Tardy A-L, Pouteau E, Marquez D, Yilmaz C, Scholey A. Vitamins and minerals for energy, fatigue and cognition: A narrative review of the biochemical and clinical evidence. *Nutrients* (2020) 12:228. doi: 10.3390/nu12010228

33. Vauléon E, Behal H, Lebellec L, Desbarbieux R, Baldacci S, Simon N, et al. Does bevacizumab increase joint pain in patients with cancer? Results of the prospective observational BEVARTHRALGIA study. *Cancer Chemother Pharmacol* (2021) 87:533–41. doi: 10.1007/s00280-020-04226-6

34. Ventriglia J, Paciolla I, Pisano C, Tambaro R, Cecere SC, Di Napoli M, et al. Arthralgia in patients with ovarian cancer treated with bevacizumab and chemotherapy. *Int J Gynecol Cancer* (2021) 31:110–3. doi: 10.1136/ijgc-2020-001540

35. Kaparelou M, Liontos M, Katsimbri P, Andrikopoulou A, Papatheodoridi A, Kyriazoglou A, et al. Retrospective analysis of bevacizumab-induced arthralgia and clinical outcomes in ovarian cancer patients. *Single center experience. Gynecol Oncol Rep* (2022) 40:100953. doi: 10.1016/j.gore.2022.100953

36. Lee S-P, Hsu H-C, Tai Y-J, Chen Y-L, Chiang Y-C, Chen C-A, et al. Bevacizumab dose affects the severity of adverse events in gynecologic Malignancies. *Front Pharmacol* (2019) 10:426. doi: 10.3389/fphar.2019.00426

37. Manso L, Moreno F, Márquez R, Castelo B, Arcediano A, Arroyo M, et al. Use of bevacizumab as a first-line treatment for metastatic breast cancer. *Curr Oncol* (2015) 22:e51–60. doi: 10.3747/co.22.2210

38. Plummer C, Michael A, Shaikh G, Stewart M, Buckley L, Miles T, et al. Expert recommendations on the management of hypertension in patients with ovarian and cervical cancer receiving bevacizumab in the UK. *Br J Cancer* (2019) 121:109–16. doi: 10.1038/s41416-019-0481-y

39. Izzedine H, Massard C, Spano JP, Goldwasser F, Khayat D, Soria JC. VEGF signalling inhibition-induced proteinuria: Mechanisms, significance and management. *Eur J Cancer* (2010) 46:439–48. doi: 10.1016/j.ejca.2009.11.001

40. Randall LM, Monk BJ. Bevacizumab toxicities and their management in ovarian cancer. *Gynecol Oncol* (2010) 117:497–504. doi: 10.1016/j.ygyno.2010.02.021

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PARP inhibitor maintenance treatment for newly diagnosed ovarian cancer patients: a real-world study from China

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Purpose: This study evaluated the efficacy and safety in a real-world population of epithelial ovarian cancer (EOC) treated with poly (ADP-ribose) polymerase inhibitor (PARPi) as first-line maintenance therapy in the largest gynecologic oncology center in Western China.

Methods: This study included patients newly diagnosed EOC who received PARPi as first-line maintenance therapy in West China Second University Hospital from August 1, 2018 to September 31, 2022. The primary endpoints were progression-free survival (PFS) and safety evaluated by Common Terminology Criteria for Adverse Events Version 5.0(CTCAE 5.0). The secondary endpoints were overall survival (OS) and prognostic factors influencing the PFS of patients in real world.

Results: Among the eligible 164 patients, 104 patients received olaparib and 60 patients received niraparib. 100 patients (61.0%) had mutations in breast cancer susceptibility gene (BRCA). 87 patients (53.0%) received primary debulking surgery (PDS) while 77 patients (47.0%) received interval debulking surgery (IDS). 94 patients (94/164, 57.3%) achieved R0 and 39 patients (23.8%) achieved R1 after PDS/IDS. 112 (68.3%) achieved complete response (CR) after first-line chemotherapy, while 49 (29.9%) achieved partial response (PR). The median follow-up time was 17.0 months (95% CI 15.6-18.4), and the median PFS has not been reached yet. Multivariate analysis demonstrated that BRCA mutations and CR/PR after platinum-based chemotherapy were independent factors associated with prolonged PFS. Hematologic toxicity was the most common grade≥3 AE. There were no incidence of myelodysplastic syndromes/acute myelogenous leukemia (MDS/AML).

Conclusion: Focusing on PARPi as first-line maintenance therapy for patients with EOC, this study represented the largest single-center real-world study in China to date. Two independent factors were identified to prolong the PFS of

patients: BRCA mutated type and CR/PR after primary treatment, which should be further confirmed with long-term follow-up and large sample sizes.

KEYWORDS

ovarian cancer, poly (ADP-ribose) polymerase inhibitors, progression-free survival, adverse events, real-world study

1 Introduction

Ovarian cancer is the third most common malignancy in the female reproductive system, with mortality rate second only to cervical cancer globally. However, in developed countries, it has the highest mortality rate among female reproductive system (1). In 2020, the World Health Organization (WHO) published global cancer burden data revealing 55,342 new cases of ovarian cancer in China, accounting for 17.62% of the global incidence. In the same year, there were 37,519 deaths in China due to ovarian cancer, accounting for 18.10% of the global deaths (1, 2). Epithelial ovarian cancer (EOC) is represented by several different histology, such as serous, endometrioid, clear cell and mucinous histology, each with its own specific genetic and clinical characteristics (3). Kurman et al. provided a dualistic model for EOC according to two different carcinogenic pathways (4). Type I EOC are suggested to be relatively indolent and genetically stable tumors which consist of clearly described precursor lesions such as low-grade serous, mucinous, endometrioid, or borderline tumors at an earlier stage. In contrast, type II EOC are proposed to be high-grade, biologically aggressive tumors from their outset, where precursor lesions are not clearly described. Most patients were diagnosed with advanced EOC and there are currently no effective early detection strategies exist. The initial treatment is crucial for patients newly diagnosed with EOC as it significantly influences comprehensive management by delaying relapse, prolonging survival, and increasing the potential for cure. First-line maintenance therapy is defined as the treatment for EOC patients who have completed initial chemotherapy and achieved complete response (CR) or partial response (PR) to platinum-based survival (5). CR requires the disappearance of all target lesions and a reduction of the short diameter of all pathological lymph nodes to less than 10 mm (6). PR indicates a reduction of at least 30% in the sum of target lesion diameters compared to baseline levels.

Currently, the main maintenance therapy drugs include bevacizumab and PARP inhibitors (PARPi). Both the ICON7 study (7) and the GOG-218 study (8) found that bevacizumab could improve progression-free survival (PFS) in patients with International Federation of Obstetrics and Gynecology (FIGO) III to IV. Following the results of the SOLO1, PRIMA, PRIME and PAOLA-1 studies, the efficacy of PARPi in first-line maintenance therapy has been validated by the vast majority of EOC patients (9–13). The SOLO-1 study (10) showed that after 7 years of follow-up, 67.0% of the patients in the olaparib group were alive and half of them did not receive any subsequent treatment. The PRIMA study (11) showed that niraparib provided different degree of benefit in the first-line maintenance treatment of advanced ovarian cancer in the general population. The PRIME study (12) which performed in Asian population and used an individualized starting dose of niraparib showed a survival advantage in niraparib group regardless of surgical residual disease and biomarker status. The PAOLA-1 study (13) showed that in the HRD-positive population, OS was longer with olaparib plus bevacizumab than placebo plus bevacizumab. At 5 years, the updated PFS also showed that a higher proportion of patients with no recurrence in olaparib plus bevacizumab group. PARPi has become the standard treatment for first-line maintenance therapy in ovarian cancer. These large randomized controlled trials (RCTs) have laid a solid foundation for clinical diagnosis and treatment. However, these studies were based on strict inclusion criteria and treatment measures, which avoided bias but inevitably differed from the clinical reality (14). The real-world study (RWS) is commonly used to evaluate the efficacy of drug in real clinical practice after large RCTs, and the conclusions from RWS have better external validity (15). However, there is a lack of RWS on the PARPi in first-line maintenance therapy, especially in Chinese population. As the largest gynecological oncology center in western China, the West China Second University Hospital of Sichuan University has annually treated 450 newly diagnosed cases of EOC. Therefore, this study aimed to collect clinicopathological data from patients with EOC receiving PARPi as first-line maintenance therapy and evaluate the efficacy and safety in a real-world population from China.

2 Methods

2.1 Patients and study design

This study followed the Declaration of Helsinki and was approved by the Ethics Committee of West China Second University Hospital (approval number: 20220129). The clinicopathological data of newly diagnosed cases of EOC from August 1, 2018 to September 31, 2022 were collected. This study included: (1) age ≥ 18 years old; (2) pathologically confirmed as EOC with complete clinical and pathological data; (3) patients receiving PARPi as first-line maintenance therapy. The patients

who refused follow-up or missed important clinical data were excluded. The clinicopathological data from medical records included demographics, histology, breast cancer susceptibility gene (BRCA) status, FIGO stage, neoadjuvant chemotherapy (NACT), residual diseases after primary surgery, platinum-based chemotherapy, first-line maintenance therapy details [baseline CA125 levels before PARPi, baseline computed tomography (CT) or magnetic resonance imaging (MRI) results, duration of treatment, and dose interruption/reduction/discontinuation]. Missing information will be supplemented through follow-up phone calls or face-to-face inquiries (if the patients are alive and accessible).

2.2 Endpoints

The primary endpoints were PFS and safety evaluated by Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE 5.0) (16). The secondary endpoints were OS and prognostic factors influencing the PFS of patients in real world. PFS was defined as the time from initiation of PARPi to radiographic progression according to response evaluation criteria in solid tumors (RECIST) version 1.1 (6), death from any cause, or study cutoff. OS was defined as the time from initiation of PARPi to death from any cause or study cutoff. The relevant factors included age, BRCA gene status, FIGO staging, histology, NACT, residual disease after surgery, response to chemotherapy and PARPi maintenance treatment. R0 was defined as no visible residual disease after surgery. R1 was defined as residual disease ≤1cm, and R2 was defined as residual disease >1cm. The response to chemotherapy was performed with RECIST 1.1.

2.3 Follow up

Follow-up was conducted through telephone interviews, outpatient visits, WeChat groups, QQ groups (an online community), and other methods to assess the survival status of patients and monitor adverse events (AEs) associated with PARPi. Safety-related data included AE terms, the highest CTCAE grade, treatment measures taken for AEs, measures taken for PARPi (dose reduction, interruption, discontinuation), and outcomes. The follow-up endpoint was disease recurrence, progression, death or the cut-off date, which was December 1, 2022.

2.4 Statistical analysis

For continuous variables that followed a normal distribution, they were presented as mean ± standard deviation (mean ± SD). If the variables did not follow a normal distribution, they were expressed as median (Q1, Q3). Categorical variables were presented as counts (n) and percentages (%). PFS and OS curves were described according to the Kaplan Meier method. The median follow-up time was calculated using the reverse Kaplan-Meier method. The univariate analysis associated with prolonged PFS was performed with the Log-rank test. Factors with a significance level of $P<0.05$ in the univariate analysis were included in the multivariate Cox analysis. A significance level of $P<0.05$ was used to define statistically significant differences. All statistical analyses were performed with SPSS version 25.0 software.

3 Results

3.1 Baseline characteristics

This study included a total of 164 patients, with 104 patients (104/164, 63.4%) receiving olaparib and 60 patients (60/164, 36.6%) receiving niraparib. The baseline characteristics of the patients are shown in Table 1. 100 patients (100/164, 61.0%) had mutations with BRCA. 77 patients (77/164, 47.0%) received NACT. 94 patients (94/164, 57.3%) achieved R0 after primary debulking surgery (PDS)/interval debulking surgery (IDS), while 17 patients (17/164, 10.4%) didn't have residual lesions after surgery. Among these, 9 patients had no description of residual lesions in the surgical records, In 3

TABLE 1 Clinicopathological characteristic in the real world.

Characteristics		Olaparib (N=104)	Niraparib (N=60)	*P*
Age (mean ± SD, years)		52.71 ± 10.33	55.42 ± 9.87	0.103
BMI (median (Q1,Q3), kg/m^2)		22.29 (20.52-24.44)	22.95 (20.83-25.21)	0.650
Complication, n (%)	Yes	34 (32.7)	19 (32.8)	0.892
	No	70 (67.3)	41 (40.6)	
Family history, n (%)	Yes	58 (36.6)	24 (31.6)	
	No	104 (63.4)	52 (68.4)	
BRCA gene, n (%)	Wild type	9 (8.7)	52 (86.7)	**0.000**
	Mutation type	92 (88.5)	8 (13.3)	
	Unknown	3 (2.9)	0	

(Continued)

TABLE 1 Continued

Characteristics		Olaparib (N=104)	Niraparib (N=60)	*P*
NACT, n (%)	Yes	53 (51.0)	24 (40.0)	0.175
	No	51 (49.00)	36 (60.0)	
The residual disease, n (%)	R0	54 (51.9)	40 (66.7)	0.277
	R1	29 (27.9)	10 (16.7)	
	R2	10 (9.6)	4 (6.7)	
	Unknown	11 (10.6)	6 (10.0)	
FIGO 2014, n (%)	I	1 (1.0)	1 (1.7)	0.929
	II	10 (9.6)	5 (8.3)	
	III	79 (76.0)	44 (73.3)	
	IV	14 (13.5)	10 (16.7)	
Histology, n (%)	HGSOC	101 (97.1)	53 (88.3)	0.057
	Endometrial	1 (1.0)	2 (3.3)	
	OCCC	0 (1.3)	2 (3.3)	
	Others	2 (1.0)	3 (5.0)	
First-line chemotherapy, n (%)	≤6 cycles	84 (80.8)	44 (73.3)	0.268
	>6 cycles	20 (19.2)	16 (26.7)	
Response to chemotherapy, n (%)	CR	69 (66.3)	43 (71.7)	0.777
	PR	32 (30.8)	17 (28.3)	
	SD	1 (1.0)	0	
	PD	2 (1.9)	0	
The interval between chemotherapy and maintenance therapy, n (%)	4-8 weeks	81 (77.9)	43 (71.6)	0.372
	>8 weeks	23 (22.1)	17 (28.4)	
Combined with bevacizumab in maintenance therapy, n (%)	Yes	22 (21.2)	4 (6.7)	**0.014**
	No	82 (78.8)	56 (93.3)	
CA125 before PARPi, n (%)	<35U/ml	101 (97.1)	54 (90.0)	0.075
	≥35U/ml	3 (2.9)	6 (10.0)	
Time of PARPi treatment, median (Q1,Q3)		15 (9-19)	9 (5-15)	**0.003**
PARPi, n (%)	Interruption	26 (25)	28 (46.7)	**0.004**
	Reduction	33 (31.7)	31 (51.7)	**0.012**
	Discontinuation	7 (6.7)	1 (1.7)	0.283

The clinicopathological data of newly diagnosed as EOC in West China Second Hospital of Sichuan University from August 1, 2018 to September 31, 2022 who took olaparib and niraparib as first-line maintenance were shown as follows. BMI, body mass index; BRCA, Breast Cancer Susceptibility Gene; NACT, neoadjuvant chemotherapy; FIGO, International Federation of Obstetrics and Gynecology; HGSOC, high-grade serous ovarian cancer; OCCC, ovarian clear cell carcinoma; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; PARPi, PARP inhibitor. The factors with a significance level of P<0.05 were bolded.

cases, the surgical records mentioned the presence of residual lesions but did not provide information about their size. Additionally, 5 patients underwent surgeries in other hospitals. In this study, 112 patients (112/164, 68.3%) achieved CR after first-line chemotherapy, while 49 patients (49/164, 29.9%) achieved PR. Three patients with advanced high-grade serous ovarian cancer (HGSOC) carrying BRCA mutations, who were evaluated as SD or PD after first-line chemotherapy, received olaparib treatment, and all three patients achieved R1 after PDS/IDS. A total of 40 patients (40/164, 16.1%) had an interval of more than 8 weeks between the end of chemotherapy and the start of PARPi treatment. 26 patients (26/164, 15.9%) received PARPi in combination with bevacizumab for maintenance treatment. 8 patients (8/164, 4.9%) experienced dose discontinuation due to AEs, including 7 patients in the olaparib group (3 with anemia, 1 with recurrent urinary tract infection, 1 with osteodynia, 1 with kidney failure and 1 with gastrointestinal reactions) and 1 patient in the niraparib group (grade ≥3 tachycardia).

3.2 Efficacy

The median follow-up time was 17.0 months (95%CI 15.6-18.4). As of December 1, 2022, the patients in this study did not reach the mPFS (see Figure 1), and the mOS was 38.9 months (95% CI 29.4-48.4). The maturity of PFS data in this study was 26.8% (44/164), and the maturity of OS data was 7.3% (12/164). There were a case of SD and 2 cases of PD after last chemotherapy, all of whom experienced disease progression and the PFS was 10.0 months, 7.0 months, and 24.4 months, respectively.

3.3 Influencing factors for PFS

Survival analysis was performed for PFS (see Tables 2, 3). The results showed that BRCA mutations (see Table 2, *P*=0.030), residual diseases after PDS/IDS(*P*=0.046), the response to last chemotherapy (*P*=0.018) were associated with PFS for patients with EOC. No significant impact was found in age, family history, complications, histology, FIGO stage, cycles of chemotherapy, bevacizumab administration, the interval between chemotherapy and maintenance therapy (*P*>0.05). Above factors with a significance level of P<0.05 were included in the multivariate analysis (see Table 3 and Figure 2). The results showed that the BRCA mutations and achieving CR or PR after first-line chemotherapy were independent factors influencing PFS for patients with EOC (BRCA, *P* =0.011; Response to last chemotherapy, *P*=0.043). However, there was no significant difference in PFS between patients who achieved CR and those who achieved PR (HR=1.448, 95%CI 0.723-2.903, *P*=0.296).

3.4 Safety

In this study, the safety characteristics of PARPi in real-world clinical practice were divided into olaparib group and niraparib group (see Table 4). In olaparib group(N=104), the most common AEs were leukopenia (67/104, 64.4%), anemia (56/104, 53.8%), loss of appetite (42/104, 40.4%) and nausea (41/104, 39.4%). The most common grade ≥3 AEs included anemia (10/104, 9.6%), thrombocytopenia (4/104, 5.3%), and leukopenia (5/104, 3.8%). In niraparib group (N=60), leukopenia (35/60, 58.3%), anemia (25/60, 41.7%) and sleeping disorders (24/60, 40.0%) were the most common AEs. Grade ≥3 AEs included thrombocytopenia (9/60, 15.0%). anemia (5/60, 8.3%), leukopenia (1/60, 1.7%), tachycardia (1/60, 1.7%) and constipation (1/60, 1.7%). There were no cases of MDS/AML.

Additionally, approximately 4.9% of patients (8/164) discontinued treatment (see Table 5). Among them, 2.4% (4/164) discontinued the medication due to grade ≥3 AEs (4 with anemia and 1 with tachycardia). Approximately 39.0% of patients (64/164) experienced dose reduction, with 7.9% (13/164) of them related to grade≥3 AEs, such as anemia(5/164, 3.0%) and thrombocytopenia (3/164, 1.8%). 32.9% (54/164) patients underwent dose interruption due to the most common AEs, including anemia (14/164, 8.5%), leukopenia (12/164, 7.3%) and thrombocytopenia (12/164, 7.3%).

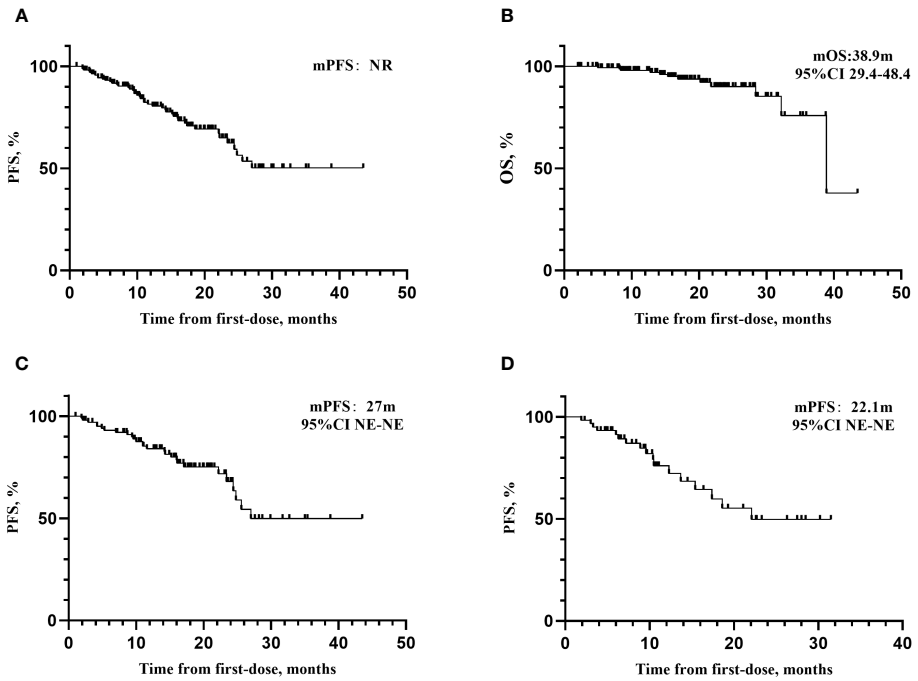

FIGURE 1
Kaplan–Meier curves for PFS and OS. **(A)** Kaplan–Meier curves for PFS. median follow-up time was 17.0 months (95%CI 15.6-18.4). As of December 1, 2022, the mPFS has not yet been reached. **(B)** Kaplan–Meier curves for OS. The mOS was 38.9 months (95%CI 29.4-48.4). **(C)** Kaplan–Meier curves for PFS of patients receiving olaparib. The mPFS for patients receiving olaparib was 27 months. **(D)** Kaplan–Meier curves for patients receiving niraparib. The mPFS for patients receiving niraparib was 22.1 months, respectively. mPFS, median progression-free survival; NE, not evaluable.

TABLE 2 Log-rank analysis of factors associated with prolonged PFS.

Characteristics		Log-Rank analysis		
		mPFS (95%CI)	χ^2	*P*
Age	<65 years	NR	2.776	0.096
	≥65 years	18.6 (NE)		
Complication	Yes	25.6 (NE)	0.218	0.640
	No	NR		
Family history	Yes	NR	2.990	0.084
	No	27.0 (NE)		
BRCA gene	Wild type	22.1 (15.9-28.3)	7.014	**0.030**
	Mutation type	NR		
	unknown	17.1 (4.8-29.4)		
NACT	Yes	25.6 (21.9-29.3)	1.607	0.205
	No	NR		
The residual disease	R0	NR	6.076	**0.046**
	≥R1	24.4 (21.8-27.0)		
	unknown	NR		
FIGO 2014	I-II	NR	1.502	0.472
	III	27.0 (NE)		
	IV	24.8 (NE)		
Histology	Serous	27.0 (NE)	0.430	0.232
	Others	NR		
First-line chemotherapy	≤6 cycles	NR	1.627	0.202
	>6 cycles	24.8 (20.4-29.2)		
Response to chemotherapy	CR	NR	8.074	**0.018**
	PR	23.4 (NE)		
	SD+PD	10.0 (5.2-14.8)		
Interval between chemotherapy and maintenance therapy	4-8 weeks	NR	1.308	0.253
	>8 weeks	24.8 (NE)		
PARPi	Olaparib	27.0 (NE)	1.986	0.159
	Niraparib	22.1 (NE)		
Combined with bevacizumab in maintenance therapy	Yes	24.4 (NE)	0.082	0.774
	No	NR		
PARPi interruption	Yes	NR	3.018	0.082
	No	27.0 (NE)		
PARPi reduction	Yes	NR	3.141	0.076
	No	24.8 (NE)		

It was found that BRCA mutation、residual diseases after primary surgery, the response to last chemotherapy were associated with PFS for patients with EOC (P<0.05). mPFS, median progression-free survival; BRCA, Breast Cancer Susceptibility Gene; NACT, neoadjuvant chemotherapy; FIGO, International Federation of Obstetrics and Gynecology; HGSOC, high-grade serous ovarian cancer; OCCC, ovarian clear cell carcinoma; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; PARPi, PARP inhibitor; NE, not evaluable; NR, not reached. The factors with a significance level of P<0.05 were bolded.

TABLE 3 Multivariate analysis of factors associated with prolonged PFS.

Clinical characteristics	Multivariate analysis							
	B	SE	Wald	df	*P*	HR	95.0% CI	
							Lower	Upper
BRCA gene			9.076	2	**0.011**			
Wild type V.S. mutation type	-0.976	0.334	8.545	1	**0.003**	0.377	0.196	0.725
Wild type V.S. unknown	-0.019	0.782	0.001	1	0.981	0.981	0.212	4.542
Residual disease			5.331	2	0.070			
R0 V.S. ≥R1	0.640	0.359	3.175	1	0.075	1.897	0.938	3.837
R0 V.S. unknown	0.982	0.490	4.021	1	**0.045**	2.670	1.022	6.971
Response to chemotherapy			6.313	2	**0.043**			
CR VS PR	0.370	0.355	1.091	1	0.296	1.448	0.723	2.903
CR VS SD+PD	1.638	0.665	6.069	1	**0.014**	5.146	1.398	18.948

The BRCA gene status and achieving CR or PR after first-line chemotherapy were independent factors influencing PFS for patients with EOC. However, there was no significant difference in PFS between patients who achieved CR and those who achieved PR. BRCA, Breast Cancer Susceptibility Gene; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease. The factors with a significance level of P<0.05 were bolded.

4 Discussion

This study is a single-center real-world study with the largest sample size in China, demonstrating the effectiveness and tolerability of PARPi as first-line maintenance therapy for patients with EOC. Based on the existing data maturity, BRCA mutations and CR or PR after first-line chemotherapy were independent factors associated with prolonged PFS, which should be further confirmed with long-term follow-up and large sample sizes.

The SOLO-1 study (10), which focused on newly diagnosed advanced EOC patients with BRCA mutations and included 10 patients from our center, showed that the mPFS for patients receiving olaparib was 56.0 months after 5-year follow-up. As of the 7th year, the olaparib group did not reach the mOS. There were 32 patients in our center included in PRIME study (12). With a follow-up of 27.5 months, in the intention-to-treat (ITT) population, the mPFS was 24.8 months in niraparib group and 8.3 months in the placebo group (HR=0.45, 95% CI: 19.2-NE). Data for OS was not mature in the ITT population. By the data cutoff, 65 patients (37 in niraparib group [56.9%] and 28 in the placebo group [43.1%]) had died (HR,0.63;95% CI,0.38-1.03), and the estimated 24-month OS rate was 87.3% for niraparib and 82.7% for placebo. PRIME study identified that for patients newly diagnosed as EOC, regardless of postoperative residual diseases or biomarker status, niraparib could reduce the risk of disease progression or death compared to placebo. Based on the PRIMA study (11), after 13.8 months of follow-up, the mPFS of niraparib group was 13.8 months, showing a 38% reduced risk of recurrence or death compared to the placebo group (HR=0.62, 95% CI 0.50-0.76, *P*<0.001) in the overall population. In this real-world study, after 17.0 months of follow-up, the mPFS has not been reached, and mOS was 38.9 months (95% CI 29.4-48.4). Among them, the mPFS of olaparib group (N=104) was 27.0 months, and that of niraparib group (N=60) was 22.1 months. The mPFS of the niraparib group is comparable to that in PRIME study (12), but better than that in PRIMA study (11). It was related

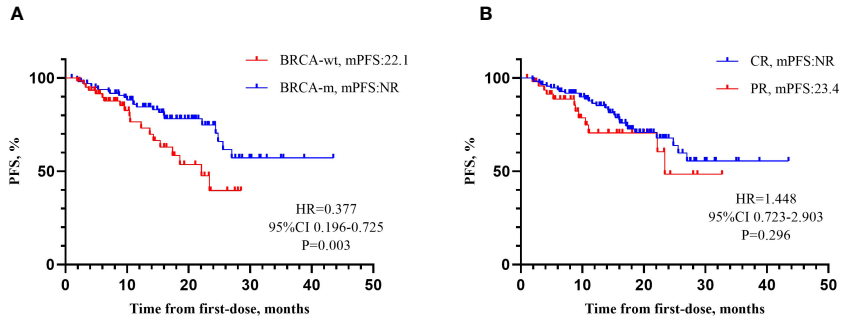

FIGURE 2
Influencing factors for PFS. **(A)** mPFS according to BRCA gene. Patients with BRCA-m demonstrated a 62.3% reduction in the risk of disease progression compared to patients with BRCA-wt (HR=0.377, 95% CI 0.196-0.725, *P*=0.003). **(B)** Response to last chemotherapy. There was no significant difference in PFS between patients who achieved CR and those who achieved PR (HR=1.448, 95% CI 0.723-2.903, *P*=0.296). It might be related to the currently immature data of PFS (26.8%).

TABLE 4 Common AEs for olaparib and niraparib in the real world.

Terms	Olaparib (N=104)		Niraparib (N=60)	
	N (%)	≥G3 (%)	N (%)	≥G3 (%)
Hematological system				
Anemia	56 (53.8)	10 (9.6)	25 (41.7)	5 (8.3)
Leukopenia	67 (64.4)	5 (3.8)	35 (58.3)	1 (1.7)
Thrombocytopenia	25 (24.0)	4 (5.3)	23 (38.3)	9 (15.0)
Gastrointestinal system				
Nausea	41 (39.4)	0	23 (38.3)	0
Vomiting	35 (33.7)	0	13 (21.7)	0
Diarrhea	8 (7.7)	0	0	0
Constipation	23 (22.1)	0	12 (20.0)	1 (1.7)
Loss of appetite	42 (40.4)	0	12 (20.0)	0
Fatigue	28 (26.9)	0	6 (10.0)	0
Infection and invasive disease				
Upper respiratory tract infection	4 (3.8)	0	1 (1.7)	0
Urinary tract infection	20 (19.2)	1 (1.0)	5 (8.3)	0
Neurological System				
Sleeping disorders	36 (34.6)	0	24 (40.0)	0
Cardiovascular System				
Tachycardia	10 (9.6)	0	11 (18.3)	1 (1.7)
Hypertension	2 (1.9)	0	5 (8.3)	0
Abdominal liver and kidney function				
Elevated transaminases	16 (15.4)	2 (1.9)	19 (31.7)	0
Elevated creatinine	19 (18.3)	0	6 (10.0)	0
Kidney failure	3 (2.9)	1 (1.0)	0	0
Others				
Muscle, skeletal and joint pain	33 (31.7)	0	12 (20.0)	0
Dermatitis, rash, photosensitivity	6 (5.8)	0	10 (16.7)	0
Oral ulcers, oral mucositis	20 (19.2)	0	5 (8.3)	0

In olaparib group, the most common AEs were leukopenia, anemia, loss of appetite and nausea. The most common grade ≥3 AEs included anemia, thrombocytopenia, and leukopenia. In niraparib group, leukopenia, anemia and sleeping disorders were the most common AEs. Grade ≥3 AEs included thrombocytopenia, anemia, leukopenia, tachycardia, and constipation. There were no cases of MDS/AML event. No additional safety signals happened.

to that PRIMA study focused on advanced patients with high risk of recurrence, among whom 35% were FIGO IV (16.7% of patients were in this study), 66% of patients underwent NACT (40.0% of patients in this study), and 99.6% of FIGO III patients still had residual lesions after primary cytoreductive surgery (30.2% in this study). The data of OS was not mature (12/164, 7.3%). Long-term follow-up is necessary to improve the comprehensiveness and reliability of survival data.

Genetic testing is considered crucial in the assessment of familial genetic risk. The first edition of the National Comprehensive Cancer Network (NCCN) Ovarian Cancer Guidelines in 2023 re-emphasized the significance of BRCA gene testing for all non-mucinous ovarian cancer patients upon their first pathologically diagnosis. It was also highlighted the necessity of HRD testing for BRCA wild type (BRCA-wt) patients (17). However, it was found that in the first-line maintenance treatment, the olaparib group had 88.5% of BRCA mutated type (BRCA-m) patients, while the niraparib group had 86.7% of BRCA-wt patients. The difference in BRCA gene status between the two groups was statistically significant ($P<0.001$). The reason for this difference is that the first edition of the NCCN guidelines in 2019 (18) recommended the use of olaparib for BRCA-m patients.

TABLE 5 Common AEs for PARPi Interruption, Reduction, and Discontinuation.

Terms	Dose interruption		Dose reduction		Dose discontinuation	
	N (%)	≥G3 (%)	N (%)	≥G3 (%)	N (%)	≥G3 (%)
	54 (32.9)	18 (11.0)	64 (39.0)	13 (7.9)	8 (4.9)	4 (2.4)
Hematological system						
Anemia	14 (8.5)	7 (4.3)	11 (6.7)	5 (3.0)	3 (1.8)	3 (1.8)
Leukopenia	12 (7.3)	2 (1.2)	17 (10.4)	1 (0.6)	0	0
Thrombocytopenia	12 (7.3)	5 (3.0)	9 (5.5)	3 (1.8)	0	0
Bone marrow suppression	6 (3.7)	2 (1.2)	6 (3.7)	2 (1.2)	0	0
Gastrointestinal system						
Nausea	0	0	5 (3.0)	0	2 (1.2)	0
Vomiting	2 (1.2)	0	1 (0.6)	0	0	0
Diarrhea	0	0	1 (0.6)	0	0	0
Constipation	1 (0.6)	0	2 (1.2)	0	0	0
Fatigue	0	0	1 (0.6)	0	0	0
Cardiovascular System						
Tachycardia	0	0	0	0	1 (0.6)	1 (0.6)
Hypertension	1 (0.6)	0	0	0	0	0
Abdominal liver and kidney function						
Elevated transaminases	2 (1.2)	1 (0.6)	2 (1.2)	1 (0.6)	0	0
Elevated creatinine	0	0	5 (3.0)	0	1 (0.6)	0
Kidney failure	1 (0.6)	1 (0.6)	2 (1.2)	1 (0.6)	0	0
Others						
Muscle, skeletal and joint pain	1 (0.6)	0	2 (1.2)	0	1 (0.6)	0
Dermatitis, rash, photosensitivity	2 (1.2)	0	0	0	0	0
Oral ulcers, oral mucositis	0	0	0	0	0	0

Approximately 39.0% of patients had experienced dose reduction, with 7.9% of them related to Grade≥3 AEs, such as anemia and thrombocytopenia. 32.9% of patients underwent dose interruption due to the most common AEs, including anemia, leukopenia and thrombocytopenia. Approximately 4.9% of patients discontinued treatment, while 2.4% discontinued the medication due to Grade ≥3 AEs (4 with anemia and 1 with tachycardia).

However, the first edition of the NCCN guidelines in 2020 (19) recommended niraparib for all newly diagnosed advanced EOC patients. It reflected a strict adherence to guidelines and the emphasis on patient education in our center. In this study, patients with BRCA-m demonstrated a 62.3% reduction in the risk of disease progression compared to patients with BRCA-wt (HR=0.377, 95% CI 0.196-0.725). In the PRIME study (12), mPFS with niraparib was not reached in patients with germline BRCA-m and 19.3 months in patients without germline BRCA-m, respectively; For patients receiving niraparib, the mPFS was not reached with homologous recombination deficient (HRD) and 16.6 months with homologous recombination proficient, respectively. In the PRIMA study (11), the mPFS for patients with BRCA-m, BRCA-wt/HRD-positive and BRCA-wt/HRD-negative were 22.1 months, 19.6 months, and 8.1 months, respectively. It demonstrated that HRD-negative patients derived significantly less benefit compared to those with BRCA-m and HRD-positive patients. Additionally, only 23% of BRCA-wt patients in this research underwent HRD testing, which could be attributed to several factors. Firstly, our center was located in a less economically developed region in the western China. The cost of HRD testing was expensive, making it unaffordable for many patients. Moreover, there were no approved HRD testing kits available for clinical use in China and some HRD tests had false-positive and false-negative results (9). Therefore, it is essential to promote greater access to HRD testing kits to support clinical practice and research.

Additionally, except for BRCA/HRD testing, technologies of proteomics play a gradually important role in ovarian cancer. Proteomics analysis of ovarian cancer, as well as their adaptive responses to therapy, can uncover new therapeutic choices, which can reduce drug resistance and potentially improve patient outcomes (20). Paulovich, et al. performed a proteogenomic analysis of untreated HGSOCs (chemotherapy-sensitive and refractory) which identified a highly specific 64-protein signature

to predict a subpopulation of refractory HGSOCs (21). In addition, they also identified 5 different HGSOC subtypes based on protein expression in the pathway, which may represent different resistance mechanisms and serve as potential therapeutic targets. Consequently, we do believe that proteomic analysis will be a dawn of a new era for the discovery of new biomarkers for diagnosis and prognosis of EOC patients.

In this study, the mPFS for patients with CR, PR, SD+PD after chemotherapy were not reached, 23.4 months, 10.0 months, respectively. CR or PR after first-line chemotherapy was an independent factor associated with prolonged PFS. However, there was no significant difference in PFS between patients who achieved CR and those who achieved PR (HR=1.448, 95% CI 0.723-2.903, *P*=0.296). It might due to the currently immature data of PFS (26.8%). In a study with 84 ovarian cancer patients in the real-world setting, there was no significant difference in PFS between patients with CR and patients with PR (HR=0.520, 95% CI 0.115-2.339, *P*=0.394) (22). Another study including 76 EOC patients found that CR after first-line chemotherapy was an independent factor influencing PFS. The PR group had a higher risk of disease progression compared to the CR group (HR=3.208, 95% CI 1.278-8.056, *P*=0.013) (23). Additionally, during the data collection process, we identified 3 BRCA-m patients with HGSOC who were assessed as SD (n=1) and PD (n=2) after first-line chemotherapy and subsequently received olaparib treatment. All three patients had R1 after PDS/IDS. At the end of the follow-up period, they all experienced disease progression, with PFS of 10.0 months, 7.0 months, and 24.4 months, respectively. It highlighted the clinical challenge of using PARPi for patients who did not achieve CR or PR after first-line platinum-based chemotherapy but had high-risk factors. For patients who do not meet the recommended scope of clinical guidelines, it is crucial to make clinical decisions based on a comprehensive evaluation of the individual clinical situation and patient-centered care.

NACT could increase the probability of satisfactory cytoreductive surgery, reduce perioperative complications, and improve the quality of life for EOC patients. However, compared to PDS, NACT followed by IDS did not significantly improve the OS of patients (24–26). In this study, 47.0% of the patients received NACT. The mPFS in the NACT+IDS group was 25.6 months, while that in the PDS group was not reached. There was no statistically significant difference in PFS between the two groups (*P*>0.05). The PRIMA study (11) showed that in patients with NACT+IDS, the mPFS of niraparib group was 13.9 months, and the risk of disease progression or death was reduced by 41% compared with the placebo group (HR=0.59, 95%CI 0.41-0.76). The mPFS in NACT group in this study was longer than that in the PRIMA study. This could be attributed to the fact that the PRIMA study enrolled patients with high risk of recurrence. Additionally, a *post hoc* analysis of the PRIMA study revealed that patients with PDS showed a mPFS of 13.7 months in the niraparib group (N=158) compared to 8.2 months in the placebo group (N=78, HR=0.67, 95%CI 0.47-0.96). In the NACT+IDS group, the mPFS of the niraparib group (N=316) were 6 months longer than that of the placebo group (N=165, 14.2 months V.S. 8.2 months, HR=0.57, 95% CI 0.44-0.73) (27). Indeed, regardless of whether NACT was administered or not, niraparib showed the ability to improve the PFS of patients. However, there was limited research on whether NACT could enhance the effectiveness of PARPi as first-line maintenance therapy or not. It is crucial to further explore the impact of NACT on the prognosis of EOC patients with larger sample sizes.

In the overall management of advanced ovarian cancer, no macroscopic residual lesions after surgical treatment (R0) is important to improve the prognosis of patients and avoid the occurrence of platinum resistance (28). In the multivariable analysis, there was no significant difference in PFS among groups with different macroscopic residual lesions (*P*=0.07). It might due to the data immaturity of PFS (26.8%) and small sample size. Additionally, 9 cases lacked descriptions of residual disease in the surgical records, 3 cases only mentioned the presence of residual disease without specifying the size, and 5 cases underwent surgeries in other hospital. Clinical physicians should be reminded to provide detailed and explicit records of the presence of residual disease, its location, size, and other relevant information after surgery. Furthermore, CR after chemotherapy may potentially weaken the impact of R0 resection on patient prognosis. Therefore, long-term follow-up is needed to confirm the results of *post hoc* analysis. Nevertheless, R0 resection remains a cornerstone in the comprehensive management of advanced ovarian cancer, which is a crucial factor in prolonging the time to disease recurrence, avoiding resistance, and improving prognosis of patients.

In the PAOLA1 study (13), after a median follow-up time of 22.9 months, the mPFS of olaparib combined with bevacizumab in the general population was 22.1 months, and the risk of disease progression or death was reduced by 41% compared with the placebo plus bevacizumab group (HR=0.59, 95% CI 0.49-0.72, *P*<0.001). The 2022 ESMO meeting updated the 5-year PFS rate of olaparib combined with bevacizumab in HRD-positive patients. The risk of disease progression or death was reduced by 59% compared with placebo combined with bevacizumab (46.1% V.S. 19.2%, HR= 0.41, 95%CI 0.32-0.54), and the 5-year OS rate of HRD-positive patients was 65.5% (HR=0.62, 95%CI 0.45-0.85) (29). The OVARIO study (30) presented its latest data at the 2022 Society of Gynecologic Oncology (SGO) conference. With a median follow-up time of 28.7 months, the combination of niraparib with bevacizumab demonstrated a mPFS of 19.6 months (95% CI 16.5-25.1) in the overall population. In this study, the patients who received a combination of PARPi and bevacizumab were specifically those with residual disease ≥R1 after surgery or those with other high-risk factors for recurrence in ovarian cancer. None of the 24 patients reached the mPFS or mOS, which suggested a potential beneficial trend of PARPi in combination with bevacizumab for patients with residual disease ≥R1 or those with high risks of recurrence. Additional RWS with larger sample sizes and longer follow-up periods are necessary in the clinical practice.

No new AEs were found in this study. Hematologic toxicity was the most common grade≥3AE, which was the main cause of dose reduction, interruption and discontinuation. It may be related to the physiological functions of PARP enzyme, except for DNA repair.

For example, PARP1 regulates cell differentiation in the bone marrow or hematopoietic system (31), while PARP2 plays a role in regulating erythropoiesis (32). Additionally, PARP1 is expressed in the megakaryocyte lineage to regulate the formation of platelets (33). The incidence of grade≥3 anemia was 9.6% in olaparib group and 8.3% in niraparib group, compared to 22.0% in SOLO1 study (34), 31.6% in PRIMA study (11), and 18.0% in PRIME study (12). The incidence of severe anemia in our center was relatively low, which could be attributed to the individualized starting dose administration, and the rigorous monitoring and management of complete blood counts. The incidence of grade ≥3 thrombocytopenia in our study was closely consistent with the data from the PRIME study (15.0%V.S. 14.1%) (12), both of which were based on the Chinese population.

There was no case of MDS/AML in this study. However, the incidence of myeloid neoplasms in SOLO1 study after 7-year follow-up was 1.5% while that in PRIMA study after 3.5-year follow-up was 1.2% and in PAOLA-1 study after 5-year follow-up was 1.7% (10, 11, 13, 35). As a delayed AE, the median latency period of the occurrence of MDS/AML after taking PARPi was 17.8 months, which was considered as a critical window period for the development of myeloid neoplasms after PARPi (36). Additionally, persistent cytopenia is considered as an early warning sign. Active surveillance, differential diagnosis, and prompt hematological referral are crucial for MDS/AML (35).

5 Limitation

1) The sample size for first-line PARPi maintenance therapy was not large enough as expected. It might be related to the fact that the center was in a less economically developed region in the western China, PARPi and BRCA testing were both expensive in the patient's cognition when PARPi were first recommended by NCCN guideline in 2019. With the indications of drugs added to medical insurance, the acceptance of PARPi has been gradually increased.
2) It was difficult to establish a control group. As a single-arm retrospective RWS, the efficacy of PARPi could only be compared with external controls, such as SOLO-1, PRIMA, PRIME studies.
3) Some patients just had a follow up of 3 months. Hence, we provided the data maturity as a reference. However, insufficient data maturity may result in less significant statistical results and inaccurate estimates of the power. Long-term follow-up is necessary to accumulate more survival-related data and further analyze the factors that influence the treatment efficacy in patients.
4) Due to the high cost of HRD testing and the lack of availability of relevant domestic HRD testing kits, some patients did not complete HRD testing. Therefore, this study did not conduct an analysis about HRD-related data.
5) The collection of safety data had some limitations, such as the investigator's assessment of the causal relationship between AEs and PARPi, and the treatment measures taken for the AEs. Further standardization is needed in the collection and administration of safety data.

6 Conclusion

This study represents the largest single-center real-world study conducted in China to date, focusing on the use of PARPi as first-line maintenance therapy for patients with EOC. The BRCA mutation status and the achievement of CR/PR in first-line chemotherapy were identified as independent factors influencing the PFS of patients. There have been no cases of MDS/AML by the study cuf-off.

Data availability statement

The datasets presented in this article are not readily available because the data generated in this study are not publicly available, which may compromise patient privacy or consent. Requests to access the datasets should be directed to RY, yinrutie@scu.edu.cn.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of West China Second University Hospital, Sichuan University. Ethical Lot Number 20220129. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from Electronic medical record system. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

JC: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. MZ: Data curation, Writing – original draft. KL: Formal analysis, Writing – original draft. YD: Investigation, Writing – original draft. JZ: Methodology, Writing – original draft. QL: Project administration, Resources, Writing – original draft. DW: Project administration, Resources, Writing – original draft. LS: Resources, Writing – original draft. QL: Conceptualization, Supervision, Writing – review & editing. RY: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020:GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China:a secondary analysis of the global cancer statistics 2020. *Chin Med J(Engl)* (2021) 134(7):783–91. doi: 10.1097/CM9.0000000000001474

3. Konstantinopoulos PA, Matulonis UA. Clinical and translational advances in ovarian cancer therapy. *Nat Cancer* (2023) 4(9):1239–57. doi: 10.1038/s43018-023-00617-9

4. Kurman RJ, Shih IM. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer–shifting the paradigm. *Hum Pathol* (2011) 42:918–31. doi: 10.1016/j.humpath.2011.03.003

5. Wen H, Zou DL, Wu XH. Chinese guidelines to the maintenance therapy for epithelial ovarian cancer (2022). *Chin J Pract Gynecol Obstet* (2022) 38(01):56–65. doi: 10.19538/j.fk2021060111

6. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). *Eur J Cancer* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026

7. Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, et al. Standard chemotherapy with or without bevacizumab for women with newlydiagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomized trial. *Lancet Oncol* (2015) 16(8):928–36. doi: 10.1016/S1470-2045(15)00086-8

8. Tewari KS, Burger RA, Enserro D, Norquist BM, Swisher EM, Brady MF, et al. Final overall survival of a randomized trial of bevacizumab for primary treatment of ovarian cancer. *J Clin Oncol* (2019) 37 (26):2317–28. doi: 10.1200/JCO.19.01009

9. Caruso G, Tomao F, Parma G, Lapresa M, Multinu F, Palaia I, et al. Poly (ADP-ribose) polymerase inhibitors (PARPi) in ovarian cancer: lessons learned and future directions. *Int J Gynecol Cancer* (2023) 33(4):431–43. doi: 10.1136/ijgc-2022-004149

10. DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim BG, Oaknin A, et al. Overall survival with maintenance olaparib at a 7-year follow-up in patients with newly diagnosed advanced ovarian cancer and a BRCA mutation: the SOLO1/GOG 3004 trial. *J Clin Oncol* (2023) 41(3):609–17. doi: 10.1200/JCO.22.01549

11. Gonzalez-Martin A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* (2019) 381(25):2391–402. doi: 10.1056/NEJMoa1910962

12. Li N, Zhu J, Yin R, Wang J, Pan LY, Kong BH, et al. Treatment with niraparib maintenance therapy in patients with newly diagnosed advanced ovarian cancer: A phase 3 randomized clinical trial. *JAMA Oncol* (2023) 9(9):1230–7. doi: 10.1001/jamaoncol.2023.2283

13. Ray-Coquard I, Leary A, Pignata S, Cropet C, González-Martín A, Marth C, et al. Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. *Ann Oncol* (2023) 34(8):681–92. doi: 10.1016/j.annonc.2023.05.005

14. Yang S, Ma LT, Zhang QQ. Chinese practice algorithm on real world study of clinical medicine. *PLA Med J* (2018) 43(01):1–6. doi: 10.11855/j.issn.0577-7402.2018.01.01

15. Wang RP. Critical points of real world clinical study classification and design. *Shanghai Med Pharm J* (2022) 43(23):63–7.

16. US Department of Health and Human Services. *Common terminology criteria for adverse events. Version 5.0. Published November 27, 2017.* (2020). Available at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.

17. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). *Ovarian Cancer, Including Fallopian Tube Cancer and Primary Peritoneal Cancer. Version 1.2023.* Fort Washington: National Comprehensive Cancer Network (2023).

18. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). *Ovarian Cancer, Including Fallopian Tube Cancer and Primary Peritoneal Cancer. Version 1.2019.* Fort Washington: National Comprehensive Cancer Network (2019).

19. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). *Ovarian Cancer, Including Fallopian Tube Cancer and Primary Peritoneal Cancer. Version 1.2020.* Fort Washington: National Comprehensive Cancer Network (2020).

20. Ghose A, Gullapalli SVN, Chohan N, Bolina A, Moschetta M, Rassy E, et al. Applications of proteomics in ovarian cancer: dawn of a new era. *Proteomes* (2022) 10 (2):16. doi: 10.3390/proteomes10020016

21. Chowdhury S, Kennedy JJ, Ivey RG, Murillo OD, Hosseini N, Song X, et al. Proteogenomic analysis of chemo-refractory high-grade serous ovarian cancer. *Cell* (2023) 186(16):3476–98.e35. doi: 10.1016/j.cell.2023.07.004

22. Zheng H, Gao Y, Guo H, Li L, Li Q, Cui H, et al. Real-world experience of olaparib treatment in patients with ovarian cancer: A Chinese multicenter study. *Mol Cancer Ther* (2021) 20(9):1735–42. doi: 10.1158/1535-7163.MCT-20-1064

23. Wang DF, Zhang J, Zhang C, Yu J, Shi Y, Xu SQ, et al. Real–world clinical data analysis of PARPi as first–line maintenance therapy in newly diagnosed epithelial ovarian cancer patients. *Chin J Obstet Gynecol* (2022) 57(9):641–52. doi: 10.3760/cma.j.cn112141-20220728-00490

24. Kehoe S, Hook J, Nankivell M, Jayson GC, Kitchener H, Lopes T, et al. Primary chemotherapy versus primary surgery for newly diagnosed advanced ovarian cancer (CHORUS):an open-label,randomized,controlled,non-inferiority trial. *Lancet* (2015) 386 (9990):249–57. doi: 10.1016/S0140-6736(14)62223-6

25. Onda T, Satoh T, Saito T, Kasamatsu T, Nakanishi T, Nakamura K, et al. Comparison of treatment invasive- ness between upfront debulking surgery versus interval debulk- ing surgery following neoadjuvant chemotherapy for stage III/IV ovarian,tubal,and peritoneal cancers in a phase III randomiszd trial:Japan Clinical Oncology Group Study JCOG060. *Eur J Cancer* (2016) 64:22–31. doi: 10.1016/j.ejca.2016.05.017

26. Yuan H, Zhang SQ, Li XP. A quick guide to the indications for neoadjuvant chemotherapy in advanced epithelial ovarian cancer(2021). *Chin J Pract Gynecol Obstet* (2021) 37(04):444–8. doi: 10.19538/j.fk2021040110

27. O'Cearbhaill RE, Pérez-Fidalgo JA, Monk BJ, Tusquets I, McCormick C, Fuentes J, et al. Efficacy of niraparib by time of surgery and postoperative residual disease status: A *post hoc* analysis of patients in the PRIMA/ENGOT-OV26/GOG-3012 study. *Gynecol Oncol* (2022) 166(1):36–43. doi: 10.1016/j.ygyno.2022.04.012

28. Nishio S, Ushijima K. Clinical significance of primary debulking surgery and neoadjuvant chemotherapy-interval debulking surgery in advanced ovarian cancer. *Jpn J Clin Oncol* (2020) 50(4):379386. doi: 10.1093/jjco/hyaa015

29. Nagao S, Harter P, Leary A, Cropet C, Pignata S, Fujiwara K, et al. Final overall survival (OS) results from the phase III PAOLA-1/ENGOT-ov25 trial evaluating maintenance olaparib (ola) plus bevacizumab (bev) in patients (pts) with newly diagnosed advanced ovarian cancer (AOC). *Ann Oncol* (2022) 33(suppl_9):S1503–14. doi: 10.1016/j.annonc.2022.10.212

30. Hardesty MM, Krivak T, Wright GS, Hamilton E, Fleming EL, Belotte J, et al. Phase 2 OVARIO study of niraparib + Bevacizumab therapy in advanced ovarian cancer following frontline platinum-based chemotherapy with bevacizumab. *Gynecol Oncol* (2022) 166(2):219–29. doi: 10.1016/j.ygyno.2022.05.020

31. Hopkins TA, Ainsworth WB, Ellis PA, Donawho CK, DiGiammarino EL, Panchal SC, et al. PARP1 trapping by PARP inhibitors drives cytotoxicity in both cancer cells and healthy bone marrow. *Mol Cancer Res* (2019) 17(2):409–19. doi: 10.1158/1541-7786.MCR-18-0138

32. Farrés J, Martín-Caballero J, Martínez C, Lozano JJ, Llacuna L, Ampurdanés C, et al. Parp-2 is required to maintain hematopoiesis following sublethal γ-irradiation in mice. *Blood* (2013) 122(1):44–54. doi: 10.1182/blood-2012-12-472845

33. De Botton S, Sabri S, Daugas E, Zermati Y, Guidotti JE, Hermine O, et al. Platelet formation is the consequence of caspase activation within megakaryocyte. *Blood* (2002) 100(4):1310–7. doi: 10.1182/blood-2002-03-0686

34. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* (2018) 379(26):2495–505. doi: 10.1056/NEJMoa1810858

35. Caruso G, Gigli F, Parma G, Lapresa M, Derio S, Palaia I, et al. Myeloid neoplasms post PARP inhibitors for ovarian cancer. *Int J Gynecol Cancer* (2023) 33 (4):598–606. doi: 10.1136/ijgc-2022-004190

36. Zeng J, Yin RT. Interpretation of Myelodysplastic syndrome and acute myeloidleukaemia in patients treated with PARP inhibitors: a safety meta-analysis of randomized controlled trials and a retrospective study of the WHO pharmacovigilance database. *Chin J Med Front* (2021) 13(07):128–32.

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Clinical efficacy of plasmid encoding p62/SQSTM1 (Elenagen) in combination with gemcitabine in patients with platinum-resistant ovarian cancer: a randomized controlled trial

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Background: The purpose of this trial is to evaluate the safety and efficacy of ELENAGEN, a novel anticancer therapeutic DNA plasmid encoding p62/SQSTM1 protein, as an adjuvant to chemotherapy with gemcitabine (GEM) in patients with advanced platinum-resistant ovarian cancer.

Methods: This open-label prospective randomized study with two arms. GEM (1000 mg/m^2) on days 1 and 8 every 3 weeks was administered in both arms: in the Chemo arm (n = 20), GEM was the only treatment, and in the ELENAGEN arm (n = 20), GEM was supplemented with ELENAGEN (2.5 mg i.m. weekly). The primary endpoint was progression-free survival (PFS), and the secondary endpoint was safety. Antitumor activity was assessed by RECIST 1.1, and criteria safety was assessed according to NCI CTCAE version 5.0.

Results: According to the cutoff data, the median follow-up was 13.8 months. There were no serious adverse events related to ELENAGEN treatment. The median PFS was 2.8 and 7.2 months in the Chemo and ELENAGEN arms, respectively (p Log-Rank = 0.03). Notably, at the time of cutoff, 9 patients (45%) in the ELENAGEN arm did not progress, with the longest PFS recorded thus far being 24 months. Subgroup analysis of patients in both arms demonstrated high efficacy of ELENAGEN in patients with worse prognostic

factors: high pretreatment levels of CA125 and progression after platinum-free interval <3 months.

Conclusions: The addition of ELENAGEN to gemcitabine is effective in patients with platinum-resistant ovarian cancer, including those with a worse prognosis.

Clinical trial registration: https://www.clinicaltrials.gov/study/NCT05979298, identifier NCT05979298, 2023-08-07.

KEYWORDS

chemotherapy, DNA vaccine, immunotherapy, chemoresistance, platinum

Background

Approximately 20 000 new cases of ovarian cancer (OC) are diagnosed in the US every year, and its overall 5-year survival rate is about 50% (1). This high lethality occurs because patients are mainly diagnosed with OC at later stages, and, following front-line therapy, tumors eventually become chemoresistant (2). Combination of platinum-based chemotherapy with taxanes still remains the standard of care for advanced and recurrent OC, but recurrent OC remains difficult to treat due to chemotherapy resistance (2). Despite introduction of antiangiogenic and poly ADP-ribose polymerase I (PARP) inhibitors in recent years, they only modestly improved patient's progression-free survival (3–5). Thus, novel OC therapeutics to improve long-term outcomes are urgently needed.

Recently, immunotherapy of cancer, especially with immune-checkpoint inhibitors (ICI), emerged as a novel treatment option for a number of solid tumors, and it was also tested in several clinical trials with OC (6). However, unlike other tumor types, the results of these trials were not encouraging. For instance, in patients with platinum-resistant OC, compared with standard chemotherapy with gemcitabine (GEM) or pegylated liposomal doxorubicin (PLD), PFS with the ICI nivolumab (anti-PDL1 antibody) was only 2.0 vs 3.8 months with GEM or PLD, and OS was 10.1 vs 12.1 months (7). Additionally, grade 3-related adverse events (AEs) occurred in 33% of patients in the nivolumab group (7). In the JAVELIN Ovarian 200 phase III trial of 566 patients with platinum-resistant OC, the addition of another anti-PD-L1 antibody, avelumab, to standard PLD treatment did not significantly increase PFS (3.7 vs 3.5 months) or OS (15.7 vs 13.1 months) (8). Furthermore, serious treatment-related adverse events occurred in 18% of patients in the combination group, compared with 11% in the PLD-only group (8). Thus, at present, the application of ICIs in the treatment of platinum-resistant OC does not appear encouraging.

We have recently developed a novel anticancer therapeutic, ELENAGEN, based on plasmid DNA encoding the p62 (SQSTM1) protein (9). p62 is a multifunctional protein that participates in selective autophagy, signal transduction, the inflammatory response and other processes (10). p62 can be a good target for anticancer vaccines since its levels are elevated in almost all human tumors tested thus far, and it increases when tumors progress (see ref ((11, 12) for review). While p62 is dispensable for normal cells, tumors require p62 for growth and metastasis (11). Importantly, p62 levels are also increased in OC and are associated with poor prognosis and platinum resistance, making p62 a good target for the immune response elicited by ELENAGEN (13, 14).

We conducted a preclinical study of the antitumor activity of ELENAGEN on several types of solid tumors in rodents. The drug showed its effectiveness on four types of solid tumors in mice (breast carcinoma, lung carcinoma, melanoma and sarcoma) as well as breast carcinoma in rats. Importantly, we observed suppression of metastasis in three different mouse models (9). Additionally, we conducted a pilot study of Elenagen in dogs with spontaneous mammary tumors, which are much closer to human breast tumors than transplantable tumors in rodents. We found that Elenagen in dogs exerted its effects in two ways: 1) in neoadjuvant settings, it made invasive and nonresectable tumors resectable, and 2) if mastectomy was impossible, tumors completely stopped growing during the period of observation (15, 16). Importantly, no toxicity of ELENAGEN was observed in either rodents or dogs (9, 15, 16).

Furthermore, we conducted a phase I/IIa clinical trial of ELENAGEN used as a monotherapy (17). In that study, ELENAGEN showed promise in treating patients with advanced disease for which all standard methods of treatment were exhausted. For example, the progression of OC was stopped for three or more months in 4 out of 6 patients. Importantly, in contrast to ICI (see above), AEs during ELENAGEN treatment were only Grade 1, and no severe AEs were observed (17). These data encouraged us to conduct a current clinical study of ELENAGEN with platinum-resistant OC.

In addition to evoking antitumor T- and B-cell immune responses (9, 15, 16), ELENAGEN can also alleviate chronic inflammation by suppressing the generation of proinflammatory cytokines such as TNF, IL-1, and IL-6 in different rodent disease models (18, 19). In contrast to acute inflammation, which is beneficial for the immune response to microbes and cancer cells, intratumoral chronic inflammation is detrimental since it disables immune cells, thus suppressing antitumor immunity (see ref (20) for review). Since most chemotherapeutics (at least partially) engage the immune system as part of their antitumoral mechanism of action (21),

chronic inflammation decreases sensitivity to chemotherapy and prevents drug delivery to tumors (22), and alleviation of chronic inflammation can enhance the effect of chemotherapy.

Therefore, two mechanisms of ELENAGEN action, as an anticancer vaccine and anti-inflammatory drug, are complimentary and can make it a unique anticancer therapeutic in combination with chemotherapeutic agents for the treatment of OC.

Patients and methods

Study design and patients

This single-country open-label prospective randomized two-center study with two arms was performed from January 2020 until August 2022.

Eligible patients were ≥18 years old; had measurable ovarian cancer per RECIST 1.1 criterion that had progressed <6 months after completion of platinum-based therapy; had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; and had adequate hematologic and organ functions.

The patients were randomly assigned in a 1:1 ratio. Forty patients underwent randomization, 20 were assigned to receive chemotherapy alone (GEM) 1000 mg/m^2 days 1,8 every 3 weeks) and 20 were assigned to receive the same chemotherapy supplemented with ELENAGEN (2.5 mg i.m. weekly).

The primary end point was progression-free survival as assessed by investigators.

The secondary endpoints were overall response rate and safety.

According to the data cutoff, the median follow-up was 13.8 months.

Assessment and endpoints

In the safety analysis set and in the efficacy-evaluable set, all patients who received ≥ 1 dose (20 patients in each arm) were included. Safety was assessed on the basis of adverse events (AEs) and serious AEs (SAEs) according to NCI Common Terminology Criteria for Adverse Events version 5.0.

Antitumor activity was assessed by the investigator according to RECIST 1.1 criteria. Evaluation of the therapeutic effect was carried out by computer tomography (CT) every 9 weeks 19-20 days after each 3rd course of chemotherapy (before the 4th, 7th, and 10th courses, on a visit for follow-up and completion of treatment, and, if necessary, on unscheduled visits).

Statistical analyses

Tumor response was evaluated according to the RECIST criteria ver. 1.1. PFS was defined as the time from randomization to objective disease progression on imaging or death from any cause and was assessed using the Kaplan–Meier method. PFS in the two treatment arms was compared using an unstratified two-sided log-rank test. A $P < 0.05$ was considered statistically significant. For the subgroup analyses, a proportional Cox regression model was used.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. The most common histological type of platinum-resistant OC in both groups was high-grade serous adenocarcinoma. More than half of the patients in both groups progressed after only one line of platinum-based chemotherapy with platinum-free intervals of 3-6 months. Additionally, the majority of patients in both groups had high levels of CA125 as well as metastases in the peritoneum (75-85%) and elsewhere (Table 1). Figure 1 represents flow diagram of PROC patients included in the analysis

Safety

Safety was assessed in all 40 patients. During the study period, one death was registered in the ELENAGEN arm without any evidence of disease progression within 2 months after randomization, and its possible cause was venous embolism. Although autopsy was not performed and the final diagnosis was not determined, this adverse event was counted as thrombosis and unrelated to the disease. One patient in the ELENAGEN arm underwent surgery due to intestinal obstruction within one month after randomization, and the subsequent cycle of the treatment was delayed for three weeks. After recovery from the surgery, the patient continued treatment without evidence of progression to the cutoff date (up to 19 months).

The majority of adverse events in the GEM and ELENAGEN arms were caused by GEM and were presented by different types of hematological toxicity. No cases of febrile neutropenia or other life-threatening complications that required hospitalization occurred. The cases of intestinal obstruction and metabolic toxicity were caused by organ compression by gross tumor mass. Only skin rash, itching and redness at the injection site were considered to be related to ELENAGEN administration. At the same time, the number of adverse events with grade <= 3 and AEs of special interest (potentially related to plasmid administration) did not significantly differ between the groups (Table 2).

A slight increase in the number of hematological adverse events in the ELENAGEN arm was apparently related to the longer GEM exposure due to increased PFS.

Efficacy

The tumor response was assessed according to the RECIST 1.1 criteria. No complete responses were observed in either group. The

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TABLE 1 Baseline Patient Characteristics.

Characteristic	Chemo		ELENAGEN	
	No	%	No	%
Age, years				
Median	54.6		54.2	
Range	33.6-65.5		32.8-69.6	
ECOG PS				
0	14	70	13	65
1	6	30	7	35
Histology at diagnosis				
Serous/adenocarcinoma	17	85	15	75
Clear cell	2	10	3	15
Adenocarcinoma	1	5	1	5
Mucinous	0	0	1	5
Histologic grade at diagnosis				
1	3	15	1	5
2	1	5	0	0
3	15	75	19	95
No data	1	5	0	0
Platinum-free interval				
Up to 3 months	7	35	8	40
3-6 months	13	65	12	60
No line of chemo for platinum sensitive ovarian cancer				
1	11	55	12	60
2	5	25	7	35
3	4	20	1	5
CA125				
Normal	5	25	4	20
High	15	75	16	80
Metastatic lesions				
Peritoneum	15	75	17	85
Peritoneal effusion	9	45	7	35
Lymph nodes	8	40	15	75
Liver	4	20	6	30
Lung	3	15	4	20
Pleural effusion	1	5	3	15
Soft tissue	5	25	3	15
Spleen	0	0	0	0
Bone	1	5	1	5

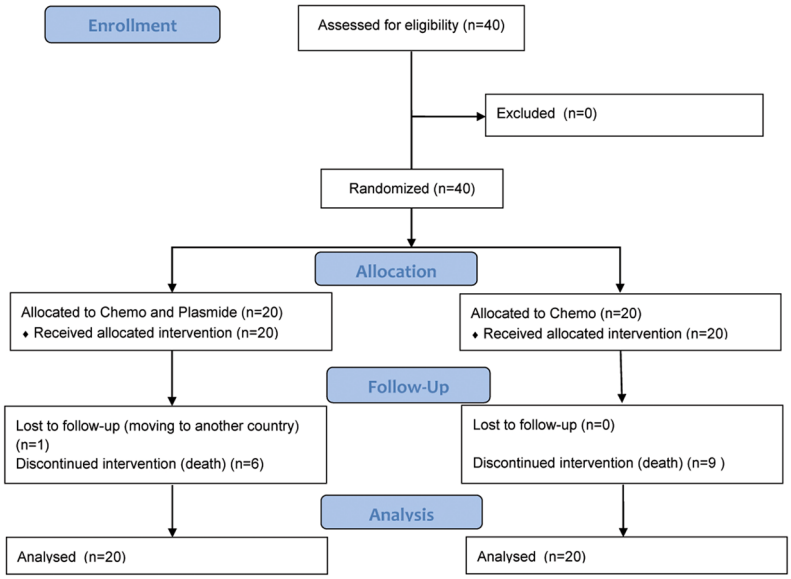

FIGURE 1
Flow diagram of patients included in the analysis.

objective response rate was higher in the ELENAGEN arm: partial response (PR) 5.9% and 26.7%, stable disease (SD) 35.3% and 53.3%, and disease progression 58.8% and 20.0% in the Chemo and ELENAGEN arms, respectively. In total, the disease control rate (PR and SD) was significantly higher in the ELENAGEN arm (80.0% vs 41.2% in the Chemo and ELENAGEN arms, respectively, p = 0,001). One patient in the ELENAGEN arm was able to undergo complete cytoreduction with no evidence of disease progression.

The median progression-free survival (PFS) was 2.8 and 7.2 months in the Chemo and ELENAGEN arms, respectively (p Log-Rank = 0.03) (Figure 2). For the lower 25th percentile (lower quartile), these numbers were 2.1 vs. 4.2 months, respectively, while for the upper quartile (75th percentile), 7.7 months, it was only possible to determine for the chemotherapy group alone.

Notably, at the time of cutoff, 9 patients (45%) in the ELENAGEN arm did not progress, with the longest PFS recorded thus far being 24 months.

Subgroup analysis

We assessed the efficacy of ELENAGEN in subgroups with different basic characteristics.

The peritoneal effusion, CA125 level (normal or high), platinum-free interval (PFI), (up to 3 months vs 3-6 months),

TABLE 2 Adverse events Grade <= 3 and of special interest.

Adverse event	Chemo arm		ELENAGEN arm	
	No	%	No	%
Neutropenia	4	20	7	35
Thrombocytopenia	2	10	4	20
Anemia	1	5	2	10
ALT/AST increase	1	5	0	0
Creatinine increase	1	5	0	0
Thrombosis	1	5	1	5
Intestinal obstruction	0	0	1	5
AE of special interest				
Skin rash G1	0	0	2	10
Itching G1	0	0	2	10

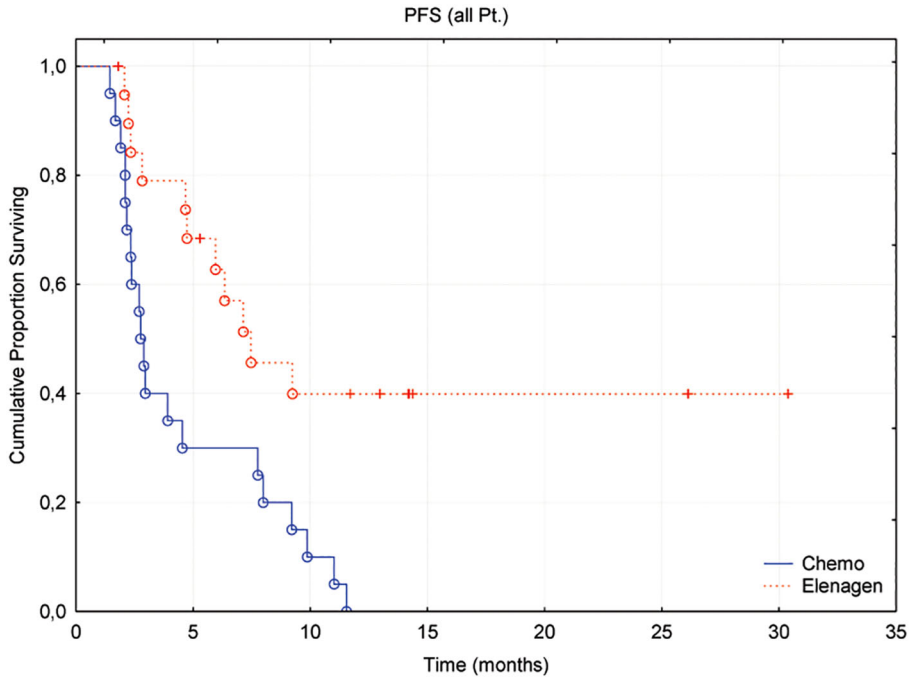

FIGURE 2
Progression-free survival of patients treated with Chemo+ELENAGEN or Chemo only.

number of treatment lines for platinum-sensitive ovarian cancer and histological type of tumor (serous vs non-serous) were chosen as potential predictive factors. Cox proportional hazards regression analyses were performed (Table 3).

The CA125 level (normal or high), platinum-free interval (up to 3 months vs 3-6 months) and histological type of tumor (serous vs non-serous) were statistically significant in the Cox model.

However, due to the low number of patients with non-serous cancer (n=5 in both groups), additional analysis for histological type was not performed, but we performed pairwise comparisons of PFS in the Chemo and ELENAGEN arms according to the identified prognostic factors CA 125 level and PFI. The initial high CA-125 level and short PFI significantly affected PFS (Table 4; Figure 3).

TABLE 3 COX regression model.

	Hazard ratio	95% CI	P Value
Peritoneal effusion	0,8	0.3 – 2.1	0,622
CA125 Level (normal vs high)	10,8	2.4 – 48.3	**0,002**
PRFI (up to 3 vs 3-6 months)	1,4	1.0 – 2.0	**0,039**
Number lines of Chemo for PSOC	1,0	0.5 – 1.9	0,889
Histology (serous vs nonserous)	1,7	1.1 – 2.6	**0,022**

PRFI, platinum-resistance free interval.
PSOC, platinum-sensitive ovarian cancer.
Bold p values are statistically significant.

Discussion

Platinum-resistant OC, even if treated with a standard therapy such as gemcitabine, PLD, paclitaxel, and topotecan, has a dismal prognosis: a medium PFS of 3-4 months and an OS of 12 months (23, 24). Therefore, a more effective therapy for this form of OC is urgently needed. Despite the success of immunotherapy with immune checkpoint inhibitors (ICIs) in some tumors (25)), such a combination of ICIs with chemotherapy in OC has not yet been successful, and this treatment was quite toxic (6, 8) (see Background). Thus, at present, the application of ICIs in the treatment of platinum-resistant OC does not appear encouraging.

Our study demonstrated that the addition of our novel plasmid drug ELENAGEN to a standard chemotherapy regimen with GEM had a profound effect on PFS, increasing it from 2.8 months to 7.2 months. Importantly, no signs of increased toxicity of this combined treatment compared to GEM alone were found. Remarkably, ELENAGEN in combination with GEM was also effective in patients with a dismal prognosis: progression after platinum therapy within 3 months and with high pretreatment

TABLE 4 Progression-free survival (PFS) in subgroups.

Subgroups	`Median PFS (months)		p Log-Rank
	Chemo	ELENAGEN	
CA125 high level	2.5 (2.1-4.1)	6.5 (2.7-NR)	p Log-Rank = 0.01
PRFI up to 3 months	2.6 (2.0-4.5)	NR	p Log-Rank = 0.03
PRFI 3-6 months	2.7 (2.1-7.0)	6.7 (4.3-NR)	p Log-Rank = 0.05

PRFI, platinum-resistance free interval.

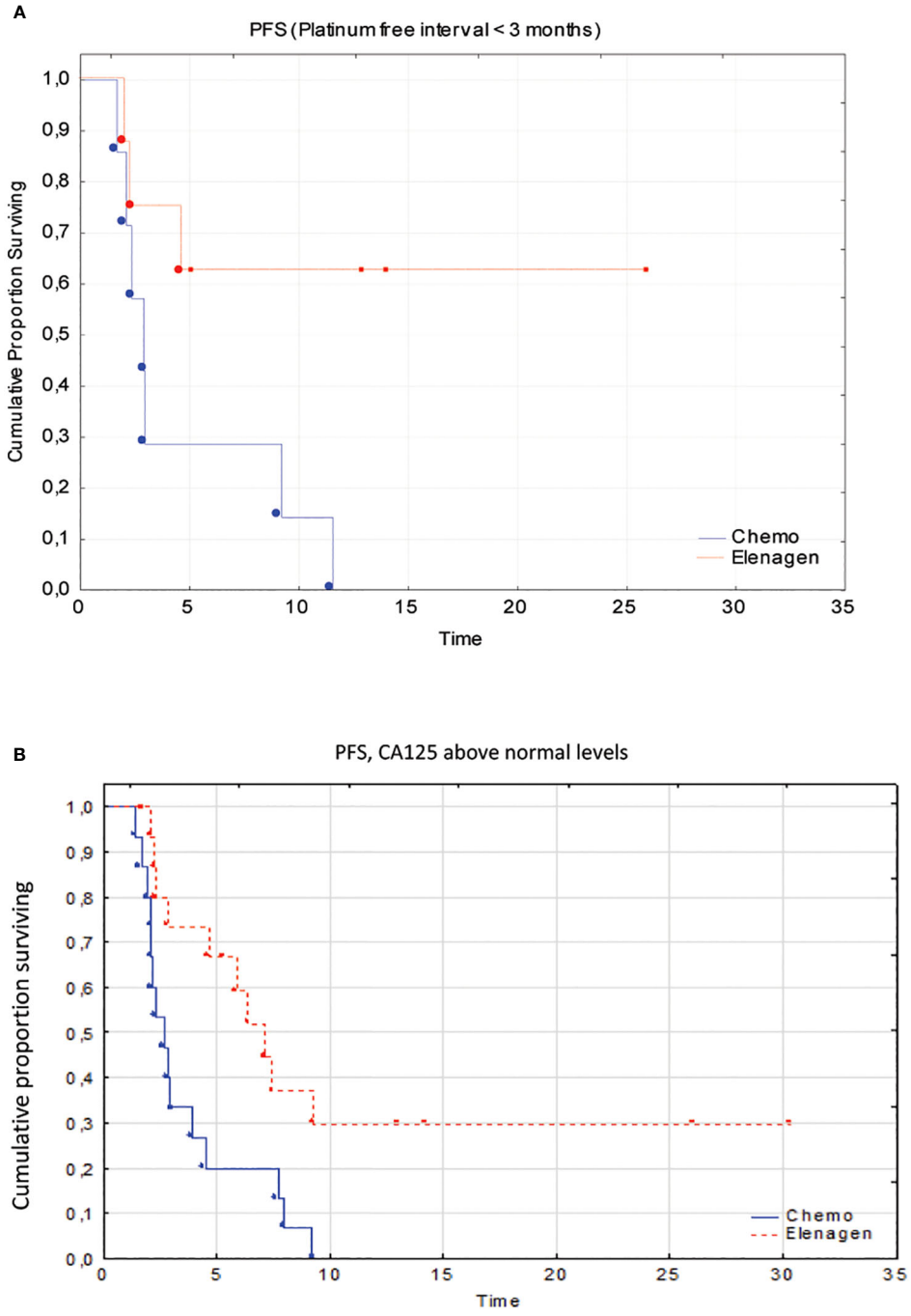

FIGURE 3
Subgroup analysis of patients with a platinum-free interval <3 months **(A)** and above normal CA125 levels **(B)**.

levels of CA125. For instance, a recent meta-analysis of data from more than 10 000 patients demonstrated that the increased serum level of CA-125 before treatment correlated with poor progression-free survival (HR=1.59, 95% CI=1.44~1.76, p<0.001) and overall survival (HR=1.62, 95% CI=1.270-2.060, p<0.001) (26). We are aware that due to a low number of patients in our subgroup analysis, these observations should be evaluated in larger trials.

ELENAGEN operates through at least two complementary mechanisms. First, ELENAGEN can work as an immunotherapeutic by activating T- and B-cellular antitumor immune responses by inducing the generation of antibodies and T-lymphocytes to p62 (9, 16) and stimulating the accumulation of T-lymphocytes in tumors (15). Since OC, especially platinum-resistant OC, has higher levels of p62 than normal tissue (13, 14, 27, 28), such an immune response to p62 may contribute to the antitumor activity of ELENAGEN. Furthermore, it is reasonable to combine elenagen with chemotherapy since anticancer drugs are currently believed to engage, at least partially, the immune system (see ref (21) for review), which may increase the antitumor activity of ELENAGEN. Indeed, the combination of chemotherapy with ICI

immunotherapy in some tumors had a greater effect than either treatment alone, and such combinations are approved by the FDA (25). Accordingly, in our previous study, we found that patients with breast and ovarian cancers achieved additional tumor stabilization for 3-7 months when subjected to chemotherapy following ELENAGEN treatment even if the tumors were initially chemoresistant (17, 29).

Second, ELENAGEN was shown to decrease chronic inflammation (30), which may hamper the effect of chemotherapy (22). Elevated levels of the proinflammatory cytokine IL-6 in the serum or ascites of OC patients correlated with chemoresistance, particularly platinum resistance (31), and higher ascites levels of IL-6 and TNF predict worse PFS in patients with OC (32). Thus, decreasing chronic inflammation ELENAGEN may promote the effect of chemotherapy in OC. Last but not least, in dogs with mammary tumors, we found that ELENAGEN treatment results in tumor shrinkage, changes in the structure of the tumor matrix and lowering the grade of the tumors (15, 16). Such tumor "normalization" may also contribute to sensitization to chemotherapy. Finally, Elenagen treatment dramatically changes the expression of collagen isoforms (16), making it easier for tumor-infiltrating lymphocytes (TILs) to enter the tumor and harder for metastatic cells to exit. Thus, these effects of elenagen make it a unique anticancer therapeutic.

In conclusion, the addition of ELENAGEN to gemcitabine is effective in patients with ovarian cancer, including those with a worse prognosis. Future studies of ELENAGEN with various tumors and chemotherapy regimens are warranted.

Data availability statement

All data generated or analyzed during this study are included in this Article. Data not shown in the manuscript are available from the corresponding author on reasonable request.

Ethics statement

The study was approved by the Ministry of Health of Belarus (#03-19) and ethical review boards of N. Alexandrov National Cancer Centre of Belarus and Minsk City Cancer Center. Informed consent were signed by all study participants.

Author contributions

SKr: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. YB: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Writing – review & editing. SP: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. EZ: Data curation, Formal Analysis, Validation, Writing – review & editing. OS: Investigation, Methodology, Project administration, Resources, Writing – review & editing. AF: Formal Analysis, Investigation, Resources, Writing – review & editing. VS: Investigation, Methodology, Writing – review & editing. SKa: Investigation, Software, Validation, Writing – review & editing. AK: Data curation, Investigation, Methodology, Project administration, Writing – review & editing. VG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Resources, Writing – original draft, Writing – review & editing. AS: Conceptualization, Supervision, Resources, Writing – review & editing.

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Conflict of interest

VG and AS are employees of CureLab Oncology, which holds the intellectual property (IP) to the Elenagen treatment. The clinical trial registration was retrospective.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: A Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

2. Pignata S, Cecere S C, Du Bois A, Harter P, Heitz F. Treatment of recurrent ovarian cancer. *Ann Oncol* (2017) 28:viii51–viii6. doi: 10.1093/annonc/mdx441

3. Rossi L, Verrico M, Zaccarelli E, Papa A, Colonna M, Strudel M, et al. Bevacizumab in ovarian cancer: A critical review of phase III studies. *Oncotarget* (2017) 8(7):12389–405. doi: 10.18632/oncotarget.13310

4. Ibrahim EM, Refae AA, Bayer AM, Sagr ER. Poly(ADP-ribose) polymerase inhibitors as maintenance treatment in patients with newly diagnosed advanced ovarian cancer: a meta-analysis. *Future Oncol* (2020) 16(10):585–96. doi: 10.2217/fon-2020-0057

5. Arora S, Narayan P, Ison G, Berman T, Suzman DL, Wedam S, et al. U.S. FDA drug approvals for gynecological Malignancies: A decade in review. *Clin Cancer Res* (2022) 28(6):1058–71. doi: 10.1158/1078-0432.CCR-21-2599

6. James NE, Woodman M, DiSilvestro PA, Ribeiro JR. The perfect combination: enhancing patient response to PD-1-based therapies in epithelial ovarian cancer. *Cancers* (2020) 12(8):2150. doi: 10.3390/cancers12082150

7. Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, et al. Safety and antitumor activity of anti–PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. *J Clin Oncol* (2015) 33(34):4015–22. doi: 10.1200/JCO.2015.62.3397

8. Pujade-Lauraine E, Fujiwara K, Ledermann JA, Oza AM, Kristeleit R, Ray-Coquard IL, et al. Avelumab alone or in combination with chemotherapy versus chemotherapy alone in platinum-resistant or platinum-refractory ovarian cancer (JAVELIN Ovarian 200): an open-label, three-arm, randomised, phase 3 study. *Lancet Oncol* (2021) 22(7):1034–46. doi: 10.1016/S1470-2045(21)00216-3

9. Venanzi F, Shifrin V, Sherman MY, Gabai V, Kisilev O, Komissarov A, et al. Broad-spectrum anti-tumor and anti-metastatic DNA vaccine based on p62-encoding vector. *Oncotarget* (2013) 4(10):1829–35. doi: 10.18632/oncotarget.1397

10. Moscat J, Diaz-Meco MT. p62: a versatile multitasker takes on cancer. *Trends Biochem Sci* (2012) 37(6):230–6. doi: 10.1016/j.tibs.2012.02.008

11. Gabai V, Shifrin V. Feasibility analysis of p62 (SQSTM1) – encoding DNA vaccine as a novel cancer immunotherapy. *Int Rev Immunol* (2014) 33(5):375–82. doi: 10.3109/08830185.2014.954699

12. Tang J, Li Y, Xia S, Li J, Yang Q, Ding K, et al. Sequestosome 1/p62: A multitasker in the regulation of Malignant tumor aggression (Review). *Int J Oncol* (2021) 59(4). doi: 10.3892/ijo.2021.5257

13. Iwadate R, Inoue J, Tsuda H, Takano M, Furuya K, Hirasawa A, et al. High expression of SQSTM1/p62 protein is associated with poor prognosis in epithelial ovarian cancer. *Acta Histochemica et cytochemica* (2014) 47(6):295–301. doi: 10.1267/ahc.14048

14. Xia M, Yu H, Gu S, Xu Y, Su J, Li H, et al. 62/SQSTM1 is involved in cisplatin resistance in human ovarian cancer cells via the Keap1-Nrf2-ARE system. *Int J Oncol* (2014) 45:2341–8. doi: 10.3892/ijo.2014.2669

15. Gabai V, Venanzi FM, Bagashova E, Rud O, Mariotti F, Vullo C, et al. Pilot study of p62 DNA vaccine in dogs with mammary tumors. *Oncotarget* (2014) 5(24):12803–10. doi: 10.18632/oncotarget.2516

16. Venanzi FM, Gabai V, Mariotti F, Magi GE, Vullo C, Sufianov AA, et al. p62-DNA-encoding plasmid reverts tumor grade, changes tumor stroma, and enhances anticancer immunity. *Aging* (2019) 11(22):10711–22. doi: 10.18632/aging.102486

17. Ponomarenko DM, Klimova ID, Chapygina YA, Dvornichenko VV, Zhukova NV, Orlova RV, et al. Safety and efficacy of p62 DNA vaccine ELENAGEN in a first-in-human trial in patients with advanced solid tumors. *Oncotarget* (2017) 8(34):56030–40. doi: 10.18632/oncotarget.16574

18. Sabbieti MG, Agas D, Capitani M, Marchetti L, Concetti A, Vullo C, et al. Plasmid DNA-coding p62 as a bone effective anti-inflammatory/anabolic agent. *Oncotarget* (2015) 6(6):3590–9. doi: 10.18632/oncotarget.2884

19. Halenova T, Savchuk O, Ostapchenko L, Chursov A, Fridlyand N, Komissarov AB, et al. P62 plasmid can alleviate diet-induced obesity and metabolic dysfunctions. *Oncotarget* (2017) 8(34):56030–40. doi: 10.18632/oncotarget.19840

20. Denk D, Greten FR. Inflammation: the incubator of the tumor microenvironment. *Trends Cancer* (2022) 8(11):901–14. doi: 10.1016/j.trecan.2022.07.002

21. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell* (2015) 28(6):690–714. doi: 10.1016/j.ccell.2015.10.012

22. Gkretsi V, Zacharia LC, Stylianopoulos T. Targeting inflammation to improve tumor drug delivery. *Trends Cancer* (2017) 3(9):621–30. doi: 10.1016/j.trecan.2017.07.006

23. Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2013) 24:vi24–32. doi: 10.1093/annonc/mdt333

24. Luvero D, Milani A, Ledermann JA. Treatment options in recurrent ovarian cancer: latest evidence and clinical potential. *Ther Adv Med Oncol* (2014) 6(5):229–39. doi: 10.1177/1758834014544121

25. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. *Cancers (Basel)* (2020) 12(3). doi: 10.3390/cancers12030738

26. Wang Q, Feng X, Liu X, Zhu S. Prognostic value of elevated pre-treatment serum CA-125 in epithelial ovarian cancer: A meta-analysis. *Front Oncol* (2022) 12. doi: 10.3389/fonc.2022.868061

27. Li S, Wei Y. Association of HMGB1, BRCA1 and P62 expression in ovarian cancer and chemotherapy sensitivity. *Oncol Lett* (2018) 15(6):9572–6. doi: 10.3892/ol.2018.8482

28. Yan X-Y, Qu X-Z, Xu L, Yu S-H, Tian R, Zhong X-R, et al. Insight into the role of p62 in the cisplatin resistant mechanisms of ovarian cancer. *Cancer Cell Int* (2020) 20(1):128. doi: 10.1186/s12935-020-01196-w

29. Ponomarenko DM, Gabai VL, Sufianov AA, Kolesnikov SI, Shneider AM. Response of a chemo-resistant triple-negative breast cancer patient to a combination of p62-encoding plasmid, Elenagen, and CMF chemotherapy. *Oncotarget* (2019) 11(3):294–9. doi: 10.18632/oncotarget.27323

30. Sabbieti MG, Marchegiani A, Sufianov AA, Gabai VL, Shneider A, Agas D. P62/SQSTM1 beyond autophagy: physiological role and therapeutic applications in laboratory and domestic animals. *Life* (2022) 12(4):539. doi: 10.3390/life12040539

31. Macciò A, Madeddu C. Inflammation and ovarian cancer. *Cytokine* (2012) 58(2):133–47. doi: 10.1016/j.cyto.2012.01.015

32. Kolomeyevskaya N, Eng KH, Khan ANH, Grzankowski KS, Singel KL, Moysich K, et al. Cytokine profiling of ascites at primary surgery identifies an interaction of tumor necrosis factor-alpha; and interleukin-6 in predicting reduced progression-free survival in epithelial ovarian cancer. *Gynecologic Oncol* (2015) 138(2):352–7. doi: 10.1016/j.ygyno.2015.05.009

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Emerging role of m6A modification in ovarian cancer: progression, drug resistance, and therapeutic prospects

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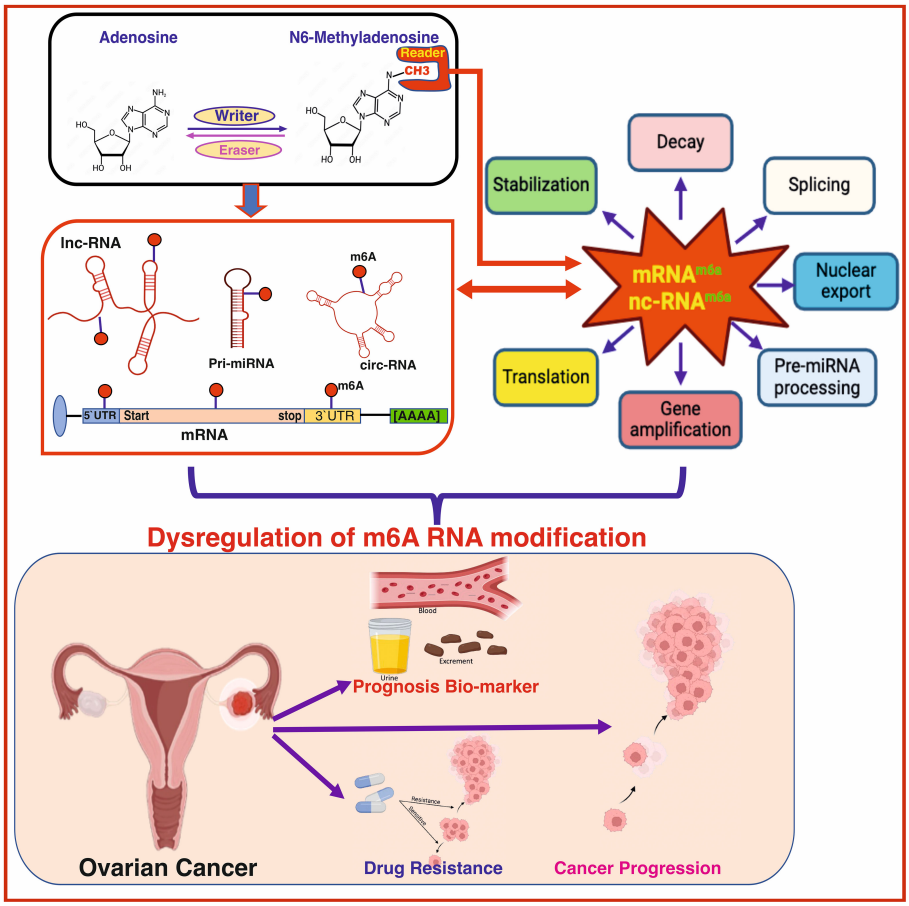
Ovarian Cancer (OC) ranks as a prominent contributor to mortality among female reproductive system associated cancers, particularly the prevalent subtype epithelial Ovarian Cancer (EOC). Despite advancements in treatment modalities, the prognosis for OC patients remains grim due to limitation of current therapeutic methodology such as high cytotoxicity of chemotherapeutic agents and tumor relapse making existing chemotherapy ineffective. Recognizing the limitations of a broad-spectrum approach to treating OC, a shift toward targeted therapies aligning with unique molecular features is imperative. This shift stems from an incomplete understanding of OC's origin, distinguishing it from extensively researched malignancies such as cervical or colon cancer. At the molecular level, postsynthetic modifications—DNA, RNA, and protein—shape transcriptional, posttranscriptional, and posttranslational processes. Posttranscriptional regulatory mechanisms, including RNA modifications are termed epitranscriptomic and play critical roles in this process. For more than five decades, 100+ RNA post-synthetic modifications, notably N6-methyladenosine (m6A), most prevalent RNA modification in mammals, dynamically regulate messenger RNA (mRNA), and non-coding RNA (ncRNA) life orchestrated via writers, erasers, and readers. The disruption of m6A modifications are found in several cancers, including OC, underscores pivotal role of m6A. This review focused on m6A modifications in coding and non-coding RNAs, emphasizing their role as prognostic markers in OC and their impact on development, migration, invasion, and drug resistance. Additionally, RNA-modified regulators have been explored as potential molecular and therapeutic targets, offering an innovative approach to combatting this challenging malignancy.

KEYWORDS

m6A RNA modifications, epitranscriptomics, cancer therapy, ovarian cancer, drug-resistance



GRAPHICAL ABSTRACT
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1 Introduction

Ovarian Cancer (OC) stands out as a prominent gynecologic malignancy, holding the first position of the fatality-inducing factor among tumors affecting the female reproductive system (1).

OC ranks as 18th, and 14th in term of incidence, and mortality respectively among different cancers (2). The composition of ovarian tumor tissue is highly intricate, with the ovary having the highest diversity of primary tumor types among all organs in the body. Various ovarian cancers exhibit significant differences in histological structure and biological behavior. The primary histological categories of OC include epithelial OC (EOC), sex cord stromal OC, and germ cell OC. Among these, EOC are the most prevalent, constituting approximately 50%-70% of cases. EOC, based on tumor cell histology, is further categorized into serous (52%), endometrioid (10%), mucinous (6%), clear cell (6%), and other diverse types (3, 4).

Based on clinicopathological and molecular genetic features, EOC is further classified into type I and type II, each exhibiting distinct characteristics. Type I tumors typically exhibit slow growth, are predominantly diagnosed at stage I clinically, and have a favorable prognosis. In contrast, type II tumors grow rapidly, are often diagnosed at advanced stages, and carry a poorer prognosis (see Figure 1A) (5–8).

OC often asymptomatic early; symptoms emerge late, usually in advanced stages with widespread metastasis to uterus, bilateral adnexa, and pelvic organs (9–11). Despite efforts to screen using serum cancer antigen 125 (CA-125) and transvaginal ultrasound (TVUS), there is no significant reduction in ovarian cancer mortality (12). Presently, no single screening test is universally endorsed for OC. The intricates molecular mechanism contributing to tumor growth in OC and potential therapeutic targets remain largely unknown (13, 14).

The high cytotoxicity and resistance of chemotherapy drug are major hurdle in OC therapeutic strategy (see Figure 1B). The four major chemotherapy drugs viz. platinum-based drug, paclitaxel, PARP inhibitors, and VEGF inhibitors exhibit drug resistance sooner or later leading to failure of chemotherapy and seeking of alternative strategy such as combinatorial chemotherapy revealing urgent need of specific, low toxic drug to treat OC patient including relapse patient (15–22). The treatment is not specific to OC leading to high cytotoxicity of chemotherapy in OC.

Recent studies have highlighted the aberrant expression of m6A modification in various cancer and their subtype causing

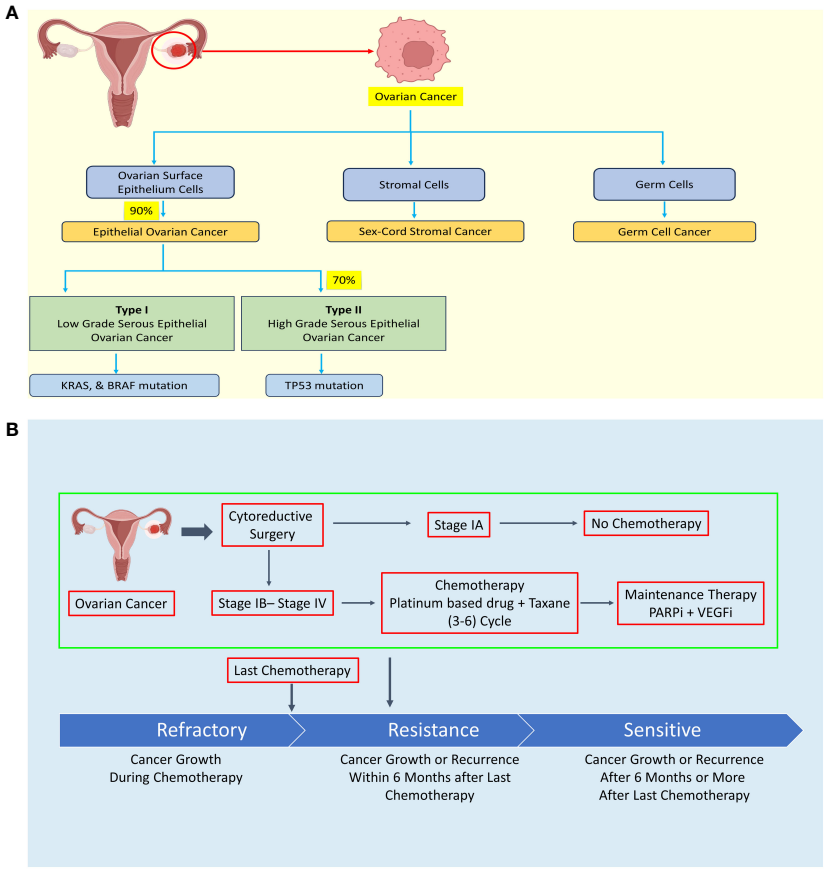

FIGURE 1
Diagram illustrating the classification of ovarian cancer (OC) according to histology, treatment approaches, and the development of resistance mechanisms. **(A)** Ovarian cancer is categorized into epithelial OC, sex-cord stromal cancer, and germ cell cancer based on histological characteristics. **(B)** Treatment of ovarian cancer typically involves debulking surgery followed by chemotherapy, with the exception of stage IA cases. However, some OC patients may develop chemoresistance during chemotherapy or experience recurrence within six months or more after their last chemotherapy session. Created with BioRender.com.

progression, and chemoresistance suggesting its role in development of personalized medicine to overcome drug resistance and high cytotoxicity due to non-specific drugs being targeted by traditional medicine and natural products (23).

At the molecular level, three primary postsynthetic chemical modifications to DNA, RNA, and protein leads to molecular changes for regulation of different cellular processes. Posttranscriptional mechanism encompassing RNA and non-coding RNAs (ncRNAs) modifications, constitutes a critical mechanism of control at translational level called as epitranscriptomics (24). In the last five decades, the identification of over 100 RNA postsynthetic changes in various types of RNA has expanded our understanding of molecular regulation such as 5-methylcytosine (25, 26), N1-methyladenosine (27–30), and 7-methylguanosine (31–34). Of these, m6A is a most common modification and has significant impact on epitranscriptomic regulation (35–37). N6-methyladenosine, commonly known as m6A, is a reversible prevalent RNA modification characterized via adenosine methylation at nitrogen-6 position at RRACH (R=G or A, and H= A or U or C) sequence. This modification is prevalent across various types of RNA, such as messenger RNA (mRNA), and ncRNA (38, 39). The significance of m6A methylation lies in its crucial role in regulating gene expression and participating in diverse cellular functions. The regulatory dynamics of this modification are orchestrated by a group of proteins classified as "writers," "erasers," and "readers" (see Figure 2).

Writers are responsible for adding the methyl group, while erasers, includes Fat Mass and Obesity-Associated Protein (FTO) and AlkB Homolog 5 (ALKBH5), play a role in removing the methyl group (40–42). The writers such as Methyltransferase-like 3 (METTL3), and Methyltransferase-like 16 (METTL16) act as a catalyst for m6A modification (43, 44), while methyltransferase-like 14 (METTL14) help to recognize the substrate via METTL3 (45). Wilms' tumor 1-associating protein (WTAP) promotes the METTL14, and METTL3 heterodimerization, and its movement to nuclear speckle (46) while vir-like m6A methyltransferase associated (VIRMA) guide the methyltransferase to specific RNA (45). RNA-binding motif protein 15 (RBM15) help in recruitment of m6A modification players to specific RNA (47), and zinc finger CCCH-type containing 13 (ZC3H13) help to bridge WTAP to mRNA binding nuclear factor Nito (48). Readers recognizes and binds to the methylated site, thereby influencing subsequent biological phenomenon such as mRNA

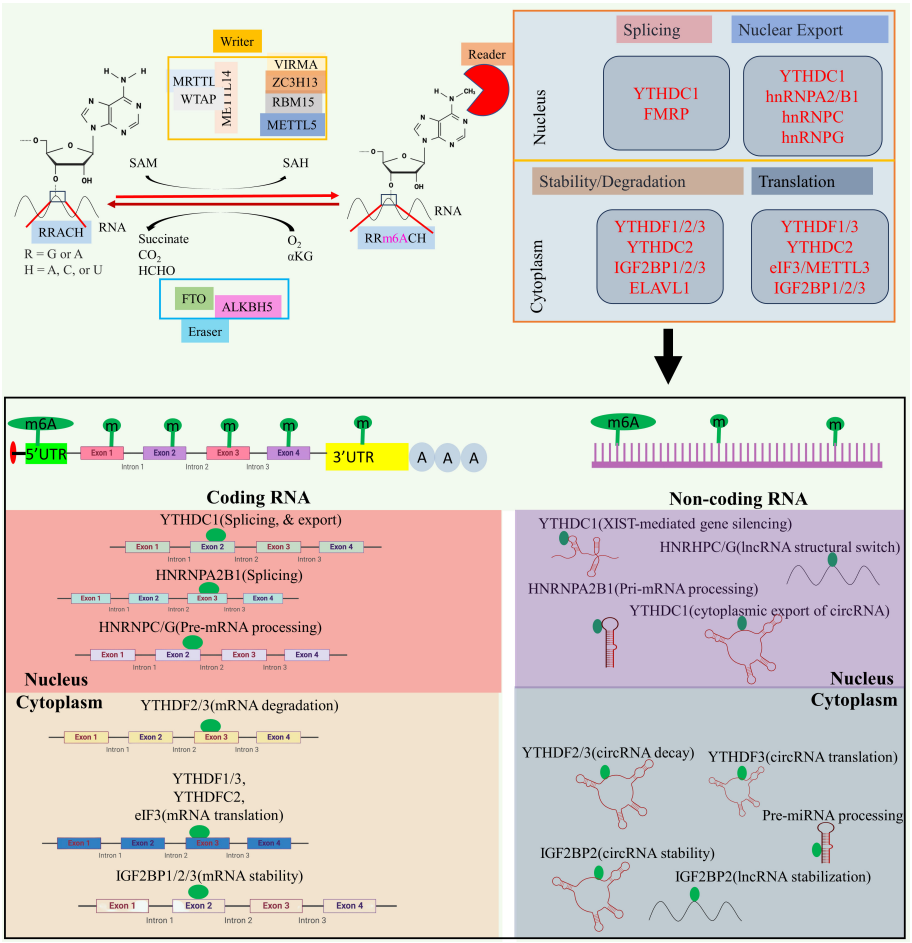

FIGURE 2
The m6A modification mechanism plays a pivotal role in regulating RNA to modulate various cellular processes. This modification, facilitated by enzymes known as writers, erasers, and readers, involves modifying both mRNA and non-coding RNA. Through this process, m6A modification enhances translation, stability, splicing, and nuclear export of RNA molecules, thereby exerting influence over cellular processes. Created with BioRender.com.

splicing, and nuclear export [YTH domain-containing proteins C1 (YTHDC1) (49–51), and heterogeneous nuclear ribonucleoprotein C (HNRNPC) (52)], promotes translation [YTH N6-methyladenosine RNA binding protein C2 (YTHDC2) (53), and YTH N6-methyladenosine RNA binding protein 1 (YTHDF1) (52)], promotes mRNA stability [insulin-like growth factor 2 mRNA binding protein 1/2/3 (IGF2BP1/2/3) (54)], decreases the mRNA stability or promotes translation [YTH N6-methyladenosine RNA binding protein 3 (YTHDF3) (55)], miRNA processing (heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1) (40, 56, 57). Aberrant expression of epitranscriptome has been implicated in different cancers such as lung cancer (58), glioblastoma (59), acute myeloid leukemia (60), colorectal cancer (61), and breast cancer (62). For example, METTL3, VIRMA, FTO, and IGF2BP1 aberrant expression linked to breast cancer, METTL3, FTO, and YTHDF1 disrupted expression promotes lung cancer, and METTL3/14, FTO, and YTHDF2 dysregulated expression promotes acute myeloid leukemia (23). Besides this, m6A modifier such as WTAP, ALKBH5, and YTHDF2 aberrant expression promotes cisplatin resistance, WTAP, and METTL3 dysregulation promotes Adriamycin resistance (63). Several inhibitors of m6A modifier has been identified such as MA2, Dac51, and FB23-2 inhibitors of FTO while IDH2 agonist for METTL3, and MPCH, and U2H1a as inhibitors for METTL3 having low IC50 value which will exhibit low cytotoxicity to overcome OC progression, and chemoresistance (63).

Recent studies have specifically highlighted the abnormal expression of m6A regulators, underscoring m6A methylation role in the incidences OC, and chemoresistance (35).

In the context of this review, we focused on m6A modifications in both coding and noncoding RNAs. We will delve into the molecular processes of these RNA modifications, emphasizing their role as OC prognostic markers and their contributions to OC development, migration, invasion, and development of drug resistance in OC. Additionally, we explored RNA-modified regulators as a promising target for therapeutic strategy for OC, adding a promising dimension to the ongoing efforts in understanding and combating this challenging malignancy.

2 m6A RNA modification enzymes as prognostic biomarkers for OC

Biomarkers for determining patient prognosis will help in monitoring patient response to treatment and response to chemotherapy in patients with tumor relapse. Recent studies underscore m6A RNA modification as a prognostic biomarker in OC. An examination of the TCGA database revealed a noteworthy association between elevated WTAP expression and notably inferior overall survival (OS) suggesting writer WTAP as a oncogenic role. The findings of Kaplan–Meier plotter reveal that upregulated alkB homolog 1 (ALKBH1), WTAP, fat mass and obesity associated (FTO), YTHDF1, alkB homolog 1 (ALKBH5), YTHDF3, and YTH N6-methyladenosine RNA binding protein 2 (YTHDF2) as well as decreased expression of METTL14, were linked to poorer OS revealing METTL14 has a tumor suppressor while others were oncogenic m6A modifier (64).

The VIRMA, IGF2BP1, and ZC3H13, prominent N6-methyladenosine modification regulators, independently predict OC prognosis. The robust predictive ability of these parameters highlights their role as significant prognostic biomarkers for OC (65). The m6A modification regulators, such as ZC3H13, insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2), methyltransferase-like protein 3 (METTL3), VIRMA, and HNRNPC are increased in OC suggesting its role in progression of OC (66). The genes IGF2BP1, VIRMA, HNRNPA2B1, and ELAV-like protein 1 (ELAVL1) are recognized as signature genes for predicting OC prognosis (66).

Decreased expression of seven m6A regulators [METTL14, YTHDC2, FTO, ALKBH5, HNRNPA2B1, VIRMA, and RNA-binding motif protein, X chromosome (RBMX)] was evident in OC tissues sample and in the advanced-stage cohort, suggesting crucial roles in tumor suppressors of OC progression. Patients with upregulated HNRNPA2B1 or downregulated VIRMA had elevated 5-year overall survival rates compared to those of controls. The VIRMA, IGF2BP1, and HNRNPA2B1 are proposed as prognostic biomarker for OC (67). m6A RNA modification regulators, such as VIRMA, HNRNPA2B1, and WTAP, have significant prognostic significance in OC and are linked with the malignant OC development (68). Elevated levels of VIRMA, a writer of m6A, and YTHDC2, a reader of m6A, were linked to an unfavorable prognosis in ovarian patients suggesting they act as a oncogene in OC (69).

The four differentially expressed RNA-modification regulatory genes (DERRG) signature, comprising the Aly/REF export factor (ALYREF), ZC3H13, WTAP, and methyltransferase like 1 (METTL1), were recognized as a self-sufficient prognostic model in OC. This model is valuable for categorizing patients, assessing patient prognosis, and predicting patient response to immunotherapy in patients with OC (70). CACNA1G-AS1, ACAP2-IT1, AC010894.3, and UBA6-AS1 were discovered as prognostic signatures in OC, and each of these genes was associated with methyltransferase-like 5 (METTL5), RBM15, IGF2BP1, and YTH N6-methyladenosine RNA-binding protein C2 (YTHDC1), respectively, suggesting that they regulate the m6A regulatory gene (71). The overexpressed KIAA1429 and YTHDC2 exhibit poor prognosis in OC suggesting its crucial role in OC development (68).

3 m6A RNA modifications role in modulating OC progression

The genes regulating tumorigenesis, invasion, and migration in OC are key players for development of OC, and epitranscriptomic regulation via m6A alteration may have a key role in revolutionizing OC treatments. The m6A modification of mRNA of genes, and non-coding RNA regulating OC progression has gained high importance within few years due to its tremendous potential as a therapeutic target (see Figure 3, Table 1).

3.1 m6A RNA modification modulating mRNA to modulate OC progression

METTL3 expression was independently correlated with poorer survival, and increased malignancy in Endometrioid EOC (EEOC). Knocking down METTL3 hindered proliferation and migration, promoting apoptosis compared to that in controls or cells with WTAP or METTL14 knockdown in CRL-11731D, and TOV-112D cell lines. Furthermore, METTL3 knockdown decreased the m6A methylation in genes linked to OC, such as CSF-1, AXL, EIF3C, and FZD10, in CRL-11731D, and TOV-112D cells. This finding suggested that METTL3-driven m6A modification is distinct from that of WTAP and METTL14 (72). Another study suggests METTL3 knockdown decreased Cyclin D1 along with reduced AKT phosphorylation (101). RPS6KA2 and JUNB were strongly linked with unfavorable prognosis of OC, and there was a positive correlation observed between RPS6KA2 and METTL3 in OC suggesting that RPS6KA is regulated through METTL3-dependent m6A modification (73). Silencing METTL3 in the endometrioid OC cell line COV362 significantly reduced proliferation, and induces G0/G1 cell cycle arrest to enhance cell death (102). OC cell growth increased in METTL3-cKO mice. OC progression was characterized by a change from macrophage polarization from M1 to M2, indicating downregulation in M1 and upregulation in M2 polarization (74). The METTL3, METTL14, IGF2BP2, FTO, and ELF3 have dysregulated expression in EOC, with METTL3 exhibiting highest upregulation. METTL3 silencing induces G0/G1 phase arrest and apoptosis. Conversely, METTL3 overexpression showed reverse effect. Sulforaphene (Sul) reversed METTL3 overexpression, reducing EOC cell viability and promoting apoptosis. Mechanistic study shows that knockdown of METTL3 result in FAS/FADD pathway activation, and altering Bax/Bcl-2 pathway. Sul promotes the apoptosis via decreasing the METTL3 expression, and inducing subsequent apoptosis pathway along with increasing the expression of IGF2BP2 and fas cell surface death receptor (FAS) and downregulating KRT8 (80). PLAA showed reduced expression in highly metastatic OC. Mechanistic study shows PLAA promotes METTL3 degradation, leading to

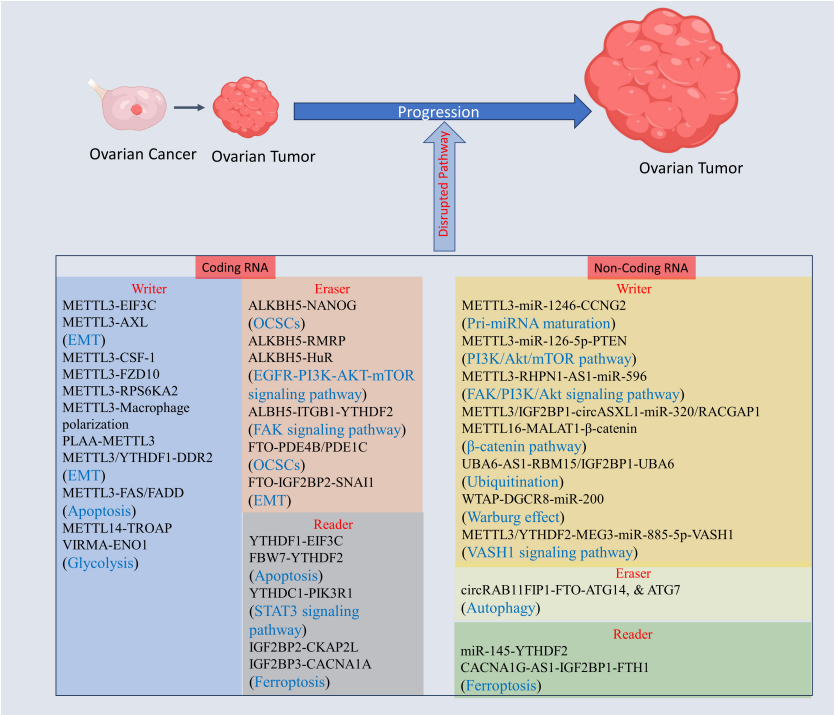

FIGURE 3
The m6A modification mechanism plays a pivotal role in regulating RNA to modulate various cellular processes. This modification, facilitated by enzymes known as writers, erasers, and readers, involves modifying both mRNA and non-coding RNA. Through this process, m6A modification enhances translation, stability, splicing, and nuclear export of RNA molecules, thereby exerting influence over cellular processes. Created with BioRender.com.

destabilization of TRPC3 which regulate intracellular calcium flux to inhibit metastasis in OC (103). The silencing of METTL3/YTHDF1 inhibit OC progression and mechanistic study reveals that METTL3/YTHDF1 axis enhanced the expression of the tumor-promoting DDR2 to foster the progression of OC (75). METTL3 is also reported to induce epithelial to mesenchymal transition (EMT) by enhancing AXL expression (76). METTL3 is shown to regulate various biological process to promote the OC progression, therefore, METTL3 inhibition will help to overcome OC mortality. The overexpressed KIAA1429(VIRMA) promotes the OC proliferation and inhibit necrosis. Mechanistic study shows KIAA1429 stabilizes the ENO1 mRNA in m6A dependent way to promote glycolysis, and proliferation of OC (86). In EOC tissues, both METTL14 expression and m6A RNA methylation levels were notably lower than those in normal tissues (82). A mechanistic study revealed that METTL14 functions as an inhibitor of EOC proliferation through the suppression of TROAP expression through a mechanism dependent on m6A RNA methylation (82). On contrary, the METTL14 was overexpressed in EOC tissues, and induces proliferation, migration, and invasion in A2780, and SKOV3 EOC cell line (104).

ALKBH5 is overexpressed in OC tissue whereas it is downregulated in cell lines. Similarly, the tumor microenvironment Toll-like receptor 4 (TLR4) exhibited this trend. Coculturing OC cells with M2 macrophages led to high expression of both ALKBH5 and TLR4. A mechanistic study revealed that upregulation of TLR4 activated nuclear factor kappa B (NF-κB) axis, causing upregulated ALKBH5, increased m6A levels and increased NANOG expression, promoting the aggressiveness of OC (87). ALKBH5 was overexpressed in EOC. In SKOV3 cells, the suppression of ALKBH5 heightened autophagy and restrained the proliferation and invasion (89). Mechanistic experimental studies reveal that ALKBH5 interacted with HuR, activating EGFR-PIK3CA-AKT-mTOR axis and promoting stabilization of BCL-2, promoting Bcl-2 and Beclin1 interaction (89). These findings further support the finding that ALKBH5 is dysregulated in OC (105). Elevated ALKBH5 expression in OC is induced by a hypoxic microenvironment, and upon inhibiting hypoxia-inducible factor (HIF)-1, ALKBH5 expression decreases concomitantly with a reduction in HIF-1 mRNA expression. ALKBH5 is overexpressed in human OC to promote OC growth and migration. A mechanistic study showed that ALKBH5 upregulates RMRP expression through demethylation and that knocking down RMRP in the OVCAR3 and SKOV-3 cell lines reduces cell growth and migration (88). ALKBH5 overexpression induced by HIF-1α promotes EOC metastasis via targeting Integrin beta 1 (ITGB1) to block YTHDF2 dependent ITGB1 degradation to induce FAK phosphorylation at Tyr397 to trigger Src kinase phosphorylation at Tyr416 (90). In OC and OC stem cells (OCSCs), FTO expression is decreased. FTO overexpression hinders the self-renewal characteristics of OCSCs and suppresses *in vivo* tumorigenesis through the demethylase activity of FTO. Mechanistically, FTO inhibits phosphodiesterases 4B (PDE4B) and 1C (PDE1C) via demethylase activity via m6A modification, resulting in cAMP accumulation and reduced stemness

TABLE 1 The role of m6A modification in OC progression, and development.

Category	M6A modification enzyme	mRNA Target Axis	Non-coding RNA Target Axis	References
Writer	METTL3	METTL3-EIF3C	METTL3-miR-1246-CCNG2 (Pri-miRNA maturation)	(29, 51, 72–81)
		METTL3-AXL (EMT)	METTL3-miR-126-5p-PTEN (PI3K/Akt/mTOR pathway),	
		METTL3-CSF-1	METTL3-RHPN1-AS1-miR-596 (FAK/PI3K/Akt signaling pathway),	
		METTL3-FZD10	METTL3/IGF2BP1-circASXL1-miR-320/RACGAP1	
		METTL3-RPS6KA2	METTL3/YTHDF2-MEG3-miR-885-5p-VASH1 (VASH1 signaling pathway)	
		METTL3-Macrophage polarization,		
		PLAA-METTL3		
		METTL3/YTHDF1-DDR2 (EMT)		
		METTL3-FAS/FADD (Apoptosis)		
	METTL14	METTL14-TROAP	–	(82)
	METTL16	–	METTL16-MALAT1-β-catenin (β-catenin pathway)	(83)
	RBM15	–	UBA6-AS1-RBM15/IGF2BP1-UBA6 (Ubiquitination)	(84)
	WTAP	–	WTAP-DGCR8-miR-200 (Warburg effect)	(85)
	VIRMA	VIRMA-ENO1 (Glycolysis)		(86)
Eraser	ALKBH5	ALKBH5-NANOG (OCSCs)	–	(87–90)
		ALKBH5-RMRP		
		ALKBH5-HuR (EGFR-PI3K-AKT-mTOR signaling pathway)		
		ALBH5-ITGB1-YTHDF2 (FAK signaling pathway)		
	FTO	FTO-PDE4B/PDE1C (OCSCs)	circRAB11FIP1-FTO-ATG14, & ATG7 (Autophagy)	(91–93)
		FTO-IGF2BP2-SNAI1 (EMT)		
Reader	YTHDF1	YTHDF1-EIF3C	–	(94)
	YTHDF2	FBW7-YTHDF2 (Apoptosis)	miR-145-YTHDF2	(95, 96)
	YTHDC1	YTHDC1-PIK3R1 (STAT3 signaling pathway)		(97)
	IGF2BP1		CACNA1G-AS1-IGF2BP1-FTH1 (Ferroptosis)	(98)
	IGF2BP2	IGF2BP2-CKAP2L	–	(99)
	IGF2BP3	IGF2BP3-CACNA1A (Ferroptosis)	–	(100)

RPS6KA2, Ribosomal protein S6 kinase alpha-2; EIF3C, Eukaryotic initiation factor 3, subunit; CFZD10, Frizzled class receptor 10; KRT8, Keratin 8; TROAP, Trophinin-associated protein; CKAP2L, cytoskeleton associated protein 2 like; CSF-1, Circulating colony stimulating factor-1; RMRP, RNA component of mitochondrial RNA processing endoribonuclease; CCNG2, Cyclin G2; MALAT1, Metastasis-associated lung adenocarcinoma transcript 1; UBA6-AS1= UBA6 antisense RNA 1; DGCR8, DiGeorge critical region-8; CACNA1G-AS1, CACNA1G antisense RNA 1; FTH1, Ferritin heavy chain 1; PTEN, Phosphatase and TENsin homolog deleted on chromosome 10; UBA6, Ubiquitin-like modifier activating enzyme 6; DDR2, Discoidin domain receptor tyrosine kinase 2; ENO1, Enolase 1; SNAI1, Snail family transcriptional repressor 1; RACGAP1, Rac GTPase activating protein 1.
-, no data available.

in OC and tumor initiation (91). FTO is reported to induce oxidative stress and apoptosis in OC, leading to suppressed tumor in nude mice (106). FTO decreased expression promotes OC progression. Mechanistic study shows FTO inhibit SNAI1 stability in an IGF2BP2 dependent manner to inhibit EMT suggesting FTO-IGF2BP2-SNAI1 axis role in OC progression (92). Therefore, FTO agonist can be used to overcome OC progression.

YTHDF1 is upregulated in OC, and linked to unfavorable prognostic outcomes in OC patients. A mechanistic study showed that YTHDF1 enhances EIF3C translation via interacting with

m6A-methylated EIF3C mRNA, suggesting that YTHDF1-EIF3C pathway is relevant for OC progression (94). FBW7 is downregulated and promote OC progression. Mechanistic study shows that FBW7 protect pro apoptotic genes BMF degradation via inhibiting YTHDF2 (95). In OC, m6A modification and IGF2BP2 expression are significantly elevated. IGF2BP2 overexpression enhanced OC growth, migration, and invasion. A mechanistic study shows that IGF2BP2 promoted translation of CKAP2L in m6A methylation dependent way without altering mRNA or protein stability. Further study revealed that overexpressing CKAP2L promoted the progression of OC cells with IGF2BP2 knockdown (99). YTHDC1 downregulated in OC while its overexpression inhibited OC development. Mechanistic study reveal YTHDC1 promotes PIK3R1 stability which decreases GANAB via STAT3 pathway (97). The overexpressed IGF2BP3 promotes the OC proliferation via inhibiting ferroptosis. Mechanistic study shows IGF2BP3 target CACNA1A. The silencing of CACNA1A promotes ferroptosis due to aberrant intracellular Ca^{2+} leading to high ROS suggesting IGF2BP3-CACNA1A axis in OC progression (100).

3.2 m6A RNA modification modulating non-coding RNA to modulate OC progression

In eukaryotic cells, ncRNAs typically lack ability to encode proteins. Instead, they carry out biological phenomenon at the RNA level. Traditionally, ncRNAs recognized as posttranscriptional regulators of gene expression. However, recent insights into RNA modifications have broadened their regulatory influence on gene expression. A notable example is m6A, a reversible epitranscriptomic alteration occurring at N6 of adenosine. This alteration is crucial in regulating RNA degradation, RNA splicing, and other biological processes. The scientific literature has previously detailed the molecular pathways that govern the role of m6A modification regulating expression of ncRNA. It is noteworthy that the levels of m6A alteration and m6A regulators expression are intricately controlled by ncRNAs (38). The ncRNA are broadly categories into housekeeping ncRNA, and regulatory ncRNA. Ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA) are known as housekeeping ncRNA while microRNA(miRNA), circular RNA (circRNA), and long non-coding RNA (lncRNA). The miRNA, circRNA, and lncRNA are classified based on their length such as miRNA are 21-23 nucleotides, circRNA are 100-10000 nucleotides, and lncRNA are greater than 200 nucleotides (107).

METTL3 was overexpressed, and hypomethylated in OC tissues and cells and displays a negative correlation with overall survival. Downregulated METTL3 hindered growth and migration of OC to induce cell death. Conversely, overexpression of METTL3 shows opposing phenotype. The underlying mechanism involved METTL3 promoting OC via targeting miR-1246, leading to suppression of CCNG2. Additionally, elevated METTL3 levels downregulated CCNG2, fostering tumors growth in mice (78). miR-126-5p is overexpressed in OC to promote proliferation, migration, and invasion. A mechanistic study revealed that METTL3 promotes miR-126-5p maturation through pri-miR-126-5p's m6A methylation, which directly binds to PTEN, leading to PI3K/Akt/mTOR pathway activation (79). RHPN1-AS1 augments growth and migration of EOC by functioning as a competing endogenous RNA (ceRNA), where it sequesters miR-596. This interaction results in increased LETM1, leading to FAK/PI3K/Akt pathway activation. Further research reveal that silencing METTL3 decreased RHPN1-AS1 expression, resulting in reduced stability of RHPN1-AS1, suggesting that RHPN1-AS1 regulation is METTL3-dependent on m6A modification (77). The circASXL1 was identified to promote the OC progression via miR-320/RACGAP1 axis. Mechanistic study reveals that METTL3/IGF2BP1 promotes the circASXL1stability in m6A dependent manner (108). METTL16 is downregulated in EOC tissue, and MALAT1 is upregulated in EOC tissue. Further study revealed that METTL16 suppressed EOC growth by facilitating MALAT1 degradation. In turn, upregulated β-catenin to facilitate its nuclear transport in EOC cells, suggesting that the METTL16-MALAT1-β-catenin axis inhibits EOC progression through METTL16 (83). Overexpressed WTAP promoted OC proliferation and invasion. Further study revealed that WTAP interaction with DGCR8 to modulate the microRNA-200 (miR-200) expression in a m6A-modification dependent manner, and the glycolysis enzyme hexokinase 2 (HK2) was found to be positively regulated by miR-200. WTAP was found to be positively regulated by HIF-1α under hypoxia in OC (85).

In EOC tissues, YTHDF2 expression was notably upregulated compared to that in normal ovarian tissues. Functional investigations shows that YTHDF2 role in enhancing EOC growth and migration while reducing overall 6-methyladenine (m6A) mRNA levels. A mechanistic study showed that miR-145 levels were inversely correlated with YTHDF2 levels, and YTHDF2 was discovered as a target of miR-145 (96). The long noncoding RNA (lncRNA) CACNA1G-AS1 was shown that it increases the growth, and migration of OC. Further support for these findings was obtained by knocking down CACNA1G-AS1, which reduced OC tumorigenesis *in vivo*. A mechanistic study showed that CACNA1G-AS1 upregulates FTH1 expression via the IGF2BP1 axis, inhibiting ferroptosis through ferritinophagy regulation (98). UBA6-AS1 was shown that it inhibits the growth, invasion, and migration of OC through its interaction with UBA6. A mechanistic study showed that UBA6-AS1 increased UBA6 mRNA's m6A methylation by enlisting RNA binding motif protein 15 (RBM15) for methylation. Additionally, insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) was found as reader protein for UBA6-AS1-RBM15 dependent UBA6 mRNA's m6A modification, thereby enhancing its stability (84). The lncRNA MEG3 was found to be downregulated in OC to promote OC malignant phenotype. Mechanistic study shows increasing the levels of MEG3 inhibited the breakdown of VASH1 via functioning as a suppressor of miR-885-5p. Further study shows that YTHDF2 enhances MEG3 degradation via METTL3 dependent (81). circRAB11FIP1 was overexpressed in SKOV3 OC cell lines to promote OC progression, Mechanistic study shows that DSC1 interact with circRAB11FIP1 to regulate its expression, and sponge miR-129 to

regulate ATG14 and ATG7 to promote autophagic flux. Further study shows circRAB11FIP1 regulate ATG14 and ATG7 via FTO dependent m6A mRNA modification (93).

4 The role of m6A RNA modifications in modulating drug resistance in OC

Despite an initially promising response to initial treatment, the chemotherapy resistance diminishes effectiveness of chemotherapy, resulting in increased relapse rates and reduced long-term survival in patients with OC. Research indicates that up to two out of three of higher stage OC patients experience relapse of tumor within eighteen months, regardless of initial therapy (109). Recent discovery shows that m6A modification has a significant contribution in drug resistance development at mRNA, and non-coding RNA level in OC (see Figure 4, Table 2).

4.1 m6A RNA modification modulating mRNA to modulate drug resistance in OC

YTHDF1, YTHDF2, WTAP, FTO, and ALKBH5 exhibited elevated expression levels in OC and were confirmed to be significant prognostic risk factors associated with decreased overall survival (OS) suggesting its oncogenic role in OC development. Downregulated YTHDC1 and upregulated RBM15 were linked to the metastatic potential of OC. In the group resistant to chemotherapy, HNRNPC, METTL3, and RBM15 exhibited decreased expression, and RMBX and METTL14 showed increased expression suggesting their role as a inhibitors, and promoter of resistance in OC respectively. Elevated HNRNPC expression reliably indicated a favorable response to paclitaxel in patients with OC (123).

RHPN1-AS1 and METTL3 are upregulated in OC, and a mechanistic study showed that METTL3 enhances stability of RHPN1-AS1 through m6A methylation, leading to overexpressed phosphorylated Akt and PI3K to confer cisplatin resistance in OC. Notably, overexpressed RHPN1-AS1 enhances cell growth, migration, invasion, and *in vivo* tumor proliferation (111). RIPK4 was upregulated in OC, fostering cisplatin resistance and tumor progression. Through mechanistic investigations, it was discovered that YTHDF1 enhances RIPK4 expression in METTL3-dependent fashion. This enhancement occurs due to inhibition of degradation of YTHDF1 mRNA. Subsequently, overexpressed RIPK4 leads to the NF-κB phosphorylation, ultimately triggering tumorigenesis and fostering resistance to cisplatin in EOC (110). PTGER2 is overexpressed in OC, and silencing PTGER2 diminishes the stemness of OC cells; reduces CD44 and CD133 expression; inhibits carboplatin resistance, migration, and invasion; increases DNA damage, as indicated by elevated γH2AX levels; and impairs EMT-related proteins such as vimentin, myc, and cyclin D1 (CCND1). Further study revealed that PTGER2 expression was elevated through METTL3-mediated m6A modification (113). IFFO1 is downregulated in OC to confer cisplatin resistance, tumor progression, and metastasis, and overexpressed IFFO1

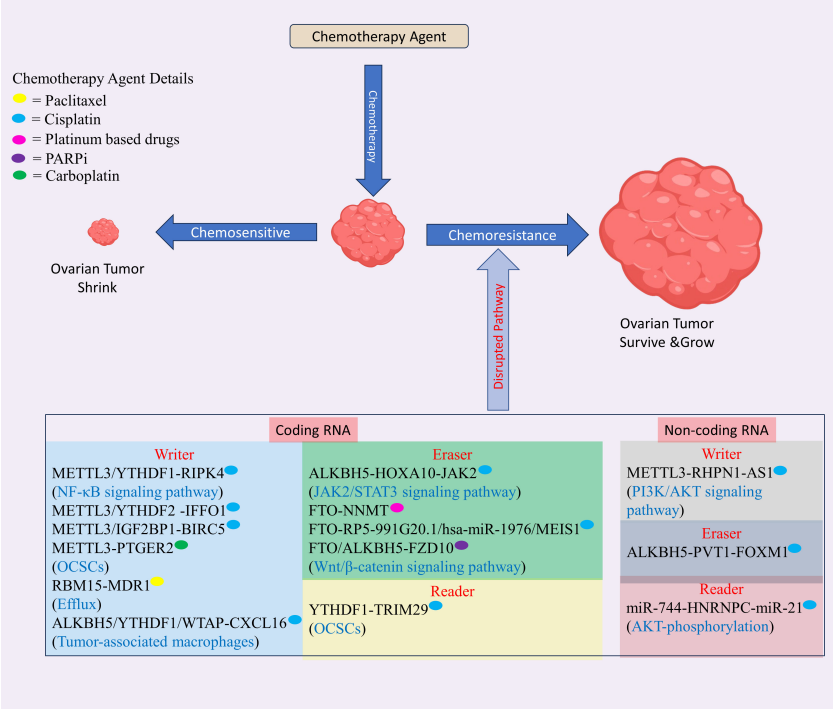

FIGURE 4
The role of m6A modification in drug resistance in ovarian cancer (OC) involves modifications occurring in both mRNA and non-coding RNA. These modifications regulate the resistance of various drugs used in OC treatment, including platinum-based compounds like cisplatin and carboplatin, and PARP inhibitors. This regulatory mechanism disrupts normal pathways, contributing to drug resistance in OC. Created with BioRender.com.

TABLE 2 The Role of m6A modification in drug resistance in OC.

Category	M6A modification enzyme	Chemotherapy drug	mRNA Target Axis (Pathway)	Non-coding RNA Target Axis (Pathway)	References
Writer	METTL3	Cisplatin	METTL3/YTHDF1-RIPK4 (NF-κB signaling pathway)	METTL3-RHPN1-AS1 (PI3K/AKT signaling pathway)	(110–112)
			METTL3/YTHDF2 -IFFO1		
			METTL3/IGF2BP1-BIRC5		
		Carboplatin	METTL3-PTGER2 (OCSCs)	–	(113)
	RBM15	Paclitaxel	RBM15-MDR1(Efflux)	–	(114)
	WTAP	Cisplatin	ALKBH5/YTHDF1/WTAP-CXCL16 (Tumor-associated macrophages)	–	(115)
Eraser	ALKBH5	Cisplatin	ALKBH5-HOXA10-JAK2 (JAK2/STAT3 signaling pathway)	ALKBH5-PVT1-FOXM1	(116, 117)
	FTO	Platinum	FTO-NNMT	–	(118)
		Cisplatin	FTO-RP5-991G20.1/hsa-miR-1976/MEIS1	–	(119)
		PARPi	FTO/ALKBH5-FZD10(Wnt/β-catenin signaling pathway)	–	(120)
Reader	YTHDF1	Cisplatin	YTHDF1-TRIM29 (OCSCs)	–	(121)
	HNRNPC	Cisplatin	–	miR-744-HNRNPC-miR-21 (AKT-phosphorylation)	(122)

RIPK4, Receptor interacting protein kinase 4; RHPN1-AS1, Rhophilin rho GTPase binding protein 1 antisense RNA 1; IFIO1, Intermediate filament family orphan 1; PTGER2, Prostaglandin E receptor 2; CXCL16, CXC chemokine ligand 16; NNMT, Nicotinamide N-methyltransferase; MEIS1, Meis Homeobox 1; PVT1, Plasmacytoma variant translocation 1; FOXM1, Forkhead box protein M1.
-, no data available.

hinders the β-catenin translocation to nucleus, resulting in reduced metastasis and enhanced sensitivity to cisplatin. A mechanistic study showed that histone deacetylase 5 (HDAC5) recruitment suppressed IFFO1 expression through yin yang 1 (YY1) and that the METTL3/YTHDF2 axis controlled IFFO1 stability through m6A modification (112).

RBM15 was discovered to be overexpressed in OC and linked with unfavorable prognosis. Silencing RBM15 was shown to decrease paclitaxel resistance and vice versa. A mechanistic study showed that RBM15 silencing reduced the m6A methylation of multidrug resistance 1 (MDR1) mRNA and that TGF–β pathway activation results in inhibition of RBM15, suggesting that TGF–β/RBM15/MDR1 is a regulatory mechanism that confers paclitaxel resistance in OC (114). Tumor-associated macrophages (TAMs) coculturing with OC cells enhance cisplatin resistance via increasing CXCL16 expression. Silencing of CXCR6 in OC or CXCL16 in TAMs inhibited cisplatin resistance observed in cells cocultured with TAMs, suggesting that CXCL16 contribute to cisplatin resistance development. A further study showed that silencing CXCL16 downregulated YTHDF1/WTAP and upregulated ALKBH5, and enhancing the expression of WTAP increased cisplatin resistance in OC, suggesting that cisplatin resistance is mediated through YTHDF1, WTAP, and ALKBH5 and can be targeted to overcome chemoresistance in OC (115).

ALKBH5 is found to be upregulated in cisplatin resistant OC to induce cisplatin resistance in OC via ALKBH5-HOXA10 loop which target JAK2 to activate JAK2/STAT3 pathway (117). FTO expression was downregulated in platinum-resistant OC, while NNMT expression was enhanced upon FTO overexpression. The sensitivity of FTO-overexpressing cells to platinum was restored upon NNMT inhibitor treatment or by silencing NNMT, suggesting that FTO mediated platinum resistance in OC (118). The MEIS1, and RP5-991G20.1 expression were significantly lower in the knockout of FTO group, while the hsa-miR-1976 level was significantly greater and negatively correlated with the FTO level. These findings suggested that FTO alterations influence RP5-991G20.1/hsa-miR-1976/MEIS1 signaling pathway. Patients with cisplatin resistance (PFS < 6 months) displayed elevated hsa-miR-1976 expression, in contrast with the decreased expression in cisplatin-sensitive patients (PFS > 6 months). Furthermore, RP5-991G20.1, FTO, and MEIS1 elevated in tumors received from patients with clinically described cisplatin sensitivity (PFS > 6 months) and reduced in tumors received from patients with clinically described cisplatin resistance (PFS < 6 months). These findings suggested that the FTO/RP5-991G20.1/hsa-miR-1976/MEIS1 axis regulate cisplatin resistance in OC. More research revealed that FTO knockdown markedly increased growth and resistance of A2780 OC cell line to cisplatin and PPARis. These studies reveal that FTO constrains the proliferation and drug resistance of OC cells, underscoring its pivotal role in reversing OC resistance (119). m6A modification was found to promote resistance to PARP inhibitors (PARPis) in BRCA-mutant EOC by enhancing Wnt/β-catenin axis through FZD10. A mechanistic study showed that silencing FTO and ALKBH5 elevated the

FZD10 mRNA's m6A methylation and decreased sensitivity to PARPis (120). Therefore, FTO, and ALKBH5 agonist are crucial to overcome PARPi resistance in OC.

The overexpressed BIRC5 promotes cisplatin resistant in OC. Mechanistic study shows METTL3/IGF2BP1 promotes BIRC5 mRNA to confer cisplatin resistance in OC (124). The TRIM29 was overexpressed in cisplatin-resistant OC and enhanced the CSC like phenotype in cisplatin resistant OC. Mechanistic study shows YTHDF1 promotes the TRIM29 translation in a m6A dependent manner to confer cisplatin resistance in OC (121).

4.2 m6A RNA modification influences non-coding RNA to modulate drug resistance in OC

The m6A modification of ncRNA also affect the drug resistance in ovarian cancer leading to failure of current chemotherapeutic treatment. Therefore, we had scrutinize the m6A modification and ncRNA related discoveries in current section. LINC02489 expression was decreased in tissue samples from metastatic and chemoresistant OC. Mechanistic study reveals that LINC02489 hinders the invasion and migration of chemoresistant OC by boosting its m6A modification and increasing PKNOX2 expression. Additionally, it regulates OC cell invasion through the PTEN/mTOR pathway, affecting the paclitaxel resistance in SKOV3. These finding suggest m6A modification regulate non-coding RNA (125).

ALKBH5 exhibited increased expression in OC. The silencing ALKBH5 resulted in reduced tumor growth and invasion, while enhancing sensitivity to cisplatin, docetaxel, and 5-FU. Mechanistically, ALKBH5 was found to promote the stability of PVT1 RNA, which, in turn, regulated FOXM1, influencing both chemosensitivity and malignant characteristics in OC (116). miRNA-744 was discovered to be downregulated in OC, while its overexpression induced apoptosis in SKOV3, OVCAR3 and cisplatin-sensitive and cisplatin-resistant A2780 cell lines. A mechanistic study showed that miR-744 downregulates HNRNPC, and nuclear factor one X (NFIX) and HNRNPC knockdown downregulate miR-21, which suppressed programmed cell death 4 (PDCD4), and PTEN suggesting its role in development of combinatorial therapy (122).

5 Clinical significance of m6A RNA modification in OC

OC remains one of the most challenging gynecological malignancies to diagnose and treat effectively. Despite advancements in treatment modalities, including surgery and chemotherapy, the prognosis for OC patients often remains poor. There's a critical need for new treatment targets and markers to enhance clinical care. In recent years, epitranscriptomic, particularly m6A RNA modification, has emerged as a key regulator of gene expression and has garnered significant attention in OC research. In this section, we discuss clinical significance of m6A modification in OC and its implications for diagnosis, prognosis, and treatment.

Recent studies have demonstrated aberrant m6A RNA modification patterns in OC tissues versus normal ovaries. These dysregulated m6A modifications have been associated with alterations in gene expression profiles that contribute to ovarian tumorigenesis. Importantly, m6A RNA modifications have shown promise as potential diagnostic biomarkers for OC (72, 95). Detection of specific m6A modified RNA transcripts in blood or tissue samples may facilitate the early detection of OC, thereby improving patient outcomes through timely intervention (79, 126, 127).

Disruption of m6A RNA modification correlates with OC advancement and metastasis. Elevated levels of m6A modification enzymes, including METTL3 and METTL14, have been linked to unfavorable clinicopathological features and poor prognoses among OC patients. Conversely, reduced expression of the m6A demethylase FTO has been correlated with improved survival outcomes. YTHDF1 has been identified as a promoter of OC cell tumorigenesis by regulating eIF3C translation via m6A-dependent way, thereby influencing global protein translation in OC (94). Furthermore, Han et al. demonstrated a significant increase in WTAP expression in ovarian tissues, with its high expression correlating with cell cycle regulation and MYC targeting (64). Aberrant RNA modification may contribute to tumor development. Jie Li et al. revealed that miR-145-mediated inhibition of YTHDF2 regulates the proliferation, apoptosis, and migration of OC (96). Takeshi Fukumoto et al. discovered that m6A modification of FZD10 regulate PARP inhibitor resistance (120). Zhang et al. found an 18% mutation rate and high expression of ZC3H13 in OC samples, with its expression negatively correlated with prognosis. METTL16 was found to be under expressed in OC tissues and positively correlated with prognosis, especially in patients under 60 years old, those in stage III-IV, and those with tumors (68). Dysregulation of m6A modification regulators has been observed in OC tissues compared to normal ovaries. Patients exhibiting high expression of KIAA1429 and YTHDC2 were found to have poorer prognoses (69). METTL3 enhanced miR-126-5p maturation, accelerating OC progression (79). Additionally, another study demonstrated that METTL3 facilitated OC growth and invasion by activating EMT (76). ALKBH5 expression was higher in EOC tissues than in normal ovarian tissues, suggesting its oncogenic potential in EOC (89). The "reader" protein IGF2BP1 enhances SRC/MAPK-driven invasive growth of OC cells, and overexpressed IGF2BP1 is linked with unfavourable prognosis in OC patients (128, 129). These findings suggest that evaluating m6A RNA modification status could serve as a prognostic indicator in OC, aiding in risk stratification and treatment decision-making.

Targeting dysregulated m6A RNA modification pathways hold promise as a novel therapeutic strategy for OC. Small molecule targeting m6A modification complex components have demonstrated efficacy in preclinical studies, inhibiting OC cell proliferation, migration, and invasion (130, 131). Moreover, modulating m6A RNA levels enhances OC cell sensitivity to therapies. YTHDF1 recruitment to m6A-modified TRIM29

accelerates translation in cisplatin-resistant OC cells, underscoring its therapeutic potential (121). These findings highlight the potential of m6A RNA modification-targeted therapies as adjuvant treatments for OC, offering new avenues for personalized medicine approaches.

Overall, the clinical significance of m6A RNA modification in OC is becoming increasingly evident. Dysregulated m6A RNA modification patterns have diagnostic, prognostic, and therapeutic implications in OC, presenting chances for novel biomarker and targeted therapy development. Further studies is warranted to elucidate underlying mechanisms of m6A modification dysregulation in OC and to translate these findings into clinical practice for improved patient outcomes.

6 Conclusion and future direction

Comprehensive exploration of m6A RNA modifications in OC reveals a nuanced landscape in which molecular intricacies intersect with clinical challenges. The multifaceted role of m6A modifications on both coding and ncRNAs has emerged as a critical determinant of the initiation, progression, and potential therapeutic intervention for OC. This review underscores the dynamic and reversible nature of m6A modifications, shedding light on their regulatory roles in gene expression at various stages of mRNA and noncoding RNA (ncRNA) life. The orchestrated interplay between writers, erasers, and readers of m6A modifications offers a compelling narrative of how these molecular actors contribute to OC development, migration, invasion, and emergence of drug resistance. The m6A-mediated modification genes YTHDF2, IGF2BP2, RBMX, METTL1, ALKBH5, METTL3, YTHDC1, METTL5, HNRNPC, METTL14, WTAP, YTHDF1, FTO, ALKBH1, YTHDF3, YTHDC2, IGF2BP1, VIRMA, ZC3H13, KIAA1429, HNRNPA2B1, ELAVL1, ALYREF, RBM15, and FTO are prognostic markers in OC. Similarly, METTL3, METTL14, ALKBH5, FTO, ELF3, IGF2BP2, IGF2BP3, YTHDF2, YTHDFC1, and YTHDF1 have been shown to modulate OC progression at mRNA level while METTL3, METTL16, WTAP, RBM15, FTO, IGF2BP1, and YTHDF2 interact with non-coding RNA to regulate OC progression. The m6A alteration role in drug resistance is very poorly understood compared to that in progression, and METTL3, RBM15, WTAP, FTO, ALKBH5, YTHDF1, METTL14, HNRNPC, RBMX, IGF2BP1, YTHDC1, and YTHDF2 have been shown to promote chemoresistance in OC at mRNA level via disruption of signaling axis such as FTO disrupt RP5-991G20.1/hsa-miR-1976/MEIS1 signaling pathway to modulate chemoresistance in OC and ALKBH5, and HNRNPC interact with non-coding RNA to confer chemoresistance in OC.

Despite significant progress in m6A modification role in OC, several challenges persist. The heterogeneity of OC poses a significant obstacle, as distinct tumor subtypes exhibit diverse m6A modification profiles. Additionally, the lack of standardized methodologies for detecting and quantifying m6A modifications hampers comparative analyses and the establishment of uniform diagnostic criteria. Similarly, some studies are not conclusive due to contradictory finding such as FTO, and ALKBH5 (64), and VIRMA, and YTHDC2 (69) are upregulated but other studies show its downregulation in OC (67). Similarly, METTL14 found as both inhibitors and promoters of OC progression (82, 104). RBM15 expression was also contradictory due to studies on different sample such as TCGA data analysis shown RBM15 downregulation while cell-based studies shows RBM15 increased expression (114, 123). Therefore, more robust studies are required to establish their role in OC development.

The m6A modification signals promising avenues for addressing different cancer types. Exploring m6A modification regulators or inhibitors offers promising therapeutic paths for OC. While certain inhibitors targeting m6A methylation have demonstrated positive effects on cancer progression (132, 133), there is a need for further investigation into novel therapeutic strategies involving m6A RNA methylation for the treatment of OC.

This review highlights the potential therapeutic avenues that could arise from targeting m6A modifications in OC. Given m6A research importance for gene expression regulation, developing strategies to modulate these modifications may open new frontiers in OC treatment. Furthermore, understanding the crosstalk between m6A modifications and other molecular pathways implicated in OC could unveil synergistic therapeutic approaches.

Integrating m6A modification data with other omics data holds great promise, and comprehensive multiomics analyses could provide a holistic view of the molecular landscape in OC, offering a more nuanced understanding of the disease and identifying novel therapeutic vulnerabilities.

In conclusion, while m6A modification's role in OC has been revealed to a significant extent, the related literature is far from complete. Addressing current challenges and seizing future opportunities will propel researchers toward a more comprehensive understanding of the role of m6A in OC, fostering the innovative and effective therapeutic strategies development for this complex and challenging malignancy.

Author contributions

PG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SA: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Global Cancer Observatory. Available online at: https://gco.iarc.fr/ (Accessed Jul. 21, 2023).

2. Cancer Today. Available online at: https://gco.iarc.fr/today/en/fact-sheets-cancers (Accessed Feb. 03, 2024).

3. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. *CA Cancer J Clin*. (2018) 68:284–96. doi: 10.3322/CAAC.21456

4. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. (2011) 61:212–36. doi: 10.3322/CAAC.20121

5. Ellenson LH, Carinelli S, Kim KR, Kupryjanczyk J, Part J, Singer G, et al. *WHO Classification of Tumours of Female Reproductive Organs*. Lyon, France: International Agency for Research on Cancer (2014).

6. Bolton KL, Ganda C, Berchuck A, Pharaoh PDP, Gayther SA. Role of common genetic variants in ovarian cancer susceptibility and outcome: progress to date from the Ovarian Cancer Association Consortium (OCAC). *J Intern Med*. (2012) 271:366–78. doi: 10.1111/j.1365-2796.2011.02509.x

7. Hunn J, Rodriguez GC. Ovarian cancer: etiology, risk factors, and epidemiology. *Clin Obstet Gynecol*. (2012) 55:3–23. doi: 10.1097/GRF.0B013E31824B4611

8. Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer,". *Arch Gynecol Obstet*. (2014) 290:839–42. doi: 10.1007/S00404-014-3364-8

9. Landrum LM, Java J, Mathews CA, Lanneau GS, Copeland LJ, Armstrong DK, et al. Prognostic factors for stage III epithelial ovarian cancer treated with intraperitoneal chemotherapy: a Gynecologic Oncology Group study. *Gynecol Oncol*. (2013) 130:12–8. doi: 10.1016/J.YGYNO.2013.04.001

10. Enakpene CA, Omigbodun AO, Goecke TW, Odukogbe AT, Beckmann MW. Preoperative evaluation and triage of women with suspicious adnexal masses using risk of Malignancy index,". *J Obstet Gynaecol Res*. (2009) 35:131–8. doi: 10.1111/j.1447-0756.2008.00869.x

11. Peres LC, Cushing-Haugen KL, Köbel M, Harris HR, Berchuck A, Rossing MA, et al. Invasive epithelial ovarian cancer survival by histotype and disease stage. *J Natl Cancer Inst*. (2019) 111:60–8. doi: 10.1093/JNCI/DJY071

12. Sölétormos G, Duffy MJ, Othman Abu Hassan S, Verheijen RHM, Tholander B, Bast RC, et al. Clinical use of cancer biomarkers in epithelial ovarian cancer: updated guidelines from the european group on tumor markers. *Int J Gynecol Cancer*. (2016) 26:43–51. doi: 10.1097/IGC.0000000000000586

13. Arend RC, Jackson-Fisher A, Jacobs IA, Chou J, Monk BJ. Ovarian cancer: new strategies and emerging targets for the treatment of patients with advanced disease. *Cancer Biol Ther*. (2021) 22:89–105. doi: 10.1080/15384047.2020.1868937

14. Kurnit KC, Fleming GF, Lengyel E. Updates and new options in advanced epithelial ovarian cancer treatment. *Obstetrics gynecology*. (2021) 137:108–21. doi: 10.1097/AOG.0000000000004173

15. Ortiz M, Wabel E, Mitchell K, Horibata S. Mechanisms of chemotherapy resistance in ovarian cancer. *Cancer Drug Resist*. (2022) 5:304–16. doi: 10.20517/cdr.2021.147

16. Sazonova EV, Kopeina GS, Imyanitov EN, Zhivotovsky B. Platinum drugs and taxanes: can we overcome resistance? *Cell Death Discov*. (2021) 7:155. doi: 10.1038/S41420-021-00554-5

17. Zhou J, Kang Y, Chen L, Wang H, Liu J, Zeng S, et al. The drug-resistance mechanisms of five platinum-based antitumor agents. *Front Pharmacol*. (2020) 11:343. doi: 10.3389/FPHAR.2020.00343

18. Alatise KL, Gardner S, Alexander-Bryant A. Mechanisms of drug resistance in ovarian cancer and associated gene targets. *Cancers (Basel)*. (2022) 14. doi: 10.3390/CANCERS14246246

19. Mai J, Yang L, Xie HJ, Li YY, Wang X, Liu XX. Molecular mechanisms of platinum-based chemotherapy resistance in ovarian cancer (Review). *Oncol Rep*. (2022) 47:1–11. doi: 10.3892/OR.2022.8293/HTML

20. Norouzi-Barough L, Sarookhani MR, Sharifi M, Moghbelinejad S, Jangjoo S, Salehi R. Molecular mechanisms of drug resistance in ovarian cancer. *J Cell Physiol*. (2018) 233:4546–62. doi: 10.1002/JCP.26289

21. Freimund AE, Beach JA, Christie EL, Bowtell DDL. Mechanisms of drug resistance in high-grade serous ovarian cancer. *Hematol Oncol Clin North Am*. (2018) 32:983–96. doi: 10.1016/j.hoc.2018.07.007

22. Marchetti C, De Felice F, Romito A, Iacobelli V, Sassu CM, Corrado G, et al. Chemotherapy resistance in epithelial ovarian cancer: Mechanisms and emerging treatments. *Semin Cancer Biol*. (2021) 77:144–66. doi: 10.1016/J.SEMCANCER.2021.08.011

23. Deng LJ, Deng WQ, Fan SR, Chen MF, Qi M, Lyu WY, et al. m6A modification: recent advances, anticancer targeted drug discovery and beyond,". *Mol Cancer*. (2022) 21:1–21. doi: 10.1186/S12943-022-01510-2

24. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA modifications in gene expression regulation. *Cell*. (2017) 169:1187–200. doi: 10.1016/J.CELL.2017.05.045

25. Motorin Y, Lyko F, Helm M. 5-methylcytosine in RNA: detection, enzymatic formation and biological functions. *Nucleic Acids Res*. (2010) 38:1415–30. doi: 10.1093/NAR/GKP1117

26. Popis MC, Blanco S, Frye M. Posttranscriptional methylation of transfer and ribosomal RNA in stress response pathways, cell differentiation, and cancer. *Curr Opin Oncol*. (2016) 28:65–71. doi: 10.1097/CCO.0000000000000252

27. Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, et al. The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. *Nature*. (2016) 530:441–6. doi: 10.1038/NATURE16998

28. Zhou H, Rauch S, Dai Q, Cui X, Zhang Z, Nachtergaele S, et al. Evolution of a reverse transcriptase to map N1-methyladenosine in human messenger RNA. *Nat Methods*. (2019) 16:1281–8. doi: 10.1038/S41592-019-0550-4

29. Liu F, Clark W, Luo G, Wang X, Fu Y, Wei J, et al. ALKBH1-mediated tRNA demethylation regulates translation. *Cell*. (2016) 167:1897. doi: 10.1016/J.CELL.2016.11.045

30. Chen Z, Qi M, Shen B, Luo G, Wu Y, Li J, et al. Transfer RNA demethylase ALKBH3 promotes cancer progression via induction of tRNA-derived small RNAs. *Nucleic Acids Res*. (2019) 47:2533–45. doi: 10.1093/NAR/GKY1250

31. Shimotohno K, Kodama Y, Hashimoto J, Miura KI. Importance of 5'-terminal blocking structure to stabilize mRNA in eukaryotic protein synthesis. *Proc Natl Acad Sci U.S.A*. (1977) 74:2734–8. doi: 10.1073/PNAS.74.7.2734

32. Trotman JB, Giltmier AJ, Mukherjee C, Schoenberg DR. RNA guanine-7 methyltransferase catalyzes the methylation of cytoplasmically recapped RNAs. *Nucleic Acids Res*. (2017) 45:10726–39. doi: 10.1093/NAR/GKX801

33. Luo Y, Yao Y, Wu P, Zi X, Sun N, He J. The potential role of N7-methylguanosine (m7G) in cancer. *J Hematol Oncol*. (2022) 15:63. doi: 10.1186/S13045-022-01285-5

34. Furuichi Y, Sekiya T. (2015). "Discovery of m7G-cap in eukaryotic mRNAs", in *Proceedings of the Japan Academy Series B: Physical and Biological Sciences*, Japan: Japan Academy, 91:394–409. doi: 10.2183/PJAB.91.394

35. Zhao S, Zhang M, Zhu X, Xing J, Zhou J, Yin X. The potential regulatory role of RNA methylation in ovarian cancer. *RNA Biol*. (2023) 20:207–18. doi: 10.1080/15476286.2023.2213915

36. Zhang Y, Geng X, Li Q, Xu J, Tan Y, Xiao M, et al. m6A modification in RNA: biogenesis, functions and roles in gliomas. *J Exp Clin Cancer Res*. (2020) 39:1–16. doi: 10.1186/S13046-020-01706-8

37. Wiener D, Schwartz S. The epitranscriptome beyond m6A. *Nat Rev Genet*. (2021) 22:119–31. doi: 10.1038/S41576-020-00295-8

38. Huang H, Weng H, Chen J. m6A modification in coding and non-coding RNAs: roles and therapeutic implications in cancer. *Cancer Cell*. (2020) 37:270. doi: 10.1016/J.CCELL.2020.02.004

39. Chang LL, Xu XQ, Liu XL, Guo QQ, Fan YN, He BX, et al. Emerging role of m6A methylation modification in ovarian cancer. *Cancer Cell Int*. (2021) 21:663. doi: 10.1186/S12935-021-02371-3

40. Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, et al. The role of m6A modification in the biological functions and diseases. *Signal Transduct Targeted Ther*. (2021) 6:1–16. doi: 10.1038/s41392-020-00450-x

41. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol Cell*. (2013) 49:18–29. doi: 10.1016/J.MOLCEL.2012.10.015

42. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol*. (2011) 7:885–7. doi: 10.1038/NCHEMBIO.687

43. Bokar JA, Shambaugh ME, Polayes D, Matera AG, Rottman FM. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. *RNA*. (1997) 3:1233.

44. Pendleton KE, Chen B, Liu K, Hunter OV, Xie Y, Tu BP, et al. The U6 snRNA m6A Methyltransferase METTL16 Regulates SAM Synthetase Intron Retention. *Cell*. (2017) 169:824–835.e14. doi: 10.1016/J.CELL.2017.05.003

45. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, et al. Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. *Cell Rep*. (2014) 8:284–96. doi: 10.1016/J.CELREP.2014.05.048

46. Agarwala SD, Blitzblau HG, Hochwagen A, Fink GR. RNA methylation by the MIS complex regulates a cell fate decision in yeast. *PloS Genet*. (2012) 8:e1002732. doi: 10.1371/JOURNAL.PGEN.1002732

47. Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, et al. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature*. (2016) 537:369–73. doi: 10.1038/NATURE19342

48. Wen J, Lv R, Ma H, Shen H, He C, Wang J, et al. Zc3h13 regulates nuclear RNA m6A methylation and mouse embryonic stem cell self-renewal. *Mol Cell*. (2018) 69:1028–1038.e6. doi: 10.1016/J.MOLCEL.2018.02.015

49. Roundtree IA, He C. Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing. *Trends Genet*. (2016) 32:320–1. doi: 10.1016/J.TIG.2016.03.006

50. Roundtree IA, Luo GZ, Zhang Z, Wang X, Zhou T, Cui Y, et al. YTHDC1 mediates nuclear export of N6-methyladenosine methylated mRNAs. *Elife*. (2017) 6. doi: 10.7554/ELIFE.31311

51. Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, et al. Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing. *Mol Cell*. (2016) 61:507–19. doi: 10.1016/J.MOLCEL.2016.01.012

52. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, et al. N(6)-methyladenosine modulates messenger RNA translation efficiency. *Cell*. (2015) 161:1388–99. doi: 10.1016/J.CELL.2015.05.014

53. Hsu PJ, Zhu Y, Ma H, Guo Y, Shi X, Liu Y, et al. Ythdc2 is an N6-methyladenosine binding protein that regulates mammalian spermatogenesis. *Cell Res*. (2017) 27:1115–27. doi: 10.1038/CR.2017.99

54. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, et al. Recognition of RNA N6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. *Nat Cell Biol*. (2018) 20:285–95. doi: 10.1038/s41556-018-0045-z

55. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, et al. YTHDF3 facilitates translation and decay of N6-methyladenosine-modified RNA. *Cell Res*. (2017) 27:315–28. doi: 10.1038/cr.2017.15

56. Liu Y, Abula A, Xiao H, Guo H, Li T, Zheng L, et al. Structural insight into HNRNP A2/B1 homodimerization and DNA recognition. *J Mol Biol*. (2023) 435:167920. doi: 10.1016/j.jmb.2022.167920

57. Wu B, Su S, Patil DP, Liu H, Gan J, Jaffrey SR, et al. Molecular basis for the specific and multivariant recognitions of RNA substrates by human HNRNP A2/B1. *Nat Commun*. (2018) 9:420. doi: 10.1038/s41467-017-02770-z

58. Diao MN, Zhang XJ, Zhang YF. The critical roles of m6A RNA methylation in lung cancer: from mechanism to prognosis and therapy. *Br J Cancer*. (2023) 129:8–23. doi: 10.1038/s41416-023-02246-6

59. Deacon S, Walker L, Radhi M, Smith S. The regulation of m6A modification in glioblastoma: functional mechanisms and therapeutic approaches. *Cancers (Basel)*. (2023) 15. doi: 10.3390/CANCERS15133307

60. Yankova E, Aspris D, Tzelepis K. The m6A RNA modification in AML. *Curr Opin Hematol*. (2021) 28:80. doi: 10.1097/MOH.0000000000000636

61. Lin Y, Shi H, Wu L, Ge L, Ma Z. Research progress of N6-methyladenosine in colorectal cancer: A review. *Medicine*. (2023) 102:E36394. doi: 10.1097/MD.0000000000036394

62. Zhou M, Dong M, Yang X, Gong J, Liao X, Zhang Q, et al. The emerging roles and mechanism of m6a in breast cancer progression. *Front Genet*. (2022) 13:983564/BIBTEX. doi: 10.3389/fgene.2022.983564

63. Liu Z, Zou H, Dang Q, Xu H, Liu L, Zhang Y, et al. Biological and pharmacological roles of m6A modifications in cancer drug resistance. *Mol Cancer*. (2022) 21:1–20. doi: 10.1186/s12943-022-01680-z

64. Han X, Liu J, Cheng G, Cui S. Gene signatures and prognostic values of m6A RNA methylation regulators in ovarian cancer. *Cancer Control*. (2020) 27:1073274820960460. doi: 10.1177/1073274820960460

65. Fan L, Lin Y, Lei H, Shu G, He L, Yan Z, et al. A newly defined risk signature, consisting of three m6A RNA methylation regulators, predicts the prognosis of ovarian cancer. *Aging*. (2020) 12:18453–75. doi: 10.18632/AGING.103811

66. Wei Q, Yang D, Liu X, Zhao H, Yang Y, Xu J, et al. Exploration of the role of m6 A RNA methylation regulators in Malignant progression and clinical prognosis of ovarian cancer. *Front Genet*. (2021) 12:650554/PDF. doi: 10.3389/fgene.2021.650554

67. Li Q, Ren CC, Chen YN, Yang L, Zhang F, Wang BJ, et al. A Risk Score Model Incorporating Three m6A RNA Methylation Regulators and a Related Network of miRNAs-m6A Regulators-m6A Target Genes to Predict the Prognosis of Patients With Ovarian Cancer. *Front Cell Dev Biol*. (2021) 9:703969/PDF. doi: 10.3389/fcell.2021.703969

68. Zhang C, Liu JH, Guo H, Hong D, Ji J, Zhang Q, et al. m6A RNA methylation regulators were associated with the Malignancy and prognosis of ovarian cancer. *Bioengineered*. (2021) 12:3159–76. doi: 10.1080/21655979.2021.1946305

69. Zhu W, Zhao L, Kong B, Liu Y, Zou X, Han T, et al. The methylation modification of m6A regulators contributes to the prognosis of ovarian cancer. *Ann Transl Med*. (2022) 10:59–9. doi: 10.21037/ATM-21-6462

70. Zheng P, Li N, Zhan X. Ovarian cancer subtypes based on the regulatory genes of RNA modifications: Novel prediction model of prognosis. *Front Endocrinol (Lausanne)*. (2022) 13:972341/PDF. doi: 10.3389/fendo.2022.972341

71. Zheng J, Guo J, Cao B, Zhou Y, Tong J. Identification and validation of lncRNAs involved in m6A regulation for patients with ovarian cancer. *Cancer Cell Int*. (2021) 21:363. doi: 10.1186/S12935-021-02076-7

72. Ma Z, Li Q, Liu P, Dong W, Zuo Y. METTL3 regulates m6A in endometrioid epithelial ovarian cancer independently of METTl14 and WTAP. *Cell Biol Int*. (2020) 44:2524–31. doi: 10.1002/CBIN.11459

73. Xu H, Li X, Wang S, Li F, Gao J, Yan L, et al. Multiomics analysis identifies key genes and pathways related to N6-methyladenosine RNA modification in ovarian cancer. *Epigenomics*. (2021) 13:1359–83. doi: 10.2217/EPI-2021-0204

74. Wang J, Ling D, Shi L, Li H, Peng M, Wen H, et al. METTL3-mediated m6A methylation regulates ovarian cancer progression by recruiting myeloid-derived suppressor cells. *Cell Biosci*. (2023) 13:202. doi: 10.1186/S13578-023-01149-6

75. Zhi D, Zhou K, Liu S, Yu W, Dong M, Yan C. METTL3/YTHDF1 m6A axis promotes tumorigenesis by enhancing DDR2 expression in ovarian cancer. *Pathol Res Pract*. (2023) 253:155047. doi: 10.1016/J.PRP.2023.155047

76. Hua W, Zhao Y, Jin X, Yu D, He J, Xie D, et al. METTL3 promotes ovarian carcinoma growth and invasion through the regulation of AXL translation and epithelial to mesenchymal transition. *Gynecol Oncol*. (2018) 151:356–65. doi: 10.1016/J.YGYNO.2018.09.015

77. Wang J, Ding W, Xu Y, Tao E, Mo M, Xu W, et al. Long non-coding RNA RHPN1-AS1 promotes tumorigenesis and metastasis of ovarian cancer by acting as a ceRNA against miR-596 and upregulating LETM1. *Aging*. (2020) 12:4558–72. doi: 10.18632/AGING.102911

78. Bi X, Lv X, Liu D, Guo H, Yao G, Wang L, et al. METTL3 promotes the initiation and metastasis of ovarian cancer by inhibiting CCNG2 expression via promoting the maturation of pri-microRNA-1246. *Cell Death Discov*. (2021) 7:237. doi: 10.1038/S41420-021-00600-2

79. Bi X, Lv X, Liu D, Guo H, Yao G, Wang L, et al. METTL3-mediated maturation of miR-126-5p promotes ovarian cancer progression via PTEN-mediated PI3K/Akt/mTOR pathway. *Cancer Gene Ther*. (2021) 28:335–49. doi: 10.1038/S41417-020-00222-3

80. Yu H-Y, Yang L, Liu Y-C, Yu A-J. Sulforaphene suppressed cell proliferation and promoted apoptosis of COV362 cells in endometrioid ovarian cancer. *PeerJ*. (2023) 11:e16308. doi: 10.7717/PEERJ.16308

81. Li Y, Lou S, Zhang J, Zhao S, Lou G. m6A methylation-mediated regulation of LncRNA MEG3 suppresses ovarian cancer progression through miR-885-5p and the VASH1 pathway. *J Transl Med*. (2024) 22:113. doi: 10.1186/s12967-024-04929-x

82. Li Y, Peng H, Jiang P, Zhang J, Zhao Y, Feng X, et al. Downregulation of methyltransferase-like 14 promotes ovarian cancer cell proliferation through stabilizing TROAP mRNA. *Front Oncol*. (2022) 12:824258/PDF. doi: 10.3389/fonc.2022.824258

83. Li C, Liu J, Lyu Y, Ling S, Luo Y. METTL16 Inhibits the Malignant Progression of Epithelial Ovarian Cancer through the lncRNA MALAT1/β-Catenin Axis. *Anal Cell Pathol (Amst)*. (2023) 2023:9952234. doi: 10.1155/2023/9952234

84. Wang Y, Chen Z. Long noncoding RNA UBA6-AS1 inhibits the Malignancy of ovarian cancer cells via suppressing the decay of UBA6 mRNA. *Bioengineered*. (2022) 13:178–89. doi: 10.1080/21655979.2021.2011640

85. Lyu Y, Zhang Y, Wang Y, Luo Y, Ding H, Li P, et al. HIF-1 α Regulated WTAP overexpression promoting the warburg effect of ovarian cancer by m6A-dependent manner. *J Immunol Res*. (2022) 2022:6130806. doi: 10.1155/2022/6130806

86. Gan L, Zhao S, Gao Y, Qi Y, Su M, Wang A, et al. N6-methyladenosine methyltransferase KIAA1429 promoted ovarian cancer aerobic glycolysis and progression through enhancing ENO1 expression. *Biol Direct*. (2023) 18:1–18. doi: 10.1186/S13062-023-00420-7

87. Jiang Y, Wan Y, Gong M, Zhou S, Qiu J, Cheng W. RNA demethylase ALKBH5 promotes ovarian carcinogenesis in a simulated tumour microenvironment through stimulating NF-κB pathway. *J Cell Mol Med*. (2020) 24:6137–48. doi: 10.1111/JCMM.15228

88. Lyu Y, Wang Y, Ding H, Li P. Hypoxia-induced m6A demethylase ALKBH5 promotes ovarian cancer tumorigenicity by decreasing methylation of the lncRNA RMRP. *Am J Cancer Res*. (2023) 13:4179–91.

89. Zhu H, Gan X, Jiang X, Diao S, Wu H, Hu J. ALKBH5 inhibited autophagy of epithelial ovarian cancer through miR-7 and BCL-2. *J Exp Clin Cancer Res*. (2019) 38:163. doi: 10.1186/S13046-019-1159-2

90. Sun R, Yuan L, Jiang Y, Wan Y, Ma X, Yang J, et al. ALKBH5 activates FAK signaling through m6A demethylation in ITGB1 mRNA and enhances tumor-associated lymphangiogenesis and lymph node metastasis in ovarian cancer. *Theranostics*. (2023) 13:833–48. doi: 10.7150/THNO.77441

91. Huang H, Wang Y, Kandpal M, Zhao G, Cardenas H, Ji Y, et al. FTO-dependent N 6-methyladenosine modifications inhibit ovarian cancer stem cell self-renewal by blocking cAMP signaling. *Cancer Res*. (2020) 80:3200–14. doi: 10.1158/0008-5472.CAN-19-4044

92. Sun M, Zhang X, Bi F, Wang D, Zhou X, Li X, et al. FTO Inhibits Epithelial Ovarian Cancer Progression by Destabilising SNAI1 mRNA through IGF2BP2. *Cancers (Basel)*. (2022) 14. doi: 10.3390/CANCERS14215218

93. Zhang Z, Zhu H, Hu J. CircRAB11FIP1 promoted autophagy flux of ovarian cancer through DSC1 and miR-129. *Cell Death Discov*. (2021) 12:219. doi: 10.1038/S41419-021-03486-1

94. Liu T, Wei Q, Jin J, Luo Q, Liu Y, Yang Y, et al. The m6A reader YTHDF1 promotes ovarian cancer progression via augmenting EIF3C translation. *Nucleic Acids Res*. (2020) 48:3816–31. doi: 10.1093/NAR/GKAA048

95. Xu F, Li J, Ni M, Cheng J, Zhao H, Wang S, et al. FBW7 suppresses ovarian cancer development by targeting the N6-methyladenosine binding protein YTHDF2. *Mol Cancer*. (2021) 20:45. doi: 10.1186/S12943-021-01340-8

96. Li J, Wu L, Pei M, Zhang Y. YTHDF2, a protein repressed by miR-145, regulates proliferation, apoptosis, and migration in ovarian cancer cells. *J Ovarian Res*. (2020) 13:111. doi: 10.1186/S13048-020-00717-5

97. Wang X, Chen Q, Bing Z, Zhou S, Xu Z, Hou Y, et al. Low expression of m6A reader YTHDC1 promotes progression of ovarian cancer via PIK3R1/STAT3/GANAB axis. *Int J Biol Sci*. (2023) 19:4672–88. doi: 10.7150/IJBS.81595

98. Jin Y, Qiu J, Lu X, Ma Y, Li G. LncRNA CACNA1G-AS1 up-regulates FTH1 to inhibit ferroptosis and promote Malignant phenotypes in ovarian cancer cells. *Oncol Res*. (2023) 31:169–79. doi: 10.32604/OR.2023.027815

99. Shi Y, Xiong X, Sun Y, Geng Z, Chen X, Cui X, et al. IGF2BP2 promotes ovarian cancer growth and metastasis by upregulating CKAP2L protein expression in an m6 A-dependent manner. *FASEB J*. (2023) 37:e23183. doi: 10.1096/fj.202202145RRR

100. Gong X, Jiang A, Shao C, Jiang Y, Du X, Kuang Y, et al. IGF2BP3 regulates CACNA1A-Mediated Ferroptosis in Ovarian Cancer Through m6A Modification. *Lancet*. (2023). doi: 10.2139/SSRN.4517182

101. Liang S, Guan H, Lin X, Li N, Geng F, Li J. METTL3 serves an oncogenic role in human ovarian cancer cells partially via the AKT signaling pathway. *Oncol Lett*. (2020) 19:3197–204. doi: 10.3892/ol

102. Yang L, Chen X, Qian X, Zhang J, Wu M, Yu A. Comprehensive analysis of the transcriptome-wide m6A methylome in endometrioid ovarian cancer. *Front Oncol*. (2022) 12:844613/PDF. doi: 10.3389/fonc.2022.844613

103. Shen Z, Gu L, Liu Y, Wang L, Zhu J, Tang S, et al. PLAA suppresses ovarian cancer metastasis via METTL3-mediated m6A modification of TRPC3 mRNA. *Oncogene*. (2022) 41:4145–58. doi: 10.1038/s41388-022-02411-w

104. Yousheng W, Desheng Y, Li L, Yan L, Xinmei Y, Wenge Z. Expression of METTL14 in epithelial ovarian cancer and the effect on cell proliferation, invasion and migration of A2780 and SKOV3 cells. *Zhonghua Fu Chan Ke Za Zhi*. (2022) 57:46–56. doi: 10.3760/CMA.J.CN112141-20210925-00553

105. Na Z, Fan L, Wang X. Gene signatures and prognostic values of N6-methyladenosine related genes in ovarian cancer. *Front Genet*. (2021) 12:542457/PDF. doi: 10.3389/fgene.2021.542457

106. Wu J, Wang X, Li X. N6-methyladenosine methylation regulator FTO promotes oxidative stress and induces cell apoptosis in ovarian cancer. *Epigenomics*. (2022) 14:1509–22. doi: 10.2217/EPI-2022-0403

107. Zhang P, Wu W, Chen Q, Chen M. Non-coding RNAs and their integrated networks. *J Integr Bioinform*. (2019) 16. doi: 10.1515/jib-2019-0027

108. Tian Q, Mu Q, Liu S, Huang K, Tang Y, Zhang P, et al. m6A-modified circASXL1 promotes proliferation and migration of ovarian cancer through the miR-320d/RACGAP1 axis. *Carcinogenesis*. (2023) 44:859–70. doi: 10.1093/CARCIN/BGAD066

109. Atallah GA, Kampan NC, Chew KT, Mohd Mokhtar N, Md Zin RR, Shafiee MNbin, et al. Predicting prognosis and platinum resistance in ovarian cancer: role of immunohistochemistry biomarkers. *Int J Mol Sci*. (2023) 24. doi: 10.3390/IJMS24031973

110. Yin X, Zhao S, Zhang M, Xing J, Zhou J, Gao W, et al. m6A-modified RIPK4 facilitates proliferation and cisplatin resistance in epithelial ovarian cancer. *Gynecol Oncol*. (2023) 180:99–110. doi: 10.1016/J.YGYNO.2023.11.034

111. Cui S. METTL3-mediated m6A modification of lnc RNA RHPN1-AS1 enhances cisplatin resistance in ovarian cancer by activating PI3K/AKT pathway. *J Clin Lab Anal*. (2022) 36:e24761. doi: 10.1002/JCLA.24761

112. Zhang Y, Qiu JG, Jia XY, Ke Y, Zhang MK, Stieg D, et al. METTL3-mediated N6-methyladenosine modification and HDAC5/YY1 promote IFFO1 downregulation in tumor development and chemo-resistance. *Cancer Lett*. (2023) 553:215971. doi: 10.1016/J.CANLET.2022.215971

113. Bin Lin Y, Xu BH. N6-methyladenosine methyltransferase METTL3 enhances PTGER2 expression to increase ovarian cancer stemness and chemoresistance. *Front Bioscience - Landmark*. (2023) 28. doi: 10.31083/j.fbl2809199

114. Yuan J, Guan W, Li X, Wang F, Liu H, Xu G. RBM15-mediating MDR1 mRNA m6A methylation regulated by the TGF-β signaling pathway in paclitaxel-resistant ovarian cancer. *Int J Oncol*. (2023) 63. doi: 10.3892/ijo

115. Hong L, Wang X, Zheng L, Wang S, Zhu G. Tumor-associated macrophages promote cisplatin resistance in ovarian cancer cells by enhancing WTAP-mediated N6-methyladenosine RNA methylation via the CXCL16/CXCR6 axis. *Cancer Chemother Pharmacol*. (2023) 92:71–81. doi: 10.1007/S00280-023-04533-8

116. Li L, Chen J, Wang A, Yi K. ALKBH5 regulates ovarian cancer growth via demethylating long noncoding RNA PVT1 in ovarian cancer. *J Cell Mol Med*. (2023) 28:e18066. doi: 10.1111/JCMM.18066

117. Nie S, Zhang L, Liu J, Wan Y, Jiang Y, Yang J, et al. ALKBH5-HOXA10 loop-mediated JAK2 m6A demethylation and cisplatin resistance in epithelial ovarian cancer. *J Exp Clin Cancer Res*. (2021) 40:284. doi: 10.1186/S13046-021-02088-1

118. Huang H, Zhao G, Cardenas H, Valdivia AF, Wang Y, Matei D. N6-methyladenosine RNA modifications regulate the response to platinum through nicotinamide N-methyltransferase. *Mol Cancer Ther*. (2023) 22:393–405. doi: 10.1158/1535-7163.MCT-22-0278

119. Chen L, Gao W, Lin L, Sha C, Li T, Chen Q, et al. A methylation- and immune-related lncRNA signature to predict ovarian cancer outcome and uncover mechanisms of chemoresistance. *J Ovarian Res*. (2023) 16:186. doi: 10.1186/S13048-023-01260-9

120. Fukumoto T, Zhu H, Nacarelli T, Karakashev S, Fatkhutdinov N, Wu S, et al. N6-methylation of adenosine of FZD10 mRNA contributes to PARP inhibitor resistance. *Cancer Res*. (2019) 79:2812–20. doi: 10.1158/0008-5472.CAN-18-3592

121. Hao L, Wang JM, Liu BQ, Yan J, Li C, Jiang JY, et al. m6A-YTHDF1-mediated TRIM29 upregulation facilitates the stem cell-like phenotype of cisplatin-resistant ovarian cancer cells. *Biochim Biophys Acta Mol Cell Res*. (2021) 1868:118878. doi: 10.1016/J.BBAMCR.2020.118878

122. Kleemann M, Schneider H, Unger K, Sander P, Schneider EM, Fischer-Posovszky P, et al. MiR-744-5p inducing cell death by directly targeting HNRNPC and NFIX in ovarian cancer cells. *Sci Rep*. (2018) 8:1–15. doi: 10.1038/s41598-018-27438-6

123. Wang Q, Zhang Q, Li Q, Zhang J, Zhang J. Clinicopathological and immunological characterization of RNA m6 A methylation regulators in ovarian cancer. *Mol Genet Genomic Med*. (2021) 9:e1547. doi: 10.1002/MGG3.1547

124. Fan Y, Pan Y, Jia L, Gu S, Liu B, Mei Z, et al. BIRC5 facilitates cisplatin-chemoresistance in a m6 A-dependent manner in ovarian cancer. *Cancer Med*. (2023) 13:e6811. doi: 10.1002/CAM4.6811

125. Xie Y, Wang L, Luo Y, Chen H, Yang Y, Shen Q, et al. LINC02489 with m6a modification increase paclitaxel sensitivity by inhibiting migration and invasion of ovarian cancer cells. *Biotechnol Genet Eng Rev*. (2023) 1–15. doi: 10.1080/02648725.2023.2167772

126. Guo J, Zheng J, Zhang H, Tong J. RNA m6A methylation regulators in ovarian cancer. *Cancer Cell Int*. (2021) 21:609. doi: 10.1186/S12935-021-02318-8

127. Song Y, Qu H. Identification and validation of a seven m6A-related lncRNAs signature predicting prognosis of ovarian cancer. *BMC Cancer*. (2022) 22:633. doi: 10.1186/S12885-022-09591-4

128. Müller S, Bley N, Glaß M, Busch B, Rousseau V, Misiak Dv, et al. IGF2BP1 enhances an aggressive tumor cell phenotype by impairing miRNA-directed downregulation of oncogenic factors. *Nucleic Acids Res*. (2018) 46:6285–303. doi: 10.1093/NAR/GKY229

129. Bley N, Schott A, Müller S, Misiak D, Lederer M, Fuchs T, et al. IGF2BP1 is a targetable SRC/MAPK-dependent driver of invasive growth in ovarian cancer. *RNA Biol*. (2021) 18:391–403. doi: 10.1080/15476286.2020.1812894

130. Huang Y, Yan J, Li Q, Li J, Gong S, Zhou H, et al. Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. *Nucleic Acids Res*. (2015) 43:373–84. doi: 10.1093/NAR/GKU1276

131. Huang Y, Su R, Sheng Y, Dong L, Dong Z, Xu H, et al. Small-molecule targeting of oncogenic FTO demethylase in acute myeloid leukemia. *Cancer Cell*. (2019) 35:677–691.e10. doi: 10.1016/J.CCELL.2019.03.006

132. Weng H, Huang H, Wu H, Qin X, Zhao BS, Dong L, et al. METTL14 Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA m6A Modification. *Cell Stem Cell*. (2018) 22:191–205.e9. doi: 10.1016/J.STEM.2017.11.016

133. Zhang J, Tsoi H, Li X, Wang H, Gao J, Wang K, et al. Carbonic anhydrase IV inhibits colon cancer development by inhibiting the Wnt signalling pathway through targeting the WTAP-WT1-TBL1 axis. *Gut*. (2016) 65:1482–93. doi: 10.1136/GUTJNL-2014-308614

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Efficacy and safety of PARP inhibitors combined with antiangiogenic agents in the maintenance treatment of ovarian cancer: a systematic review and meta-analysis with trial sequential analysis of randomized controlled trials

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Background: Poly (ADP-ribose) polymerase (PARP) inhibitor and antiangiogenic agent monotherapy have shown to be effective as maintenance treatment in patients with ovarian cancer (OC). However, there is currently a lack of evidence-based study to directly compare the effects of combination therapy with these two drugs. Therefore, this study aimed to compare the efficacy and safety of combination therapy with PARP inhibitors and antiangiogenic agents in women with OC using a meta-analysis.

Methods: An exhaustive search of literature was undertaken using multiple databases, including PubMed, Web of Science, Embase, and the Cochrane Library to identify pertinent randomized controlled trials (RCTs) published up until 17 December 2023. The data on progression-free survival (PFS), overall survival (OS), and adverse events (AEs) were pooled. We computed the pooled hazard ratios (HRs) and their 95% confidence intervals (CIs) for PFS and OS, along with the relative risks (RRs) and 95% CIs for AEs. Trial sequential analysis, heterogeneity test, sensitivity analysis, and publication bias assessment were performed. Stata 12.0 and Software R 4.3.1 were utilized for all analyses.

Results: This meta-analysis included 7 RCTs with a total of 3,388 participants. The overall analysis revealed that combination therapy of PARP inhibitors and antiangiogenic agents significantly improved PFS (HR = 0.615, 95% CI = 0.517–0.731; 95% PI = 0.379–0.999), but also increased the risk of AEs, including urinary tract infection (RR = 1.500, 95% CI = 1.114–2.021; 95% PI = 0.218–10.346), fatigue (RR = 1.264, 95% CI = 1.141–1.400; 95% PI = 1.012–1.552), headache (RR = 1.868, 95% CI = 1.036–3.369; 95% PI = 0.154–22.642), anorexia (RR = 1.718, 95% CI = 1.320–2.235; 95% PI = 0.050–65.480), and hypertension (RR = 5.009, 95% CI = 1.103–22.744; 95% PI = 0.016–1580.021) compared with PARP inhibitor or antiangiogenic agent monotherapy. Our study has not yet

confirmed the benefit of combination therapy on OS in OC patients (HR = 0.885, 95% CI = 0.737–1.063). Additionally, subgroup analyses further showed that combination therapy resulted in an increased risk of AEs, encompassing thrombocytopenia, vomiting, abdominal pain, proteinuria, fatigue, headache, anorexia, and hypertension (all $p < 0.05$).

Conclusion: Our study demonstrated the PFS benefit of combination therapy with PARP inhibitors and antiangiogenic agents in patients with OC. The OS result need to be updated after the original trial data is mature. Clinicians should be vigilant of AEs when administering the combination therapy in clinical practice.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42023494482.

KEYWORDS

PARP inhibitors, antiangiogenic agents, olaparib, bevacizumab, ovarian cancer, combination therapy, meta-analysis

1 Introduction

Ovarian cancer (OC) is a prevalent gynecologic malignancy and the leading cause of mortality among females facing gynecological malignancies (Siegel et al., 2020). Given the difficulty in detecting OC during its early stages, a significant number of patients receive their diagnosis at an advanced stage, leading to a reduced 5-year relative survival rate (Wang et al., 2021). Treatment for advanced OC typically involves cytoreductive surgery and platinum-based chemotherapy. However, despite its initial efficacy, approximately 70% of patients experience a recurrence post-primary treatment, gravely impacting survival duration (Giornelli, 2016; Capriglione et al., 2017; Coleridge et al., 2021). Researches have indicated the efficacy of maintenance chemotherapy in extending remission periods (Markman et al., 2003; Markman et al., 2009; Abaid et al., 2010). Presently, novel targeted treatments are being explored to manage OC and prevent its recurrence. Foremost among these are poly (ADP-ribose) polymerase (PARP) inhibitors and antiangiogenic agents.

PARP inhibitors have surfaced as a notable category of drugs for women experiencing recurrent OC in various contexts, such as treating BRCA mutation-associated relapsed conditions or as maintenance therapy in platinum-sensitive cases after responding to platinum-based treatments (Liu et al., 2019). PARP inhibitors have demonstrated their ability to induce DNA damage through the catalytic inhibition of PARP enzyme and entrapping DNA-PARP complexes, fostering synthetic lethality in cells impaired in homologous recombination repair, thereby enhancing the destruction of tumor cells (Ding et al., 2018; O'Sullivan et al., 2014). Currently, multiple PARP inhibitors (e.g., olaparib, niraparib, rucaparib, veliparib, and talazoparib) are undergoing trials in different phases of development, either in combination with other drug categories or as a standalone agent (Hopkins et al., 2019). The pairing of PARP inhibitors with antiangiogenic agents is a growing area of interest in OC research. Antiangiogenic medications hinder tumor vascularization and impede tumor cells from accessing nutrients by inflicting damage on established tumor blood vessels and obstructing the formation of new ones (Abdalla et al., 2018; Jászai and Schmidt, 2019). As a result, antiangiogenic agents have evolved into a promising drug class for OC patients. Furthermore, the potential for therapeutic synergy is particularly notable when combining PARP inhibitors with antiangiogenic agents. The hypoxia triggered by antiangiogenic treatments may escalate DNA damage and genetic instability (Chan and Bristow, 2010a), culminating in defective homologous recombination that could heighten sensitivity to PARP inhibitors (Hegan et al., 2010).

Although several high-quality randomized, phase II/III trials in recent years have shown that maintenance combination therapy with PARP inhibitors (olaparib or niraparib) and antiangiogenic agents (bevacizumab or cediranib) significantly improved progression-free survival (PFS) *versus* PARP inhibitor or antiangiogenic agent monotherapy after first-line treatment for OC (Liu et al., 2019; Mirza et al., 2019; Ray-Coquard et al., 2023), the conclusions derived from the randomized controlled trials (RCTs) remain a subject of debate (Vergote et al., 2021; Liu et al., 2022). Moreover, combination therapy might be more susceptible to adverse events (AEs) compared to monotherapy (Ray-Coquard et al., 2019). Consequently, this study conducted a systematic review and meta-analysis of RCTs to determine the clinical efficacy and safety of maintenance combination therapy of PARP inhibitors and antiangiogenic agents *versus* PARP inhibitor or antiangiogenic agent monotherapy in patients with OC.

2 Materials and methods

2.1 Study design

In compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, this meta-analysis was carried out (Page et al., 2021). Concurrently, the protocol for this study was registered in anticipation with the PROSPERO database, under the identifier CRD42023494482.

2.2 Literature search strategy

We undertook a comprehensive search of databases such as PubMed, Web of Science, Embase, and the Cochrane Library for

pertinent studies published prior to 17 December 2023. The primary search treatment-related retrieval fields included: "angiogenesis inhibitors", "tyrosine kinases inhibitors", "bevacizumab", "cediranib", "recentin", "avastin", "aflibercept", "votrient", "sunitinib" AND "PARP inhibitors", "olaparib", "lynparza", "rucaparib", "talazoparib", "niraparib", "veliparib", "rubraca", "talzenna". The cancer-related retrieval fields included: "ovarian cancer", "ovary cancer", "ovarian neoplasm", "cancer of ovary". No additional restrictions were imposed, encompassing language. Furthermore, to uncover more pertinent studies, we also scoured the reference lists of all relevant review articles. A detailed search strategy was presented in Supplementary Material S1.

2.3 Inclusion and exclusion criteria

The selection process for relevant literature involved a rigorous screening protocol based on the following inclusion criteria: (i) RCTs; (ii) patients must have a histologically or cytologically confirmed diagnosis of ovarian, primary peritoneal, or fallopian tube cancer; (iii) intervention: PARP inhibitors plus antiangiogenic agents; (iv) comparison: PARP inhibitors or antiangiogenic agents as a single agent; (v) outcomes: PFS, overall survival (OS), or AEs. Studies were excluded if they (i) were not RCTs; (ii) failed to report on the outcomes of interest; (iii) included trial populations with overlaps; (iv) were case reports, editorial comments, animal studies, conference abstracts, or reviews.

2.4 Data extraction and endpoint

Two independent reviewers conducted the data extraction process, with any discrepancies in study eligibility being settled through mutual agreement. We collated the following information from the selected studies: first author's name, publication year, abbreviation of RCT, trial phase, disease setting, treatment line, regimen details in experimental and control arm, number and age of patients allocated for each arm, follow-up duration, and outcomes. The primary endpoints for this meta-analysis were PFS and OS, while secondary endpoints included AEs like fatigue, hypertension, and nausea. In cases where multiple publications reported results from the same trial, we prioritized the most recent or comprehensive publication that provided the relevant information. For studies where PFS or OS data could not be directly extracted, we utilized Engauge Digitizer 10.8 (http://markummitchell.github.io/engauge-digitizer/) and the methodology proposed by Tierney et al. (Tierney et al., 2007) to extract data from the Kaplan-Meier curves.

2.5 Risk of bias assessment

The assessment of the included RCTs' quality was conducted using the modified Jadad scale (Jadad et al., 1996). Each study was independently appraised by two reviewers on aspects, including the randomization procedure, concealment of allocation, implementation of double-blinding, and the reporting of withdrawals and dropouts. Any divergences in assessment were settled through consensus. Trials were scored and classified as either high quality (4-7 points) or low quality (0–3 points).

2.6 Statistical analysis

We computed the pooled hazard ratios (HRs) and their 95% confidence intervals (CIs) for PFS and OS, along with the relative risks (RRs) and 95% CIs for AEs. HR (or RR) > 1 was interpreted as favoring the control group, whereas HR (or RR) < 1 indicated preference for the intervention group. To assess the heterogeneity across studies, we employed the Cochrane Q-test, I^2 statistics, and 95% prediction interval (PI) (Bowden et al., 2011; IntHout et al., 2016). Based on these heterogeneity outcomes, we applied either the Mantel-Haenszel fixed-effects model or the DerSimonian-Laird random-effects model to derive the pooled effects. The threshold for employing a random-effects model was set at $I^2 > 50\%$ or p-value <0.10, indicating moderate to high heterogeneity; otherwise, a fixed-effects model was utilized (Higgins and Thompson, 2002). We performed subgroup analysis based on specific PARP inhibitors and antiangiogenic drugs. Publication bias was assessed through funnel plots and Begg's and Egger's tests (Begg and Mazumdar, 1994; Egger et al., 1997), with the trim-and-fill method adjusting for any detected bias (Duval and Tweedie, 2000). We conducted a sensitivity analysis by excluding each study in turn to assess changes in the combined HR or RR. All statistical analyses were carried out using R software 4.3.1 and Stata 12.0 (Stata Corp. College Station, Texas, United States). A two-sided $p < 0.05$ was considered statistically significant.

2.7 Trial sequential analysis

In our pursuit to rigorously evaluate the efficacy and safety of the combination of PARP inhibitors with antiangiogenic agents in OC patients, we employed trial sequential analysis (TSA). This methodology was applied to PFS and OS data using Stata software version 12.0 and R software version 4.3.1, while AEs were scrutinized using TSA software version 0.9.5.10 Beta (www.ctu.dk/tsa). TSA aimed to determine whether the current data suffices for a conclusive evidence base, known as the required information size (RIS) (Wetterslev et al., 2017). We utilized the "metacumbounds" and "rsource" functions within Stata 12.0, and the "foreign" and "ldbounds" packages in R software to conduct TSA for PFS and OS, adopting an *a priori* information size (APIS) approach (Xie et al., 2022). For the analysis of AEs, the TSA software was harnessed to calculate the RIS and establish the O'Brien-Fleming α-spending boundaries, adhering to 5% type I error and 20% type II error, both set as two-sided thresholds. A crossing of the cumulative Z-curve over the RIS or the trial sequential monitoring boundary signaled that additional studies were redundant, providing substantial evidence to either support or reject the effect of intervention.

3 Results

3.1 Study selection

The preliminary search identified 4,362 records, from which 964 were discarded as duplicates. The subsequent step involved a careful review of the titles and abstracts of the remaining

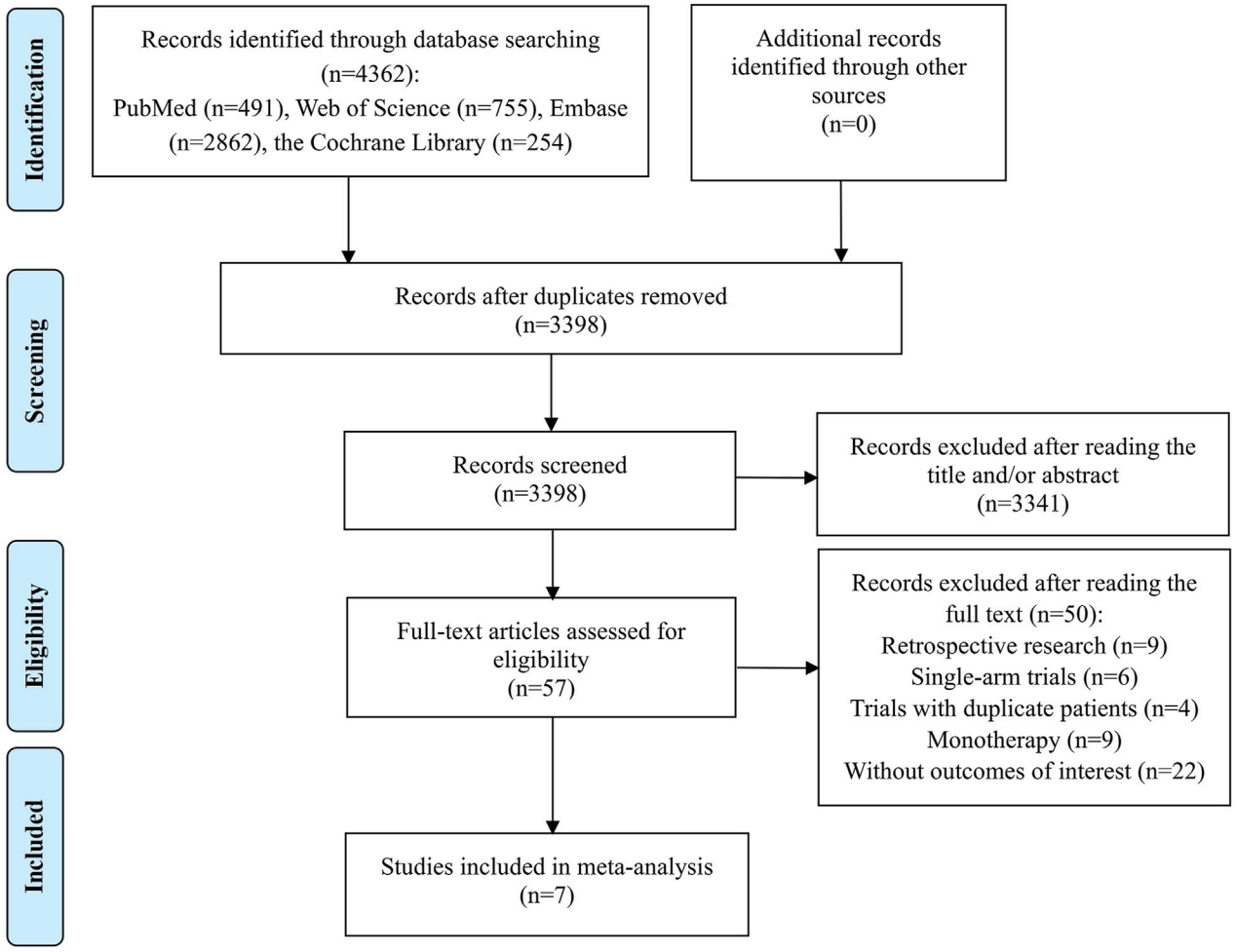

FIGURE 1
Flow diagram of the process of study selection.

3,398 studies, leading to the elimination of 3,341 papers that did not align with our research topic. Of the remaining 57 studies deemed potentially relevant, a full-text review was conducted, resulting in the exclusion of 50 studies for the following reasons: 9 were retrospective research; 6 were single-arm trials; 4 trials contained duplicate patients; 9 studies focused solely on monotherapy for OC; and 22 articles did not provide the required outcome data. Ultimately, 7 studies met the criteria and were included in the meta-analysis (Liu et al., 2019; Mirza et al., 2019; Ray-Coquard et al., 2019; Vergote et al., 2021; Liu et al., 2022; Ray-Coquard et al., 2023; Sabatier et al., 2023). The process of study identification and selection was depicted in Figure 1.

3.2 Characteristics and quality assessment of included studies

The characteristics of these included 7 RCTs (2 phase II trials and 5 phase III trials) were shown in Table 1. The research articles were published from 2019 to 2023 in English. The interventions in each study were maintenance therapies administered to OC patients following first-line treatment. A total of 2,043 OC patients were assigned to a combination of PARP inhibitors and antiangiogenic agents, whereas 1,345 patients received either PARP inhibitors alone or antiangiogenic agents with placebo. 4 trials investigated the combination therapy of olaparib and bevacizumab, 2 trials examined the pairing of olaparib and cediranib, and one study specifically explored the combination of niraparib and bevacizumab. All studies included in this analysis were deemed to be of high quality. A significant methodological shortcoming observed was the absence of double blinding in the trial design. More information on the quality assessment can be located in Supplementary Material S2.

3.3 Meta-analysis of efficacy outcomes

6 RCTs analyzed PFS outcome. The trials demonstrated significant heterogeneity (I^2 = 54.8%, Tau^2 = 0.0227), prompting the adoption of a random-effects model for analysis. The results revealed that combination therapy with PARP inhibitors and antiangiogenic drugs resulted in a significantly better pooled PFS than PARP inhibitor or antiangiogenic monotherapy (HR = 0.615, 95% CI = 0.517–0.731; 95% PI = 0.379–0.999) (Table 2; Figure 2A). Subgroup analysis based on the specific drugs of PARP inhibitors

TABLE 1 Characteristics of the included RCTs.

First author (Year)	Trial	Study phase	Disease setting	Line	Sample size (E/C)	Age [median (range), years]	Experimental arm	Control arm	Median duration of follow-up (months)	Meta-analysis end-points
Sabatier et al. (2023)	PAOLA-1/ENGOT-ov25	III	Newly diagnosed advanced, high-grade ovarian cancer	Maintenance after first-line platinum-taxane-bevacizumab triplet treatment	537/269	26–87	Olaparib 300 mg twice daily + Bevacizumab	Placebo twice daily + Bevacizumab	22.1	AEs
Ray-Coquard et al. (2023)	PAOLA-1/ENGOT-ov25	III	Newly diagnosed advanced stage, high-grade serous or endometrioid ovarian cancer	Maintenance after first-line platinum-based chemotherapy plus bevacizumab treatment	537/269	E: 61 (32–87); C: 60 (26–85)	Olaparib 300 mg twice daily + Bevacizumab	Placebo twice daily + Bevacizumab	E: 61.7; C: 61.9	PFS, OS
Liu et al. (2022)	NRG-GY004	III	Platinum-sensitive relapsed high-grade serous or high-grade endometrioid ovarian, primary peritoneal, or fallopian tube cancer	Maintenance after first-line platinum-based chemotherapy	189/189	>18 years	Olaparib 200 mg tablets twice daily + Cediranib 30 mg tablet once daily	Olaparib 300 mg tablets twice daily	24 (Mean)	PFS, AEs
Liu et al. (2019)	NCT01116648	II	Relapsed platinum-sensitive ovarian cancer	Maintenance after anti-angiogenic therapy in the first-line setting	44/46	E: 57.8 (41.9–85.6); C: 58.1 (32.7–81.9)	Cediranib 30 mg orally daily + Olaparib capsules 200 mg orally twice daily	Olaparib capsule monotherapy 400 mg orally twice daily	46	PFS, OS, AEs
Vergote et al. (2021)	Pooled analysis of SOLO1 and PAOLA-1/ENGOT-ov25	III	Newly diagnosed, advanced BRCA-mutated ovarian cancer	Maintenance after first-line treatment with platinum-based chemotherapy or platinum-based chemotherapy plus bevacizumab	151/254	E: 54.3 (mean age); C: 53.6 (mean age)	Olaparib 300 mg twice daily + Bevacizumab	Olaparib 300 mg twice daily	E: 22.7; C: 40.7	PFS, AEs
Mirza et al. (2019)	NSGO-AVANOVA2/ENGOT-ov24	II	Platinum-sensitive recurrent ovarian cancer	Maintenance after first-line platinum-based chemotherapy	48/49	E: 67 (IQR: 59–70); C: 66 (IQR: 58–70)	Niraparib 300 mg once daily + Bevacizumab 15 mg/kg every 3 weeks	Niraparib 300 mg once daily	16.9	PFS, AEs
Ray-Coquard et al. (2019)	PAOLA-1	III	Newly diagnosed advanced, high-grade serous or endometrioid ovarian cancer, primary peritoneal cancer, or fallopian-tube cancer	Maintenance after first-line platinum-taxane chemotherapy plus bevacizumab treatment	537/269	E: 61 (32–87); C: 60 (26–85)	Olaparib 300 mg twice daily + Bevacizumab	Placebo twice daily + Bevacizumab	22.9	PFS, AEs

E, Experimental group; C, Control group; PFS, progression-free survival; AEs, adverse events; OS, overall survival; IQR: interquartile range.

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TABLE 2 Pooled effect of the efficacy and safety outcomes of PARP inhibitors combined with antiangiogenic agents for ovarian cancer.

Outcomes	Number of studies	Meta-analysis				Heterogeneity	
		HR/RR	95% CI	*p*-value	95% PI	I^2, Tau^2	*p*-Value
PFS	6	0.615	0.517–0.731	<0.001	0.379–0.999	54.8%, 0.0227	0.050
OS	2	0.885	0.737–1.063	0.193	-	29.9%, 0.0197	0.232
Anemia	5	1.106	0.490–2.498	0.809	0.048–25.480	95.5%, 0.7989	<0.001
Leukopenia	3	1.293	0.732–2.285	0.376	0.002–712.363	68.9%, 0.1624	0.040
Neutropenia	5	1.054	0.833–1.332	0.662	0.706–1.597	1.6%, 0.0014	0.397
Thrombocytopenia	5	1.427	0.832–2.449	0.197	0.248–8.227	62.7%, 0.2271	0.030
Nausea	5	1.210	0.818–1.788	0.340	0.268–5.459	94.7%, 0.1845	<0.001
Vomiting	5	1.264	0.756–2.115	0.372	0.196–8.139	86.4%, 0.2735	<0.001
Diarrhea	5	1.757	0.739–4.177	0.202	0.073–42.406	94.7%, 0.8056	<0.001
Abdominal pain	4	1.156	0.952–1.402	0.143	0.364–3.778	46.2%, 0.0456	0.134
Constipation	4	0.976	0.791–1.204	0.822	0.410–2.347	27.3%, 0.0217	0.248
Urinary tract infection	3	1.500	1.114–2.021	0.008	0.218–10.346	0%, 0	0.981
Proteinuria	4	7.195	0.235–219.980	0.258	-	90.7%, 10.6739	<0.001
Fatigue	4	1.264	1.141–1.400	<0.001	1.012–1.552	0%, 0	0.671
Headache	4	1.868	1.036–3.369	0.038	0.154–22.642	76.0%, 0.2457	0.006
Anorexia	3	1.718	1.320–2.235	<0.001	0.050–65.480	22.3%, 0.0382	0.276
Dyspnea	3	1.272	0.928–1.742	0.135	0.004–443.951	34.8%, 0.1164	0.216
Hypertension	5	5.009	1.103–22.744	0.037	0.016–1580.021	98.2%, 2.6730	<0.001

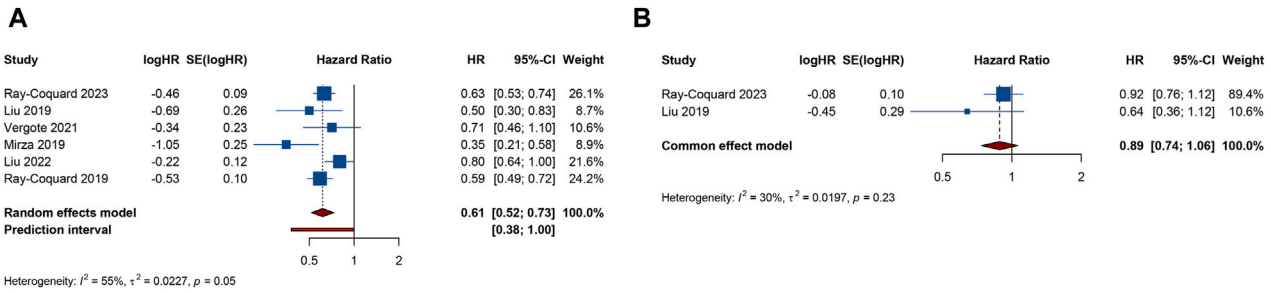

FIGURE 2
Forest plot of efficacy outcomes after combination therapy with PARP inhibitors and antiangiogenic drugs for ovarian cancer. **(A)** progression-free survival; **(B)** overall survival.

and antiangiogenic therapy showed that the combination therapy of olaparib and bevacizumab yielded a significant PFS benefit (HR = 0.613, 95% CI = 0.540–0.695; I^2 = 0%, Tau^2 = 0) over bevacizumab monotherapy (Table 3; Supplementary Figure S1).

2 RCTs addressed OS outcome. There was no significant heterogeneity observed across trials (I^2 = 29.9%, Tau^2 = 0.0197). The results, derived from a fixed-effects model, indicated that compared with PARP inhibitor or antiangiogenic monotherapy, combination therapy led to an improvement in OS, but with no statistical significance (HR = 0.885, 95% CI = 0.737–1.063) (Table 2; Figure 2B). The constricted inclusion of merely two trials in the pooled analysis precluded the possibility of conducting a subgroup analysis for OS outcome.

3.4 Meta-analysis of safety outcomes

3.4.1 Hematologic AEs

5 studies documented the AEs of anemia, neutropenia, or thrombocytopenia, while leukopenia was examined in 3 trials. The overall analysis proposed that PARP inhibitors plus antiangiogenic agents did not elevate the occurrence of

TABLE 3 Subgroup analysis of the efficacy and safety outcomes of PARP inhibitors combined with antiangiogenic agents for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis			Heterogeneity	
		HR/ RR	95% CI	*p*-value	I2, Tau2	*p*-Value
PFS						
Olaparib plus Bevacizumab vs. Bevacizumab	2	0.613	0.540–0.695	<0.001	0%, 0	0.614
Cediranib plus Olaparib vs. Olaparib	2	0.670	0.429–1.047	0.079	63.4%, 0.0700	0.099
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	0.503	0.252–1.007	0.052	76.9%, 0.1922	0.038
Anemia						
Cediranib plus Olaparib vs. Olaparib	2	0.538	0.249–1.163	0.115	63.0%, 0.2126	0.100
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	1.053	0.844–1.314	0.647	0%, 0	0.881
Leukopenia						
Cediranib plus Olaparib vs. Olaparib	2	0.932	0.633–1.372	0.721	0%, 0	0.369
Neutropenia						
Cediranib plus Olaparib vs. Olaparib	2	1.273	0.809–2.003	0.297	0%, 0	0.475
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	0.744	0.453–1.220	0.241	0%, 0	0.358
Thrombocytopenia						
Cediranib plus Olaparib vs. Olaparib	2	2.051	1.302–3.229	0.002	0%, 0	0.602
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	0.733	0.432–1.243	0.249	0%, 0	0.335
Nausea						
Cediranib plus Olaparib vs. Olaparib	2	1.113	0.987–1.256	0.081	13.1%, 0.0016	0.283
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	0.950	0.613–1.473	0.818	81.6%, 0.0832	0.020
Vomiting						
Cediranib plus Olaparib vs. Olaparib	2	1.305	1.024–1.663	0.031	0%, 0	0.799
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	1.031	0.297–3.580	0.961	89.3%, 0.7240	0.002
Diarrhea						
Cediranib plus Olaparib vs. Olaparib	2	9.912	0.515–190.778	0.129	89.2%, 4.1064	0.002
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	0.605	0.429–0.853	0.004	22.0%, 0.0396	0.257
Abdominal pain						
Cediranib plus Olaparib vs. Olaparib	2	1.414	1.088–1.837	0.010	44.3%, 0.1663	0.180
Constipation						
Cediranib plus Olaparib vs. Olaparib	2	1.618	0.363–7.218	0.529	75.6%, 0.9284	0.043

(Continued on following page)

TABLE 3 (*Continued*) Subgroup analysis of the efficacy and safety outcomes of PARP inhibitors combined with antiangiogenic agents for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis			Heterogeneity	
		HR/ RR	95% CI	*p*-value	I2, Tau2	*p*-Value
Proteinuria						
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	33.136	4.711–233.077	<0.001	0%, 0	0.685
Fatigue						
Cediranib plus Olaparib vs. Olaparib	2	1.244	1.110–1.394	<0.001	0%, 0	0.351
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	1.295	1.071–1.566	0.008	0%, 0	0.491
Headache						
Cediranib plus Olaparib vs. Olaparib	2	2.862	1.106–7.409	0.030	70.6%, 0.3500	0.065
Anorexia						
Cediranib plus Olaparib vs. Olaparib	2	1.942	0.946–3.988	0.071	52.9%, 0.1705	0.145
Dyspnea						
Cediranib plus Olaparib vs. Olaparib	2	2.684	0.261–27.651	0.407	66.3%, 2.1025	0.085
Hypertension						
Cediranib plus Olaparib vs. Olaparib	2	14.608	0.951–224.473	0.054	75.4%, 3.1047	0.044
Bevacizumab plus Olaparib (or Niraparib) vs. Olaparib (or Niraparib)	2	5.722	1.037–31.579	0.045	93.4%, 1.4185	<0.001

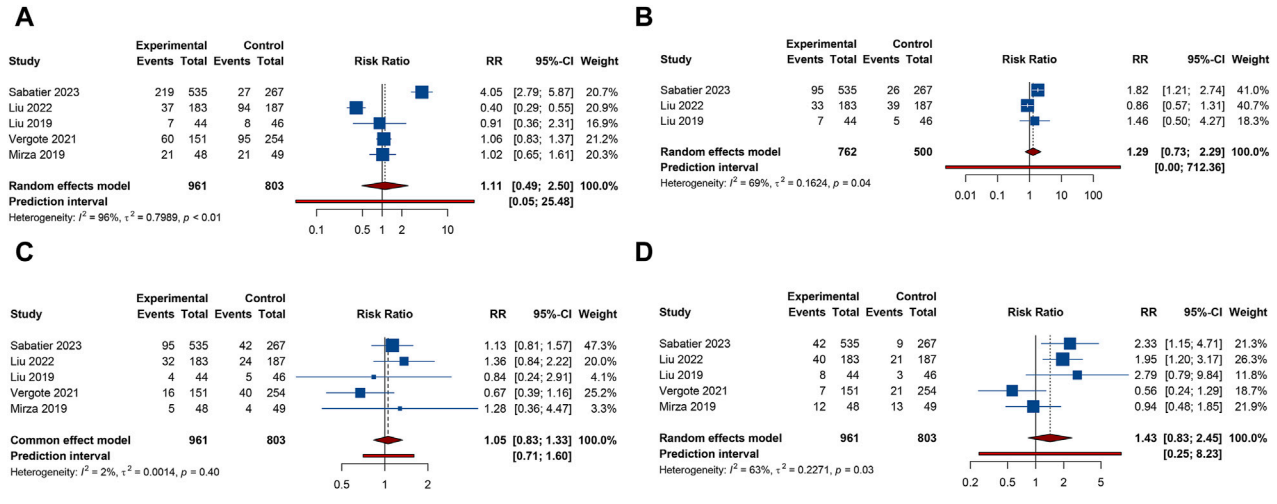

FIGURE 3
Forest plot of hematologic adverse events after combination therapy with PARP inhibitors and antiangiogenic drugs for ovarian cancer. **(A)** Anemia; **(B)** Leukopenia; **(C)** Neutropenia; **(D)** Thrombocytopenia.

anemia (RR = 1.106, 95% CI = 0.490–2.498; 95% PI = 0.048–25.480; I^2 = 95.5%, Tau^2 = 0.7989), leukopenia (RR = 1.293, 95% CI = 0.732–2.285; 95% PI = 0.002–712.363; I^2 = 68.9%, Tau^2 = 0.1624), neutropenia (RR = 1.054, 95% CI = 0.833–1.332; 95% PI = 0.706–1.597; I^2 = 1.6%, Tau^2 = 0.0014), and thrombocytopenia (RR = 1.427, 95% CI = 0.832–2.449; 95%

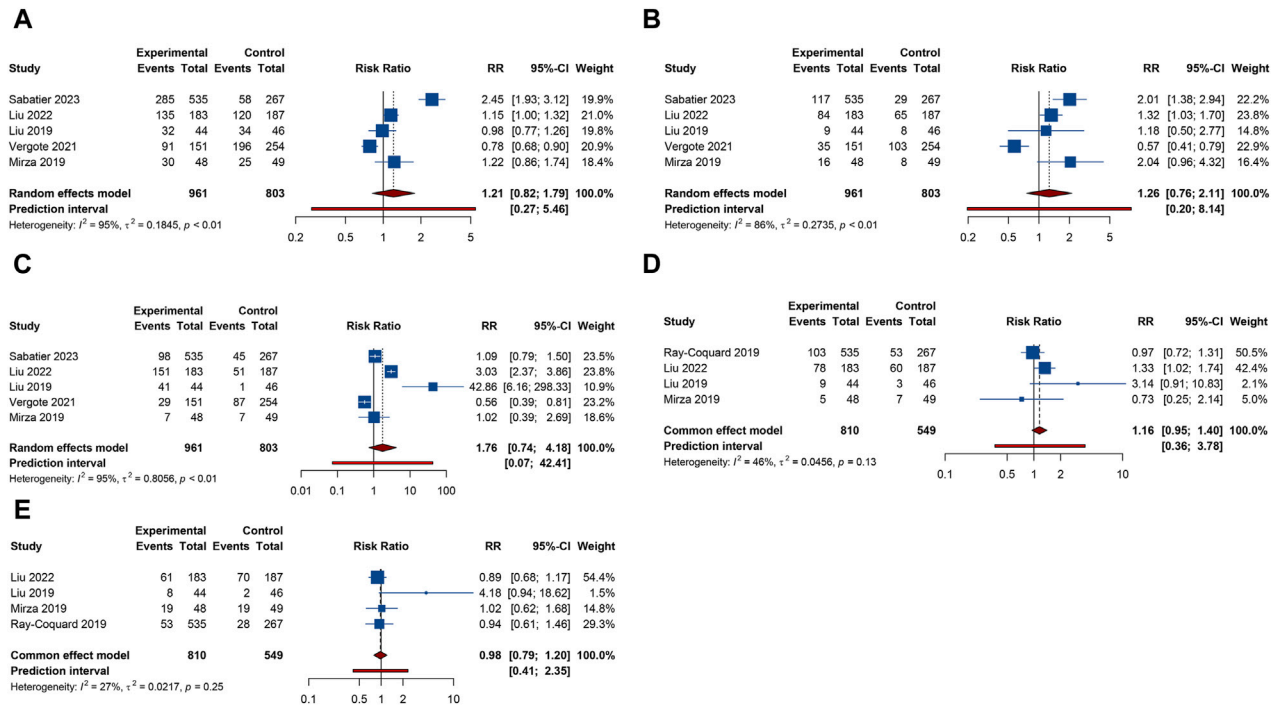

FIGURE 4
Forest plot of gastrointestinal adverse events after combination therapy with PARP inhibitors and antiangiogenic drugs for ovarian cancer. **(A)** Nausea; **(B)** Vomiting; **(C)** Diarrhea; **(D)** Abdominal pain; **(E)** Constipation.

PI = 0.248–8.227; I^2 = 62.7%, Tau^2 = 0.2271) relative to the isolated application of either PARP inhibitors or antiangiogenic medications (Table 2; Figure 3). However, the subgroup analysis indicated that cediranib plus olaparib posed a higher risk for thrombocytopenia (RR = 2.051, 95% CI = 1.302–3.229; I^2 = 0%, Tau^2 = 0) compared with olaparib monotherapy (Table 3; Supplementary Figure S2).

3.4.2 Gastrointestinal AEs

5 RCTs furnished data on gastrointestinal AEs, including nausea, vomiting, or diarrhea. The overall analysis revealed that compared with PARP inhibitor or antiangiogenic monotherapy, combination therapy with PARP inhibitors and antiangiogenic drugs did not raise the risks of nausea (RR = 1.210, 95% CI = 0.818–1.788; 95% PI = 0.268–5.459; I^2 = 94.7%, Tau^2 = 0.1845), vomiting (RR = 1.264, 95% CI = 0.756–2.115; 95% PI = 0.196–8.139; I^2 = 86.4%, Tau^2 = 0.2735), and diarrhea (RR = 1.757, 95% CI = 0.739–4.177; 95% PI = 0.073–42.406; I^2 = 94.7%, Tau^2 = 0.8056) (Table 2; Figures 4A–C). Subgroup analysis indicated that compared with olaparib monotherapy, combination therapy with cediranib and olaparib escalated vomiting risk (RR = 1.305, 95% CI = 1.024–1.663; I^2 = 0%, Tau^2 = 0). Additionally, the combination of bevacizumab and olaparib (or niraparib) was associated with a reduced likelihood of diarrhea relative to the monotherapeutic application of olaparib (or niraparib) (RR = 0.605, 95% CI = 0.429–0.853; I^2 = 22.0%, Tau^2 = 0.0396) (Table 3; Supplementary Figure S3).

4 trials provided information on abdominal pain or constipation. The findings from these studies suggested that the combination therapy of PARP inhibitors and antiangiogenic agents was not associated with an increased incidence of abdominal pain (RR = 1.156, 95% CI = 0.952–1.402; 95% PI = 0.364–3.778; I^2 = 46.2%, Tau^2 = 0.0456) and constipation (RR = 0.976, 95% CI = 0.791–1.204; 95% PI = 0.410–2.347; I^2 = 27.3%, Tau^2 = 0.0217) compared with PARP inhibitor or antiangiogenic drug monotherapy (Table 2; Figures 4D,E). Nonetheless, the combination of bevacizumab and olaparib was linked with a considerable increase in the risk of abdominal pain relative to the use of olaparib alone (RR = 1.414, 95% CI = 1.088–1.837; I^2 = 44.3%, Tau^2 = 0.1663) (Table 3; Supplementary Figure S3).

3.4.3 Renal and urinary AEs

Urinary tract infection was reported in 3 RCTs. Patients receiving combination therapy with PARP inhibitors and antiangiogenic drugs exhibited a statistically significant increase in the incidence of urinary tract infection compared with monotherapy (RR = 1.500, 95% CI = 1.114–2.021; 95% PI = 0.218–10.346; I^2 = 0%, Tau^2 = 0) (Table 2; Figure 5A). Subgroup analysis based on the specific drugs of PARP inhibitors and antiangiogenic therapy was not available for urinary tract infection. Proteinuria outcome was examined in 4 RCTs. The overall analysis indicated that PARP inhibitors plus antiangiogenic agents did not escalate the occurrence of proteinuria relative to monotherapy (RR = 7.195, 95% CI = 0.235–219.980; I^2 = 90.7%, Tau^2 = 10.6739) (Table 2; Figure 5B). Yet, subgroup analysis demonstrated that compared with olaparib (or niraparib) monotherapy, bevacizumab plus olaparib (or niraparib) therapy significantly heightened proteinuria risk (RR = 33.136, 95% CI = 4.711–233.077; I^2 = 0%, Tau^2 = 0) (Table 3; Supplementary Figure S4).

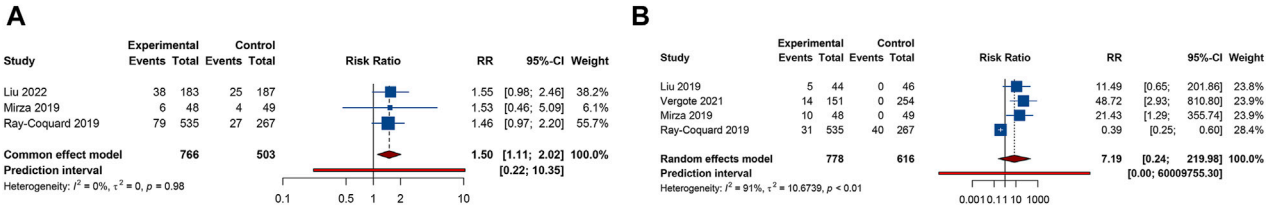

FIGURE 5
Forest plot of renal and urinary adverse events after combination therapy with PARP inhibitors and antiangiogenic drugs for ovarian cancer. **(A)** Urinary tract infection; **(B)** Proteinuria.

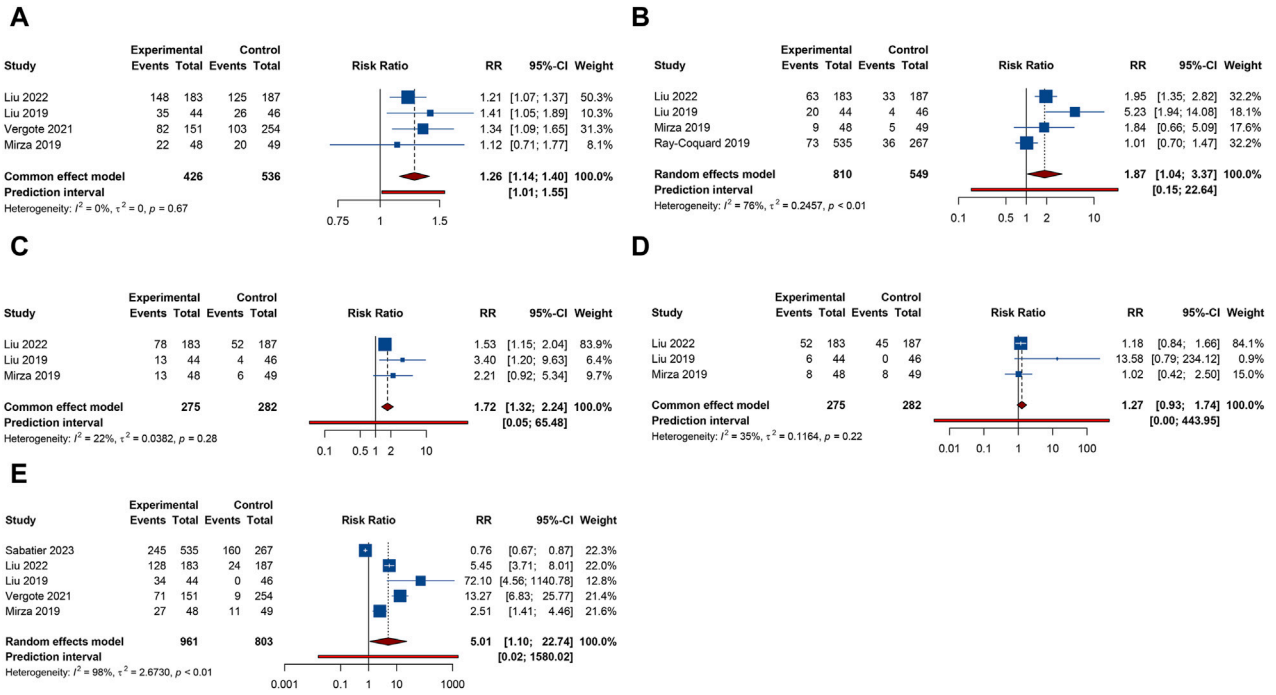

FIGURE 6
Forest plot of other adverse events after combination therapy with PARP inhibitors and antiangiogenic drugs for ovarian cancer. **(A)** Fatigue; **(B)** Headache; **(C)** Anorexia; **(D)** Dyspnea; **(E)** Hypertension.

3.4.4 Other AEs

4 trials analyzed fatigue or headache. The overall analysis suggested that combination treatment of PARP inhibitors and antiangiogenic drugs significantly increased the risks of fatigue (RR = 1.264, 95% CI = 1.141–1.400; 95% PI = 1.012–1.552; I^2 = 0%, Tau^2 = 0) and headache (RR = 1.868, 95% CI = 1.036–3.369; 95% PI = 0.154–22.642; I^2 = 76.0%, Tau^2 = 0.2457) (Table 2; Figures 6A,B). Subgroup analysis showed that combination therapy with cediranib and olaparib was related to an increased risk of fatigue (RR = 1.244, 95% CI = 1.110–1.394; I^2 = 0%, Tau^2 = 0) and headache (RR = 2.862, 95% CI = 1.106–7.409; I^2 = 70.6%, Tau^2 = 0.3500) compared to olaparib monotherapy. Similarly, bevacizumab plus olaparib (or niraparib) therapy was found to heighten fatigue risk compared to olaparib (or niraparib) monotherapy (RR = 1.295, 95% CI = 1.071–1.566; I^2 = 0%, Tau^2 = 0) (Table 3; Supplementary Figure S5).

3 RCTs investigated anorexia or dyspnea. The incidence of anorexia was notably higher in patients receiving combined PARP inhibitor and antiangiogenic therapy than in those on either treatment alone (RR = 1.718, 95% CI = 1.320–2.235; 95% PI = 0.050–65.480; I^2 = 22.3%, Tau^2 = 0.0382). However, this combination did not correlate with a higher rate of dyspnea (RR = 1.272, 95% CI = 0.928–1.742; 95% PI = 0.004–443.951; I^2 = 34.8%, Tau^2 = 0.1164) (Table 2; Figures 6C, D). Further examination of subgroups did not reveal a significant link between cediranib plus olaparib therapy and the onset of anorexia and dyspnea (all p > 0.05) (Table 3; Supplementary Figure S5).

5 RCTs focused on hypertension outcome. The combined therapy of PARP inhibitors and antiangiogenic agents was found to escalate hypertension risk (RR = 5.009, 95% CI = 1.103–22.744; 95% PI = 0.016–1580.021; I^2 = 98.2%, Tau^2 = 2.6730) (Table 2; Figure 6E). Subgroup analysis demonstrated that the combined treatment with bevacizumab and either olaparib or niraparib led to a significant increase in hypertension incidence relative to

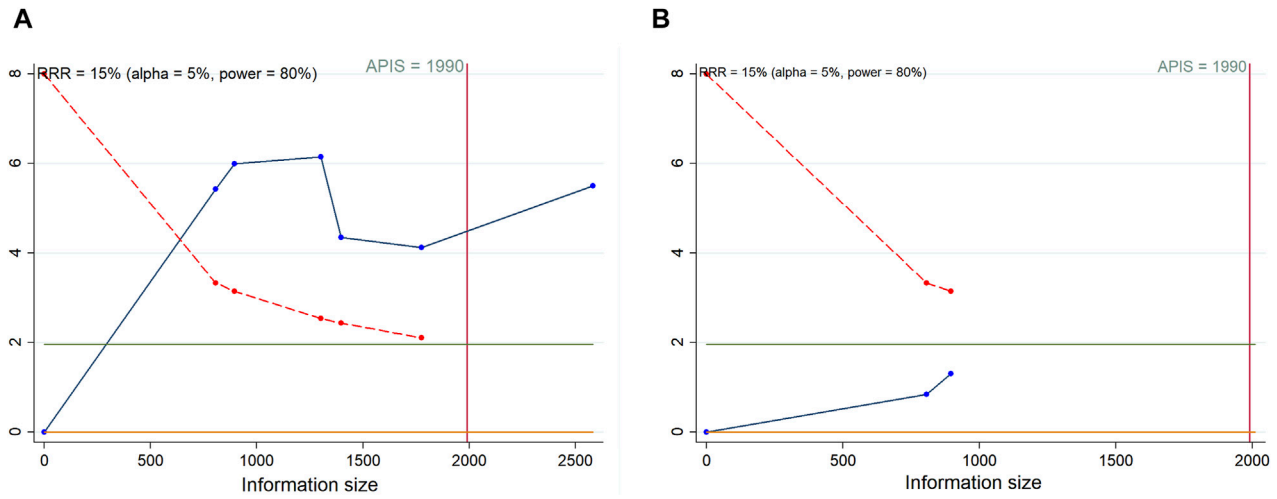

FIGURE 7
Trial sequential analysis of progression-free survival **(A)** and overall survival **(B)** after combination therapy with PARP inhibitors and antiangiogenic drugs for ovarian cancer. Red inward-sloping line to the left represents trial sequential monitoring boundary. Blue line represents evolution of cumulative Z-score. Horizontal green lines represent the conventional boundaries for statistical significance. Heterogeneity-adjusted required information size to demonstrate or reject 15% relative risk (*a priori* estimate) of mortality risk (with alpha of 5% and beta of 20%) is 1990 patients for PFS and OS (vertical red line). Cumulative Z-curve crossing the trial sequential monitoring boundary or the APIS boundary provides firm evidence of effect.

olaparib or niraparib monotherapy (RR = 5.722, 95% CI = 1.037–31.579; I^2 = 93.4%, Tau^2 = 1.4185) (Table 3; Supplementary Figure S5).

3.5 Sensitivity analysis and publication bias

Given the limited number of studies incorporated into the pooled analyses, which might impact the robustness of sensitivity analysis and the evaluation of publication bias, we only carried out these assessments for PFS, the outcome with the largest number of studies included. To ensure the reliability of our findings, we employed the leave-one-out method for the sensitivity analysis. This approach confirmed the stability of the pooled PFS result (Supplementary Figure S6). Begg's and Egger's tests were applied to evaluate publication bias. The results indicated no significant publication bias in PFS outcome (Begg's test: $p = 0.452$, Egger's test: $p = 0.420$). The funnel plots were presented in Supplementary Figure S7.

3.6 Trial sequential analysis results

As shown in Figure 7, we calculated a RIS of 1990 for PFS and OS. The cumulative Z-curve for PFS traversed both the RIS boundary and the trial sequential monitoring boundary, implying a relatively definitive result for PFS. Conversely, the cumulative Z-curve for OS failed to cross either boundary, suggesting that a solid conclusion regarding OS cannot be drawn due to potential false positive. Regarding AEs, definitive conclusions can be inferred for urinary tract infection, fatigue, and anorexia, as only their cumulative Z-curves managed to cross the trial sequential monitoring boundary or RIS boundary (Supplementary Figures S8–S11).

4 Discussion

PARP inhibitors and antiangiogenic agents, both demonstrating promising efficacy as standalone treatments, have garnered particular attention to their combination due to minimal overlapping toxicities (Mirza et al., 2016; Coleman et al., 2017; Moore et al., 2018; González-Martín et al., 2019). The groundbreaking PAOLA-1/ENGOT-ov25 trial, which released its findings in 2019, included 806 patients who were divided in a 2: 1 ratio to either receive a combination of bevacizumab and olaparib or placebo as the first-line maintenance treatment following response to a regimen of chemotherapy and bevacizumab. The addition of maintenance olaparib yielded a significant benefit in terms of PFS (HR = 0.59, 95% CI = 0.49–0.72) (Ray-Coquard et al., 2019). However, a subsequent joint analysis of the SOLO1 and PAOLA-1/ENGOT-ov25 trials indicated that the addition of bevacizumab to olaparib did not appear to enhance PFS compared with olaparib alone (HR = 0.71, 95% CI = 0.45–1.09) (Vergote et al., 2021). Despite previous network meta-analysis reporting significant benefit of PARP inhibitor and angiogenesis inhibitor monotherapy in improving PFS compared to placebo (Feng et al., 2019), there is currently still a lack of meta-analysis directly comparing the efficacy and safety of combined therapy with these two drugs *versus* monotherapy for patients with OC. Therefore, we performed a systematic review and meta-analysis of previous RCTs, and the pooled results demonstrated that combination therapy with PARP inhibitors and antiangiogenic drugs significantly improved PFS, but also increased the risks of AEs such as urinary tract infection, fatigue, headache, anorexia, and hypertension compared with monotherapy with either a PARP inhibitor or an antiangiogenic agent. Given the immature OS outcome in several trials, this meta-analysis obtained OS data from only two RCTs, and the combined results did not confirm the OS benefits of combination therapy compared to monotherapy.

Experimental studies have indicated pathways through which the joint administration of PARP inhibitors and antiangiogenic treatments could enhance outcomes in OC (Lim et al., 2014; Ivy et al., 2016). The study suggested a synergistic effect, with direct and indirect modulation of the tumor cell genome-chiefly through alterations in the tumor microenvironment-potentially underpinning the improved therapeutic efficacy (Ivy et al., 2016). One such mechanism involves the hypoxic conditions induced by antiangiogenic agents (Ueda et al., 2017), which have been observed to attenuate the expression and functionality of the homologous recombination protein RAD51 in neoplastic cells (Chan et al., 2010b). This downregulation of RAD51 under hypoxic conditions was further validated *in vivo* through immunofluorescent imaging of mouse model tumors (Bindra et al., 2004). Additionally, the suppression of VEGFR3 in OC cells has been correlated with reduced levels of the tumor suppressor proteins BRCA1 and BRCA2 (Lim et al., 2014). On the flip side, PARP1-deficient mice exhibited impaired angiogenic responses to growth factors (Tentori et al., 2007). Preclinical models also revealed that high levels of PARP1 expression enhance angiogenesis in epithelial OC by modulating VEGF-A (Wei et al., 2016). The silencing of PARP1 in SKOV3 cells markedly lowered VEGF-A mRNA and protein levels, thus supporting the rationale for the combination of both agents (Le Saux et al., 2021). Nonetheless, the precise biological underpinnings of these therapeutic combinations remain elusive, potentially differ with each antiangiogenic agents, and have yet to be confirmed in clinical settings. Further research is needed to precisely delineate the mechanisms by which this combination exerts its antineoplastic effects. Beyond demonstrating PFS benefit from combination therapy of PARP inhibitors and antiangiogenic agents, our further subgroup analysis validated that combination therapy with olaparib and bevacizumab improved PFS compared with bevacizumab monotherapy. The PAOLA-1 study, a randomized, double-blind, phase III trial, compared the efficacy of olaparib-bevacizumab combined treatment against bevacizumab-placebo in OC patients. The PFS outcome from this study lent credence to the proposition that olaparib, when added to bevacizumab as an initial maintenance therapy, could offer substantial clinical benefit. These findings have led to the authorization by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) of the olaparib-bevacizumab combination for maintenance therapy in the OC patients (Ray-Coquard et al., 2019; Salutari et al., 2024). Updated analysis from the PAOLA-1/ENGOT-ov25 trial further corroborated that olaparib plus bevacizumab combination therapy significantly prolonged PFS compared with bevacizumab plus placebo treatment (HR = 0.63, 95% CI = 0.53–0.74) (Ray-Coquard et al., 2023).

Our study did not substantiate an OS benefit of combination therapy with PARP inhibitors and antiangiogenic drugs in OC patients. While several trials have included OS as an exploratory endpoint, conclusive results on OS have not been realized owing to the insufficient follow-up time up to the data cutoff point (Mirza et al., 2019; Liu et al., 2022). Additionally, RCTs analyzing the outcome of OS reported no significant effect regarding the combined treatment of PARP inhibitors and antiangiogenic agents for OS (Liu et al., 2019; Ray-Coquard et al., 2023). A more recent analysis from a phase II randomized, open-label trial compared the median OS of patients treated with the cediranib-olaparib combination (44.2 months) against those receiving olaparib as a single agent (33.3 months). The HR for this comparison stood at 0.64 with a 95% CI ranging from 0.36 to 1.11, indicating no substantial improvement (Liu et al., 2019). Comprehensive OS results from the PAOLA-1/ENGOT-ov25 trial suggested a slight, non-significant trend towards better OS for patients treated with the combination of olaparib and bevacizumab compared to those receiving bevacizumab with placebo (HR = 0.92, 95% CI = 0.76–1.12) (Ray-Coquard et al., 2023). Besides significant disparities in follow-up duration, the included two RCTs also exhibited considerable differences in the number of patients included in the combination therapy and monotherapy groups. Such variations could potentially influence the pooled results for OS to a certain extent. Consequently, the conclusions drawn from this meta-analysis on the impact of combination therapy on OS in OC patients will require updates in light of forthcoming results from mature OS outcome.

Numerous phase II/III randomized trials have highlighted the therapeutic gains of combining PARP inhibitors with antiangiogenic agents (Liu et al., 2014; Liu et al., 2020; Lorusso et al., 2020; Mirza et al., 2020; Hardesty et al., 2021), yet the elevated risk of AEs warrants attention. The safety profiles for such combined therapies align broadly with those observed for each treatment in isolation, with common all-grade AEs including fatigue, diarrhea, hypertension, and nausea (Alvarez Secord et al., 2021). Our study demonstrated that OC patients receiving combination therapy of PARP inhibitors and antiangiogenic agents experienced a higher occurrence of urinary tract infection, fatigue, headache, anorexia, and hypertension than those on PARP inhibitor or antiangiogenic agent monotherapy. AEs were typically controlled with supportive care and dosage modifications, rarely necessitating cessation of therapy (Pujade-Lauraine et al., 2017; Moore et al., 2018; Ray-Coquard et al., 2019). Notably, myelosuppression stands out as a significant clinical concern with PARP inhibitor combinations due to its potential severity and life-threatening nature, with hematological toxicities being predominant (Ren et al., 2021). Further analysis within our study revealed an increased risk of thrombocytopenia with the cediranib-olaparib combination compared to olaparib alone, underscoring the necessity for thorough blood evaluations and vigilant monitoring for blood-related toxicities in patients undergoing this treatment. In addition, our subgroup analysis indicated that the combination of cediranib and olaparib increased the incidence of vomiting, abdominal pain, fatigue, and headache compared with olaparib monotherapy. Similarly, bevacizumab combined with olaparib (or niraparib) increased the risk of proteinuria, fatigue, and hypertension compared with olaparib (or niraparib) monotherapy. Cediranib and bevacizumab exhibit distinct safety profiles reflective of their differing mechanisms of action, with the most common AEs for cediranib being fatigue and vomiting (Ledermann et al., 2016), while hypertension is frequently reported with bevacizumab maintenance (Burger et al., 2011; Perren et al., 2011). Proteinuria also merits attention as an AE of interest in bevacizumab treatment (Alvarez Secord et al., 2021). Patients on either cediranib or bevacizumab often require management strategies for hypertension, including antihypertensive medications, and should have their blood pressure closely monitored (Ivy et al., 2016). Intriguingly, our

subgroup analysis also revealed that the combination therapy of bevacizumab and olaparib (or niraparib) was associated with a lower incidence of diarrhea, suggesting differential pathways of AE manifestation whose mechanisms remain to be elucidated. Our findings accentuate the necessity for clinicians to be vigilant of AEs such as thrombocytopenia, vomiting, abdominal pain, urinary tract infection, proteinuria, fatigue, headache, anorexia, and hypertension when administering combinatorial PARP inhibitors and antiangiogenic therapy in clinical practice. It is also critical to acknowledge the heightened costs linked to combination treatments, which stem not only from the drugs themselves but also from the necessary healthcare services to administer the treatment and manage any associated toxicities (Hockings and Miller, 2023).

However, AEs that have not been statistically confirmed in our study should not be overlooked, as the wide 95% CIs for the RRs suggests instability in the results (such as diarrhea, proteinuria, constipation, etc.). Therefore, in addition to the various AEs confirmed by this study, it is still necessary in clinical practice to promptly observe and identify any AEs caused by the combination therapy of PARP inhibitors and antiangiogenic agents, and to take timely measures for treatment and control.

There are still several undeniable limitations in present research. First, despite an exhaustive search strategy, the number of studies incorporated into our analysis remains limited. This paucity is likely due to the formidable difficulties encountered in enlisting individuals with OC. Second, the heterogeneity observed across the studies in terms of PFS and majority of AEs may be attributed to variable confounding factors, including disease setting, treatment line, the stage of disease, follow-up duration, therapy modality, treatment duration, drug dosage and diverse ethnic backgrounds of the participants treated with PARP inhibitors and antiangiogenic agents. These confounding factors may also exert an impact on the combined efficacy and safety results. Third, the outcomes of TSA indicated a need for a broader sample size to lend credence to the conclusions drawn regarding OS and the majority of AEs. Furthermore, the limited number of participants in the monotherapy group may lead to instability in the final results, resulting in a wide 95% CI. This issue could be addressed by increasing the sample size. Fourth, the constrained volume of studies that met the inclusion criteria restricts a more nuanced assessment of how combination therapies influence PFS, OS, and AEs across various OC subtypes, such as those delineated by BRCA mutation or homologous recombination deficiency (HRD) status.

5 Conclusion

Through a meta-analysis of RCTs, our research demonstrated that combination therapy with PARP inhibitors and antiangiogenic agents significantly improved PFS compared with PARP inhibitor or antiangiogenic agent monotherapy. However, the present pooled analysis failed to substantiate an OS benefit of combination treatment, since the original trial data concerning OS were immature. Moreover, the combination of PARP inhibitors and antiangiogenic drugs increased the risks of AEs, including thrombocytopenia, vomiting, abdominal pain, urinary tract infection, proteinuria, fatigue, headache, anorexia, and hypertension.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

YW: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing–original draft. LH: Data curation, Formal Analysis, Writing–original draft. TL: Methodology, Writing–review and editing. TG: Conceptualization, Data curation, Formal Analysis, Writing–review and editing. CX: Formal Analysis, Methodology, Writing–original draft. JJ: Conceptualization, Investigation, Methodology, Software, Writing–original draft, Writing–review and editing. YL: Conceptualization, Data curation, Formal Analysis, Writing–review and editing. JL: Data curation, Formal Analysis, Methodology, Writing–original draft. JF: Investigation, Methodology, Writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1372077/full#supplementary-material

References

Abaid, L. N., Goldstein, B. H., Micha, J. P., Rettenmaier, M. A., Brown, J. V., 3rd, and Markman, M. (2010). Improved overall survival with 12 cycles of single-agent paclitaxel maintenance therapy following a complete response to induction chemotherapy in advanced ovarian carcinoma. *Oncology* 78 (5-6), 389–393. doi:10.1159/000320579

Abdalla, A. M. E., Xiao, L., Ullah, M. W., Yu, M., Ouyang, C., and Yang, G. (2018). Current challenges of cancer anti-angiogenic therapy and the promise of nanotherapeutics. *Theranostics* 8 (2), 533–548. doi:10.7150/thno.21674

Alvarez Secord, A., O'Malley, D. M., Sood, A. K., Westin, S. N., and Liu, J. F. (2021). Rationale for combination PARP inhibitor and antiangiogenic treatment in advanced epithelial ovarian cancer: a review. *Gynecol. Oncol.* 162 (2), 482–495. doi:10.1016/j.ygyno.2021.05.018

Begg, C. B., and Mazumdar, M. (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics* 50 (4), 1088–1101. doi:10.2307/2533446

Bindra, R. S., Schaffer, P. J., Meng, A., Woo, J., Måseide, K., Roth, M. E., et al. (2004). Down-regulation of Rad51 and decreased homologous recombination in hypoxic cancer cells. *Mol. Cell Biol.* 24 (19), 8504–8518. doi:10.1128/mcb.24.19.8504-8518.2004

Bowden, J., Tierney, J. F., Copas, A. J., and Burdett, S. (2011). Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Med. Res. Methodol.* 11, 41. doi:10.1186/1471-2288-11-41

Burger, R. A., Brady, M. F., Bookman, M. A., Fleming, G. F., Monk, B. J., Huang, H., et al. (2011). Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N. Engl. J. Med.* 365 (26), 2473–2483. doi:10.1056/NEJMoa1104390

Capriglione, S., Luvero, D., Plotti, F., Terranova, C., Montera, R., Scaletta, G., et al. (2017). Ovarian cancer recurrence and early detection: may HE4 play a key role in this open challenge? A systematic review of literature. *Med. Oncol.* 34 (9), 164. doi:10.1007/s12032-017-1026-y

Chan, N., and Bristow, R. G. (2010a). Contextual" synthetic lethality and/or loss of heterozygosity: tumor hypoxia and modification of DNA repair. *Clin. Cancer Res.* 16 (18), 4553–4560. doi:10.1158/1078-0432.Ccr-10-0527

Chan, N., Pires, I. M., Bencokova, Z., Coackley, C., Luoto, K. R., Bhogal, N., et al. (2010b). Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. *Cancer Res.* 70 (20), 8045–8054. doi:10.1158/0008-5472.Can-10-2352

Coleman, R. L., Brady, M. F., Herzog, T. J., Sabbatini, P., Armstrong, D. K., Walker, J. L., et al. (2017). Bevacizumab and paclitaxel-carboplatin chemotherapy and secondary cytoreduction in recurrent, platinum-sensitive ovarian cancer (NRG Oncology/Gynecologic Oncology Group study GOG-0213): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* 18 (6), 779–791. doi:10.1016/s1470-2045(17)30279-6

Coleridge, S. L., Bryant, A., Kehoe, S., and Morrison, J. (2021). Chemotherapy versus surgery for initial treatment in advanced ovarian epithelial cancer. *Cochrane Database Syst. Rev.* 2 (2), Cd005343. doi:10.1002/14651858.CD005343.pub5

Ding, L., Kim, H. J., Wang, Q., Kearns, M., Jiang, T., Ohlson, C. E., et al. (2018). PARP inhibition elicits STING-dependent antitumor immunity in brca1-deficient ovarian cancer. *Cell Rep.* 25 (11), 2972–2980. doi:10.1016/j.celrep.2018.11.054

Duval, S., and Tweedie, R. (2000). Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56 (2), 455–463. doi:10.1111/j.0006-341x.2000.00455.x

Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315 (7109), 629–634. doi:10.1136/bmj.315.7109.629

Feng, Y., Huang, H., Wan, T., Zhang, C., Tong, C., and Liu, J. (2019). Comparison of PARPis with angiogenesis inhibitors and chemotherapy for maintenance in ovarian cancer: a network meta-analysis. *Adv. Ther.* 36 (12), 3368–3380. doi:10.1007/s12325-019-01106-1

Giornelli, G. H. (2016). Management of relapsed ovarian cancer: a review. *Springerplus* 5 (1), 1197. doi:10.1186/s40064-016-2660-0

González-Martín, A., Pothuri, B., Vergote, I., DePont Christensen, R., Graybill, W., Mirza, M. R., et al. (2019). Niraparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 381 (25), 2391–2402. doi:10.1056/NEJMoa1910962

Hardesty, M., Krivak, T., Wright, G., Hamilton, E., Fleming, E., Belotte, J., et al. (2021). Phase II OVARIO Study of niraparib + bevacizumab therapy in advanced ovarian cancer following front-line platinum-based chemotherapy with bevacizumab. *Gynecol. Oncol.* 162, S17. doi:10.1016/s0090-8258(21)00679-x

Hegan, D. C., Lu, Y., Stachelek, G. C., Crosby, M. E., Bindra, R. S., and Glazer, P. M. (2010). Inhibition of poly(ADP-ribose) polymerase down-regulates BRCA1 and RAD51 in a pathway mediated by E2F4 and p130. *Proc. Natl. Acad. Sci. U. S. A.* 107 (5), 2201–2206. doi:10.1073/pnas.0904783107

Higgins, J. P., and Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21 (11), 1539–1558. doi:10.1002/sim.1186

Hockings, H., and Miller, R. E. (2023). The role of PARP inhibitor combination therapy in ovarian cancer. *Ther. Adv. Med. Oncol.* 15, 17588359231173183. doi:10.1177/17588359231173183

Hopkins, T. A., Ainsworth, W. B., Ellis, P. A., Donawho, C. K., DiGiammarino, E. L., Panchal, S. C., et al. (2019). PARP1 trapping by PARP inhibitors drives cytotoxicity in both cancer cells and healthy bone marrow. *Mol. Cancer Res.* 17 (2), 409–419. doi:10.1158/1541-7786.Mcr-18-0138

IntHout, J., Ioannidis, J. P., Rovers, M. M., and Goeman, J. J. (2016). Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open* 6 (7), e010247. doi:10.1136/bmjopen-2015-010247

Ivy, S. P., Liu, J. F., Lee, J. M., Matulonis, U. A., and Kohn, E. C. (2016). Cediranib, a pan-VEGFR inhibitor, and olaparib, a PARP inhibitor, in combination therapy for high grade serous ovarian cancer. *Expert Opin. Investig. Drugs* 25 (5), 597–611. doi:10.1517/13543784.2016.1156857

Jadad, A. R., Moore, R. A., Carroll, D., Jenkinson, C., Reynolds, D. J., Gavaghan, D. J., et al. (1996). Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin. Trials* 17 (1), 1–12. doi:10.1016/0197-2456(95)00134-4

Jászai, J., and Schmidt, M. H. H. (2019). Trends and challenges in tumor anti-angiogenic therapies. *Cells* 8 (9), 1102. doi:10.3390/cells8091102

Ledermann, J. A., Embleton, A. C., Raja, F., Perren, T. J., Jayson, G. C., Rustin, G. J. S., et al. (2016). Cediranib in patients with relapsed platinum-sensitive ovarian cancer (ICON6): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 387 (10023), 1066–1074. doi:10.1016/s0140-6736(15)01167-8

Le Saux, O., Vanacker, H., Guermazi, F., Carbonnaux, M., Roméo, C., Larrouquère, L., et al. (2021). Poly(ADP-ribose) polymerase inhibitors in combination with anti-angiogenic agents for the treatment of advanced ovarian cancer. *Future Oncol.* 17 (18), 2291–2304. doi:10.2217/fon-2021-0059

Lim, J. J., Yang, K., Taylor-Harding, B., Wiedemeyer, W. R., and Buckanovich, R. J. (2014). VEGFR3 inhibition chemosensitizes ovarian cancer stemlike cells through down-regulation of BRCA1 and BRCA2. *Neoplasia* 16 (4), 343–353. e1-2. doi:10.1016/j.neo.2014.04.003

Liu, J. F., Barry, W. T., Birrer, M., Lee, J. M., Buckanovich, R. J., Fleming, G. F., et al. (2014). Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. *Lancet Oncol.* 15 (11), 1207–1214. doi:10.1016/s1470-2045(14)70391-2

Liu, J. F., Barry, W. T., Birrer, M., Lee, J. M., Buckanovich, R. J., Fleming, G. F., et al. (2019). Overall survival and updated progression-free survival outcomes in a randomized phase II study of combination cediranib and olaparib versus olaparib in relapsed platinum-sensitive ovarian cancer. *Ann. Oncol.* 30 (4), 551–557. doi:10.1093/annonc/mdz018

Liu, J. F., Brady, M. F., Matulonis, U. A., Miller, A., Kohn, E. C., Swisher, E. M., et al. (2020). A phase III study comparing single-agent olaparib or the combination of cediranib and olaparib to standard platinum-based chemotherapy in recurrent platinum-sensitive ovarian cancer. *J. Clin. Oncol.* 38 (15), 6003. doi:10.1200/jco.2020.38.15_suppl.6003

Liu, J. F., Brady, M. F., Matulonis, U. A., Miller, A., Kohn, E. C., Swisher, E. M., et al. (2022). Olaparib with or without cediranib versus platinum-based chemotherapy in recurrent platinum-sensitive ovarian cancer (NRG-GY004): a randomized, open-label, phase III trial. *J. Clin. Oncol.* 40 (19), 2138–2147. doi:10.1200/jco.21.02011

Lorusso, D., Lotz, J. P., Harter, P., Cropet, C., Pérez, M. J. R., Schauer, C., et al. (2020). Maintenance olaparib plus bevacizumab (bev) after platinum-based chemotherapy plus bev in patients (pts) with newly diagnosed advanced high-grade ovarian cancer (HGOC): efficacy by BRCA1 or BRCA2 mutation in the phase III PAOLA-1 trial. *J. Clin. Oncol.* 38 (15), 6039. doi:10.1200/jco.2020.38.15_suppl.6039

Markman, M., Liu, P. Y., Moon, J., Monk, B. J., Copeland, L., Wilczynski, S., et al. (2009). Impact on survival of 12 versus 3 monthly cycles of paclitaxel (175 mg/m2) administered to patients with advanced ovarian cancer who attained a complete response to primary platinum-paclitaxel: follow-up of a Southwest Oncology Group and Gynecologic Oncology Group phase 3 trial. *Gynecol. Oncol.* 114 (2), 195–198. doi:10.1016/j.ygyno.2009.04.012

Markman, M., Liu, P. Y., Wilczynski, S., Monk, B., Copeland, L. J., Alvarez, R. D., et al. (2003). Phase III randomized trial of 12 versus 3 months of maintenance paclitaxel in patients with advanced ovarian cancer after complete response to platinum and paclitaxel-based chemotherapy: a Southwest Oncology Group and Gynecologic Oncology Group trial. *J. Clin. Oncol.* 21 (13), 2460–2465. doi:10.1200/jco.2003.07.013

Mirza, M. R., Åvall Lundqvist, E., Birrer, M. J., dePont Christensen, R., Nyvang, G. B., Malander, S., et al. (2019). Niraparib plus bevacizumab versus niraparib alone for platinum-sensitive recurrent ovarian cancer (NSGO-AVANOVA2/ENGOT-ov24): a randomised, phase 2, superiority trial. *Lancet Oncol.* 20 (10), 1409–1419. doi:10.1016/s1470-2045(19)30515-7

Mirza, M. R., Monk, B. J., Herrstedt, J., Oza, A. M., Mahner, S., Redondo, A., et al. (2016). Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N. Engl. J. Med.* 375 (22), 2154–2164. doi:10.1056/NEJMoa1611310

Mirza, M. R., Nyvang, G. B., Lund, B., Christensen, R. D., Werner, T. L., Malander, S., et al. (2020). Final survival analysis of NSGO-AVANOVA2/ENGOT-OV24: combination of niraparib and bevacizumab versus niraparib alone as treatment of recurrent platinum-sensitive ovarian cancer-A randomized controlled chemotherapy-free study. *J. Clin. Oncol.* 38 (15), 6012. doi:10.1200/jco.2020.38.15_suppl.6012

Moore, K., Colombo, N., Scambia, G., Kim, B. G., Oaknin, A., Friedlander, M., et al. (2018). Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 379 (26), 2495–2505. doi:10.1056/NEJMoa1810858

O'Sullivan, C. C., Moon, D. H., Kohn, E. C., and Lee, J. M. (2014). Beyond breast and ovarian cancers: PARP inhibitors for BRCA mutation-associated and BRCA-like solid tumors. *Front. Oncol.* 4, 42. doi:10.3389/fonc.2014.00042

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj* 88, 105906. doi:10.1016/j.ijsu.2021.105906

Perren, T. J., Swart, A. M., Pfisterer, J., Ledermann, J. A., Pujade-Lauraine, E., Kristensen, G., et al. (2011). A phase 3 trial of bevacizumab in ovarian cancer. *N. Engl. J. Med.* 365 (26), 2484–2496. doi:10.1056/NEJMoa1103799

Pujade-Lauraine, E., Ledermann, J. A., Selle, F., Gebski, V., Penson, R. T., Oza, A. M., et al. (2017). Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 18 (9), 1274–1284. doi:10.1016/s1470-2045(17)30469-2

Ray-Coquard, I., Leary, A., Pignata, S., Cropet, C., González-Martín, A., Marth, C., et al. (2023). Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. *Ann. Oncol.* 34 (8), 681–692. doi:10.1016/j.annonc.2023.05.005

Ray-Coquard, I., Pautier, P., Pignata, S., Pérol, D., González-Martín, A., Berger, R., et al. (2019). Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N. Engl. J. Med.* 381 (25), 2416–2428. doi:10.1056/NEJMoa1911361

Ren, N., Zhang, L., Yu, J., Guan, S., Dai, X., Sun, L., et al. (2021). Efficacy and safety of PARP inhibitor combination therapy in recurrent ovarian cancer: a systematic review and meta-analysis. *Front. Oncol.* 11, 638295. doi:10.3389/fonc.2021.638295

Sabatier, R., Rousseau, F., Joly, F., Cropet, C., Montégut, C., Frindte, J., et al. (2023). Efficacy and safety of maintenance olaparib and bevacizumab in ovarian cancer patients aged ≥65 years from the PAOLA-1/ENGOT-ov25 trial. *Eur. J. Cancer* 181, 42–52. doi:10.1016/j.ejca.2022.11.029

Salutari, V., Giudice, E., and Lorusso, D. (2024). Maintenance therapy for newly and recurrent epithelial ovarian cancer: current therapies and future perspectives. *Curr. Opin. Obstet. Gynecol.* 36 (1), 9–17. doi:10.1097/gco.0000000000000931

Siegel, R. L., Miller, K. D., and Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J. Clin.* 70 (1), 7–30. doi:10.3322/caac.21590

Tentori, L., Lacal, P. M., Muzi, A., Dorio, A. S., Leonetti, C., Scarsella, M., et al. (2007). Poly(ADP-ribose) polymerase (PARP) inhibition or PARP-1 gene deletion reduces angiogenesis. *Eur. J. Cancer* 43 (14), 2124–2133. doi:10.1016/j.ejca.2007.07.010

Tierney, J. F., Stewart, L. A., Ghersi, D., Burdett, S., and Sydes, M. R. (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 8, 16. doi:10.1186/1745-6215-8-16

Ueda, S., Saeki, T., Osaki, A., Yamane, T., and Kuji, I. (2017). Bevacizumab induces acute hypoxia and cancer progression in patients with refractory breast cancer: multimodal functional imaging and multiplex cytokine analysis. *Clin. Cancer Res.* 23 (19), 5769–5778. doi:10.1158/1078-0432.Ccr-17-0874

Vergote, I., Ray-Coquard, I., Anderson, D. M., Cantuaria, G., Colombo, N., Garnier-Tixidre, C., et al. (2021). Population-adjusted indirect treatment comparison of the SOLO1 and PAOLA-1/ENGOT-ov25 trials evaluating maintenance olaparib or bevacizumab or the combination of both in newly diagnosed, advanced BRCA-mutated ovarian cancer. *Eur. J. Cancer* 157, 415–423. doi:10.1016/j.ejca.2021.08.023

Wang, Z., Huang, Y., Long, L., Zhou, L., Huang, Y., Gan, L., et al. (2021). Apatinib treatment efficiently delays biochemical-only recurrent ovarian cancer progression. *J. Ovarian Res.* 14 (1), 91. doi:10.1186/s13048-021-00843-8

Wei, W., Li, Y., Lv, S., Zhang, C., and Tian, Y. (2016). PARP-1 may be involved in angiogenesis in epithelial ovarian cancer. *Oncol. Lett.* 12 (6), 4561–4567. doi:10.3892/ol.2016.5226

Wetterslev, J., Jakobsen, J. C., and Gluud, C. (2017). Trial Sequential Analysis in systematic reviews with meta-analysis. *BMC Med. Res. Methodol.* 17 (1), 39. doi:10.1186/s12874-017-0315-7

Xie, M., Zhong, Y., Yang, Y., Shen, F., and Nie, Y. (2022). Extended adjuvant endocrine therapy for women with hormone receptor-positive early breast cancer: a meta-analysis with trial sequential analysis of randomized controlled trials. *Front. Oncol.* 12, 1039320. doi:10.3389/fonc.2022.1039320

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Body composition and inflammation variables as the potential prognostic factors in epithelial ovarian cancer treated with Olaparib

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Background: Epithelial ovarian cancer (EOC) is a significant cause of mortality among gynecological cancers. While Olaparib, a PARP inhibitor, has demonstrated efficacy in EOC maintenance therapy, individual responses vary. This study aims to assess the prognostic significance of body composition and systemic inflammation markers in EOC patients undergoing initial Olaparib treatment.

Methods: A retrospective analysis was conducted on 133 EOC patients initiating Olaparib therapy. Progression-free survival (PFS) was assessed through Kaplan-Meier analysis and Cox proportional hazards regression. Pre-treatment computed tomography images were utilized to evaluate body composition parameters including subcutaneous adipose tissue index (SATI), visceral adipose tissue index (VATI), skeletal muscle area index (SMI), and body mineral density (BMD). Inflammatory markers, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), serum albumin, and hemoglobin levels, were also measured.

Results: The median follow-up duration was 16 months (range: 5-49 months). Survival analysis indicated that high SATI, high VATI, high SMI, high BMD, low NLR, and low PLR were associated with decreased risk of disease progression (all $p < 0.05$). Multivariate analysis identified several factors independently associated with poor PFS, including second or further lines of therapy (HR = 2.16; 95% CI = 1.09-4.27, p = 0.027), low VATI (HR = 3.79; 95% CI = 1.48-9.70, p = 0.005), low SMI (HR = 2.52; 95% CI = 1.11-5.72, p = 0.027), low BMD (HR = 2.36; 95% CI = 1.22-4.54, p = 0.010), and high NLR (HR = 0.31; 95% CI = 0.14-0.69, p = 0.004). Subgroup analysis in serous adenocarcinoma patients revealed distinct prognostic capabilities of SATI, VATI, SMI, PLR, and NLR

Conclusion: Body composition and inflammation variables hold promise as predictors of therapeutic response to Olaparib in EOC patients. Understanding their prognostic significance could facilitate tailored treatment strategies, potentially improving patient outcomes.

KEYWORDS

epithelial ovarian cancer, poly (ADP-ribose) polymerase inhibitors, body composition, inflammation variables, progression free survival

Introduction

Ovarian cancer ranked as the third most prevalent gynecological cancer in the global cancer statistics of 2020. The worldwide incidence of new cases reached 313,959, with 207,252 resulting in fatalities (1). In China, the statistics for 2022 reported 57,090 new cases and 24,494 deaths (2), which demonstrate only a slight decline compared to the 2015 data (3). The high mortality rate can be attributed to the advanced stage at the time of ovarian cancer diagnosis (4). For decades, the conventional treatment approach for ovarian cancer has been radical debulking surgery followed by platinum-based combination chemotherapy, which has proven to be the most effective and widely used method (5). However, within five years, approximately 70% of patients experience recurrence (6). The efficacy of subsequent lines of chemotherapy diminishes with each relapse, resulting in a minority of advanced-stage ovarian cancer patients surviving for five years with traditional treatment (7).

The synthetic lethal approach of targeting the DNA repair pathway is the mechanism of Poly (ADP-ribose) polymerase (PARP) inhibitors as maintenance therapy in ovarian cancers (8). With increasing evidence supporting the use of maintenance therapy, Olaparib has become popular due to its longer progression-free survival (PFS) and overall survival (OS) in the SOLO1 trial (9). This trial treated patients with BRCA1/2 mutation diagnosed with high-grade serous/endometrioid ovarian cancer with Olaparib, which resulted in a 70% lower risk of disease progression or death. In the PAOLA-1 trial, Olaparib treatment for homologous recombination deficient (HRD) tended to extend the PFS and OS (10). There is also strong evidence that relapsed platinum-sensitive-ovarian cancer responds well to maintenance drugs such as Olaparib (11–13). Undoubtedly, the PFS and OS are the reliable terms of predictive treatment outcomes, who receive PARP inhibitors. However, not every patient benefits from Olaparib as maintenance therapy, and the outcomes of PARP inhibitors for the specific patients cannot be determined until progression. Therefore, the reliable and validated biomarkers from patients are needed to predict their response to these drugs.

Abdominal adipose tissue, especially the distributions of visceral adipose tissue (VAT)and subcutaneous adipose tissue (SAT) measured by quantitative computer tomography (QCT), have been acknowledged as a good prognostic biomarker for PFS and OS after surgery, radiation, or classical chemotherapy (14, 15). Overweight has been identified as a high-risk factor for several cancers (16, 17), such as prostate, breast and colorectal cancers. Emerging evidence also suggests that sarcopenic obesity, characterized by severe obesity and low skeletal muscle area (SMA), might be a predictor of cancer (18). Many observational studies have shown that sarcopenic obesity as the biomarker predicts a poor OS in cancer patients (19), as well as the loss of body mineral density (BMD) (20). Furthermore, research has focused on the body composition as a predictor of response and toxicity to cancer immune checkpoint inhibitors (21). Meanwhile, the efficacy of apatinib as vascular endothelial growth factor (VEGF)-targeted therapy in predicting the outcome of ovarian cancer patients by evaluating the distinct adipose tissue has been reported (22).

In addition to the patient's body composition, systemic inflammation is believed to play an important role in the progression of ovarian cancers (23). Inflammation-based prognostic indicators, such as neutrophil-to-lymphocyte ratio (NLR) (24) and the platelet-to-lymphocyte ratio (PLR) (25), have been reported in various cancers. The level of hemoglobin and serum albumin can also reflect nutritional status, which has been investigated as a prognostic factor in cancers (26).

We aimed to explore whether CT-based body composition (VAT, SAT, SMA, and BMD), systemic inflammation (NLR and PLR), and nutritional status could serve as prognostic predictors for epithelial ovarian cancer (EOC) patients treated with Olaparib.

Methods

Patients

In this retrospective analysis, we examined patients diagnosed with Stage IIB-IV EOC as classified by the International Federation of Gynecology and Obstetrics (27). These individuals exhibited either BRCA1/2 mutations (germline and/or somatic mutations) and/or were identified as HRD-positive. Following optimal debulking surgery, they underwent first-line platinum-based

chemotherapy. Subsequently, they received an initial treatment with Olaparib (300 mg bid) at our institution between November 2018 and December 2021. The maximum duration of Olaparib maintenance therapy extended to 2 years, with no instances of treatment discontinuation attributed to side effects. Discontinuation events were solely linked to early cessation prompted by disease progression. For individuals undergoing Olaparib maintenance therapy for epithelial ovarian cancer, common side effects, including nausea, fatigue, anemia, thrombocytopenia, insomnia, leucopenia, constipation, diarrhea, and joint pain, were typically mild to moderate (grades 1-3). Notably, bone marrow suppression, such as anemia, platelet reduction, and leucopenia, often fell within this range. Additionally, other side effects were generally of grade 1 severity. Additionally, patients with platinum-sensitive, relapsed epithelial ovarian cancer who had received 2 or more lines of treatment initially treated with Olaparib were also included. The inclusion criteria were as follows: (a) individuals who had undergone a diagnostically acceptable abdominal CT within 1 month before initiating Olaparib treatment; (b) those with histologically confirmed EOC. The exclusion criteria were as follows: (1) incomplete clinical follow-up data; (2) poor quality CT scans; (3) absence of routine hematological and biochemical examinations within 7 days before the initial Olaparib treatment; (4) combined with bevacizumab as maintenance therapy; (5) individuals receiving steroids or other immunomodulatory agents within 1 month prior to starting Olaparib treatment or those diagnosed with infections or immunodeficiencies. PFS was defined as the time (in months) from the initiation of Olaparib treatment to disease progression or the last follow-up in December 2022.

Clinical and pathological data, including age, weight, height, tumor grading and histology type, lines of treatment, pre-treatment complete blood counts (neutrophil, lymphocyte, and platelet counts), serum albumin, and hemoglobin, were extracted from retrospective medical records at the time of Olaparib initiation and before administering the first dose (300 mg bid). NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, and PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. Serum hemoglobin increased ≥110 g/L was defined as normal, and a serum albumin <40 g/l was defined as hypoalbuminemia. Height and weight measurements acquired within 14 days before the treatment. Body mass index (BMI) was calculated using the formula weight/height2 (kilograms per square meter). Patients were classified into four weight categories: underweight (BMI < 18.5 kg/m^2), normal weight (18.5 kg/m^2 ≤ BMI ≤ 22.9 kg/m^2), overweight (23 kg/m^2 ≤ BMI ≤ 24.9 kg/m^2), and obese (BMI ≥ 25 kg/m^2).

CT analysis

Abdominal CT images were obtained before initiating Olaparib treatment (within a month). CT examinations were performed in the axial plane with 5-mm-thick sections using a 64-row CT scanner (Somatom definition AS large-aperture, Siemens Healthcare, Germany) and a 256-row CT scanner (revolution, GE Healthcare, USA). A single slice of each patient's baseline CT image was selected at the third lumbar vertebra (L3) as the standard for assessing body composition. The segmentation of SAT, VAT and SMA were performed by using 3D Slicer software (version 4.11.2; Boston, MA, USA) (Figure 1A) and the area of interest were manually calculated. The threshold for adipose tissue was set between -190 and -30 Hounsfield units (HU) (SAT: ranging from -190 to -30 HU; VAT: ranging from -150 to -50 HU). SMA was measured within the range of -29 to +150 HU (18) (Figure 1B). The cross-sectional area values were normalized for height, and the measurements were labeled as SATI, VATI, SMI following previously published methods [$(cm^2)/(m^2)$] (28). Additionally, BMD values were calculated at the L2 vertebra level and the area of the interest was approximately 4 cm^2 (29) (Figure 1C).

Statistical analyses

R software (Version 4.2.3) was used to perform all data analyses. Continuous variables were expressed as mean ± standard error. Categorical variables were compared using the chi-square test. The optimal cutoff value for continuous variables (including NLR, PLR, VATI, SATI, SMI, and BMD) was determined using the surv_cutpoint function based on the previously published methods (30, 31). Kaplan-Meier survival curves and log-rank tests

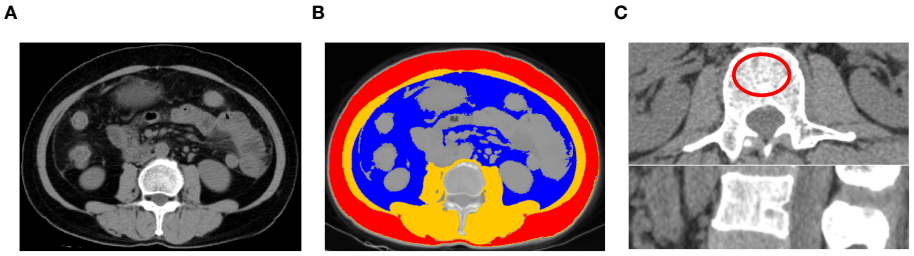

FIGURE 1
An example of segmentation of body composition. **(A)** original image; **(B)** Subcutaneous adipose tissue (red), visceral adipose tissue (blue), and skeletal muscle area (Brown) from an axial image at the level of L3 vertebra of a CT scan; **(C)** Measurement of bone mineral density of L2 vertebra a CT scan.

were conducted using the "survival" and "survminer" R packages to illustrate the survival differences between the two groups. To identify potential independent prognostic factors, univariate analyses were performed initially, and a multivariate Cox proportional hazards regression (stepwise model) analysis was subsequently conducted, including all variables with a p-value less than 0.05 from the univariate analysis. To reduce the potential confounding and selection bias, propensity score matching (PSM) analysis was carried out and 1:1 nearest-neighbor matching. Propensity scores were calculated using logistic regression models with the clinical, body composition and inflammation variables. Statistical significance was defined as $p < 0.05$.

Results

Patients characteristics

Between November 2018 and December 2021, a total of 168 patients underwent screening, of whom 35 patients were excluded (Figure 2). Ultimately, 133 patients were included in this study, with a mean age of 54.32 ± 8.29 years (range: 28-71), mean serum albumin of 43.10 ± 3.61 g/l, mean hemoglobin of 111.25 ± 15.30 g/L, mean NLR of 2.69 ± 1.82, and mean PLR of 159.75 ± 91.82. The median follow-up duration was 16 months (range: 5-49 months). Serous adenocarcinoma was the most common subtype, accounting for 84.9% (113/133) of the total patients. Fifty-seven out of 133 (42.8%) patients received first-line treatment. The clinical characteristics of the patients are summarized in Table 1. The optimal cut-off values for NLR, PLR, determined using the surv_cutpoint R function, were 2.11, and 192, respectively. To facilitate further analysis, patients were categorized into high or low groups based on these cut-off values (NLR ≤ 2.11 and > 2.11; PLR ≤ 192 and > 192). Kaplan-Meier curve analysis for PFS demonstrated clear differentiation between the two groups for NLR and PLR (both $p<0.001$), indicating a significant association between decreased NLR, decreased PLR, and favorable PFS (Figures 3A, B). However, serum hemoglobin and albumin were not significantly associated with PFS (Figures 3C, D).

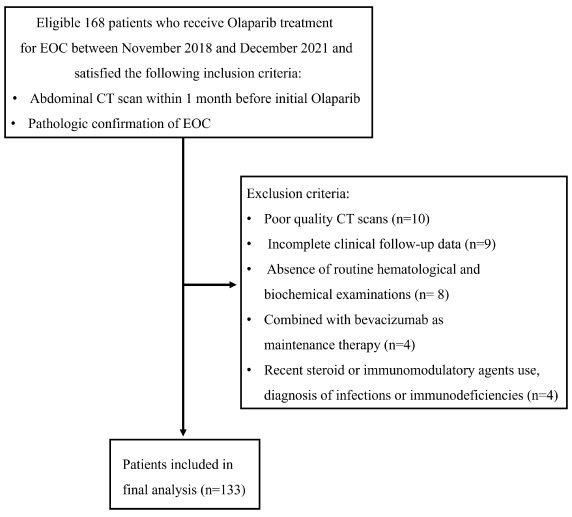

FIGURE 2
Flow diagram depicting patient selection process.

Body composition and serum inflammation factors associated with progression-free survival

High intra-observer consistency was observed for the measurement of SAT, VAT, SM, and BMD, with pretreatment

TABLE 1 Patient characteristics: Demographics.

Parameters	N
Age (year), mean ± SD	54.32 ± 8.29
≥60	32
<60	101
BMI (kg/m2), mean ± SD	23.23 ± 2.94
BMI range*	
Underweight (<18.5)	7
Normal (18.5–22.9)	57
Overweight (23.0–24.9)	35
Obesity (≥25.0)	34
Histology types	
Serous	113
Endometroid	2
Clear cell	2
Mucinous	1
Others	15
Tumor grading	
Well-Moderate differentiated	10
Low differentiated	123
Number of previous chemotherapy lines	
1 line	57
2-3 lines	67
>3 lines	9
FIGO staging	
I-II	18
III-IV	115
Laboratory Tests	
Neutrophil count (10^9/L)	3.15 ± 1.69
Lymphocyte count (10^9/L)	1.34 ± 0.53
Platelet count (10^9/L)	180.61 ± 72.60

(Continued)

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TABLE 1 Continued

Parameters	N
Laboratory Tests	
NLR	2.69 ± 1.82
PLR	159.75 ± 91.82
Hemoglobin (g/L)	111.25 ± 15.30
Albumin (g/L)	43.10 ± 3.61
Body Composition Parameters, mean ± SD	
SATI (cm^2/m^2)	61.33 ± 18.95
VATI (cm^2/m^2)	28.17 ± 14.24
SMI (cm^2/m^2)	41.20 ± 5.93
BMD (HU)	153.96 ± 49.31

FIGO, The International Federation of Gynecology and Obstetrics; BMI, body mass index; SATI, subcutaneous adipose tissue index; VATI, visceral adipose tissue index; SMI, skeletal muscle area index; BMD, bone mineral density; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte; SD, standard deviation.

intraclass association coefficients of 0.906, 0.873, 0.864, 0.836, respectively. After normalizing for height, the average values for subcutaneous adipose tissue index (SATI), visceral adipose tissue index (VATI), and skeletal muscle area index (SMI) were 61.33 ± 18.95, 28.17 ± 14.24, and 41.20 ± 5.93 $(cm^2)/(m^2)$, respectively (Table 1). Patients were divided into high or low groups based on cut-off values of 50.7 cm^2/m^2 for SATI, 35.7 cm^2/m^2 for VATI, 37.0 cm^2/m^2 for SMI, and 163 HU for BMD (Table 2). The risk of disease progression in the high group was further analyzed. Kaplan-Meier curve analysis revealed that patients with high SATI (Figure 4A), high VATI (Figure 4B), high SMI (Figure 4C), and high BMD (Figure 4D) had a lower risk of disease progression compared to those with low SATI ($p = 0.036$), low VATI ($p = 0.0006$), low SMI ($p < 0.001$), and low BMD ($p = 0.023$), respectively. SMI was the strongest prognostic factor for disease progression.

Based on PSM analysis, we obtained matched patients for SATI, VATI, SMI, BMD, NLR, and PLR variables respectively at 1:1 ratio. We then performed the survival analysis to evaluate prognosis outcomes. Kaplan-Meier curve of SATI, VATI, SMI, BMD, NLR, and PLR could clearly distinguish two groups (high vs low) (all $p < 0.05$), consistent with previous results of whole patients (Supplementary Figure 1).

Univariable Cox proportional hazard analysis was conducted to assess the association between clinical parameters (including tumor grading, histology type, chemotherapy lines, body composition and serum inflammation factors) and progression-free survival in patients. NLR, PLR and SMI were found to be the strongest prognostic parameter for progression-free survival ($p < 0.001$) (Table 2). Second or further lines therapy, high SATI, high VATI, and high BMD were associated with decreased progression-free survival compared to the corresponding group ($p < 0.05$) (Table 2). Multivariable Cox proportional hazard models for progression-free survival were also presented in Table 2. In the multivariate analysis, chemotherapy lines, three body composition parameters and one serum inflammation factor were identified as independent factors associated with poor PFS: second or further lines (HR = 2.16; 95%

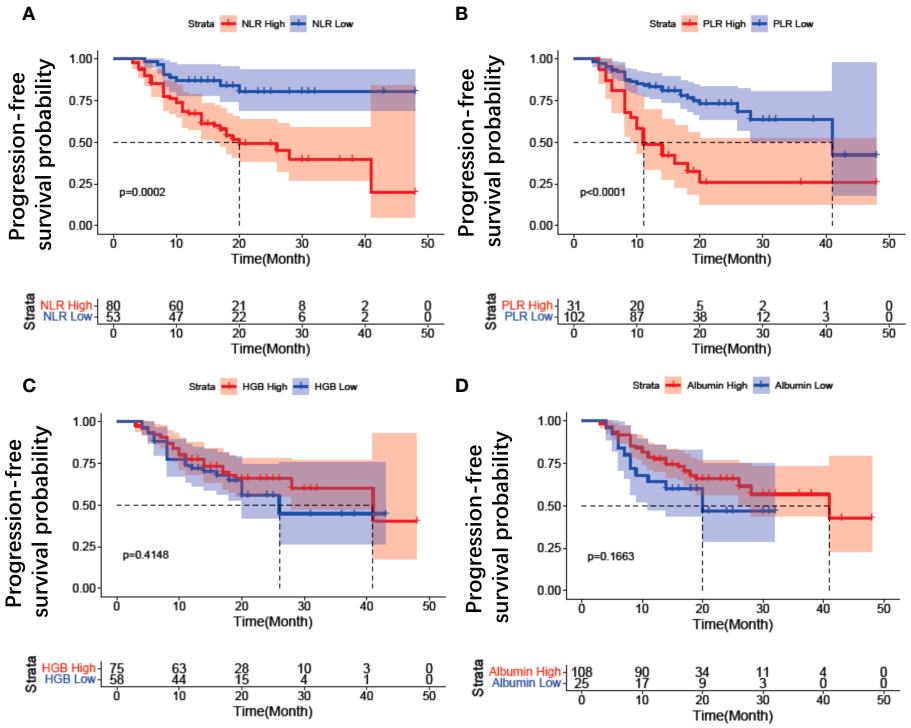

FIGURE 3
Kaplan–Meier estimates of progression free survival for inflammation variables in patients with EOC treated with Olaparib. **(A)** NLR change, **(B)** PLR change, **(C)** HGB change, **(D)** Albumin change. NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; HGB, hemoglobin.

TABLE 2 Cox proportional hazard models for progression-free survival of patients with epithelial ovarian cancer during Olaparib maintenance treatment.

	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (<60 years)	0.87 (0.45-1.67)	0.668		
BMI (<23 kg/m2)	1.31 (0.74-2.32)	0.351		
FIGO staging (I-II)	0.85 (0.36-2.01)	0.716		
Histology type (non-Serous)	0.76 (0.37-1.58)	0.455		
Tumor grading (low differentiated)	1.22 (0.48-3.10)	0.671		
Chemotherapy lines (second or further lines)	2.19 (1.16-4.14)	0.016	2.16 (1.09-4.27)	0.027
NLR (<2.11)	0.28 (0.13- 0.58)	<0.001	0.31 (0.14-0.69)	0.004
PLR (<192)	0.28 (0.16- 0.50)	<0.001	0.50 (0.25-1.00)	0.050
HGB (<110 g/L)	1.27 (0.72-2.25)	0.40		
Albumin (<40 g/L)	1.59 (0.82-3.05)	0.17		
SATI (<50.7 cm2/m2)	1.82 (1.03-3.22)	0.038	0.60 (0.28-1.29)	0.190
VATI (<35.7 cm2/m2)	2.61 (1.22-5.58)	0.013	3.79 (1.48-9.70)	0.005
SMI (<37.0 cm2/m2)	3.34 (1.85-6.02)	<0.001	2.52 (1.11-5.72)	0.027
BMD (<163 HU)	2.06 (1.09-3.89)	0.027	2.36 (1.22-4.54)	0.010

BMI, body mass index; FIGO, The International Federation of Gynecology and Obstetrics; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; HGB, hemoglobin; SATI, subcutaneous adipose tissue index; VATI, visceral adipose tissue index; SMI, skeletal muscle area index; BMD, bone mineral density; HR, hazard ratio; CI confidence interval.

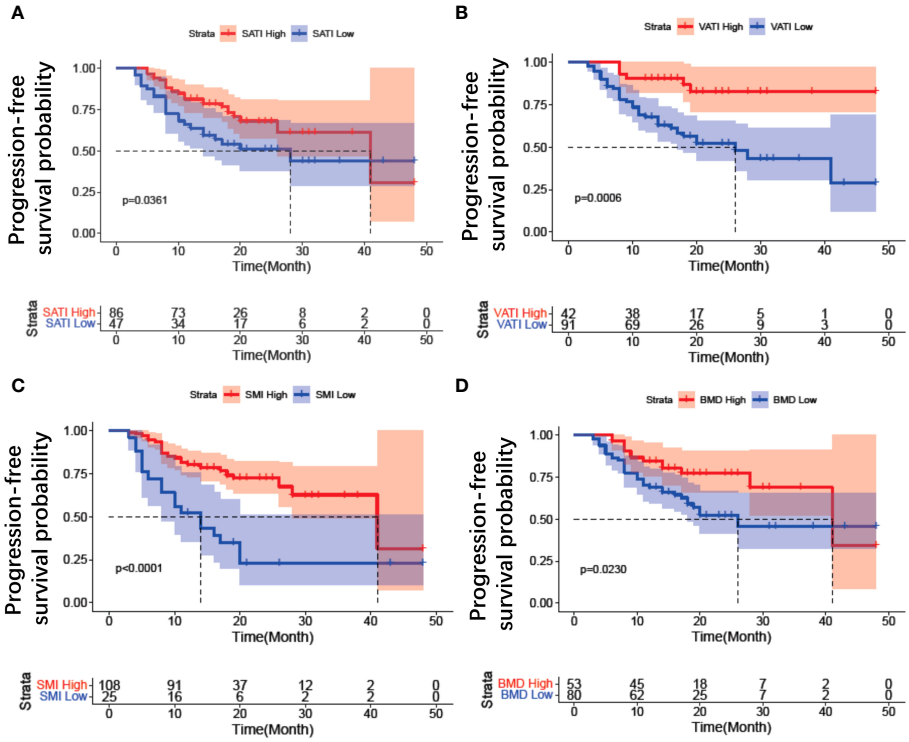

FIGURE 4
Kaplan–Meier estimates of progression free survival for body composition in patients with EOC treated with Olaparib. **(A)** SATI change; **(B)** VATI change; **(C)** SMI change; **(D)** BMD change. SATI, subcutaneous adipose tissue index; VATI, visceral adipose tissue index; SMI, skeletal muscle area index; BMD, bone mineral density.

CI = 1.09-4.27, p = 0.027), low VATI (HR = 3.79; 95% CI = 1.48-9.70, p = 0.005), low SMI (HR = 2.52; 95% CI = 1.11-5.72, p = 0.027), low BMD (HR = 2.36; 95% CI = 1.22-4.54, p = 0.010), and high NLR (HR = 0.31; 95% CI = 0.14-0.69, p = 0.004).

To remove the difference of histological subtype in the results, Kaplan-Meier curve analysis was further performed only for the population with serous adenocarcinoma. The results revealed that patients with high SATI (p = 0.0182) (Figure 5A), high VATI (p = 0.002) (Figure 5B), high SMI (p = 0.0038) (Figure 5C), low NLR (p = 0.001) (Figure 5D), and low PLR (p<0.0001) (Figure 5E) had a lower risk of disease progression. The Kaplan-Meier curves of other clinical parameters, including chemotherapy lines and BMD, could not distinguish two groups. Furthermore, we analyzed the variables between patients with first line maintenance or relapse maintenance. There were no differences in body composition and inflammation variables between these two groups (Supplementary Table 1).

Discussion

PARP enzymes are expressed in various metabolic tissues and organs, including skeletal muscle, endocrine glands, and adipose tissue (32). It is plausible that PARP plays a role in facilitating DNA repair in adipocytes, thus improving metabolic imbalances associated with obesity (33). Moreover, studies have reported that PARP inhibitors can enhance skeletal muscle function by promoting mitochondrial biogenesis and protecting against diet-induced obesity (34). Notably, Olaparib, one of the PARP inhibitors, can also influence adipocyte formation (35). PARP inhibitors are closely associated with the metabolism of tissues such as muscle and fat. Numerous studies have shown that assessing body composition through imaging techniques can predict the efficacy of drugs in cancer treatment. In this context, our study aims to elucidate the effectiveness of Olaparib in patients with EOC.

In this study, we investigated the use of Olaparib as a maintenance drug for epithelial ovarian cancer patients who had BRCA mutations or HRD positive as the first-line therapy and experienced platinum-sensitive recurrence. Advanced epithelial ovarian cancer (AEOC) is a heterogeneous disease (36) with varying responses to Olaparib. Our study is the first to demonstrate the clinical significance of body composition and serum inflammatory indexes in predicting the outcomes of patients treated with Olaparib. We found that the adipose tissue index, skeletal muscle mass index, and bone density measured by QCT were associated with the prognosis of EOC patients treated with Olaparib. Univariate and multivariable logistic regression analyses revealed that decreased VATI, SMI, and BMD were independent predictors of poor progression-free survival.

Accumulating evidence suggests that visceral adipose tissue not only functions as an energy storage organ but also plays a role in tumor development (37). Several studies have demonstrated an association between adipose tissue and various types of cancers. In some cases, lower visceral adipose tissue has been linked to the development of gastrointestinal cancer and head and neck squamous cell carcinoma (38, 39), which aligns with our findings. However, higher VAT values have been associated with worse outcomes in metastatic colorectal cancer (40). Moreover, clinically observable indicators like adipose tissue could serve as reliable markers for evaluating the efficacy of targeted drugs. For instance, in AEOC patients treated with anti-angiogenic therapy such as bevacizumab, adipose tissue levels were significantly associated with overall survival (41). Similarly, adipose tissue has been identified as a predictor of the efficacy of VEGF receptor inhibitors in ovarian cancer (22). These findings support the hypothesis that adipose tissue could be a potential predictor of clinical drug outcomes.

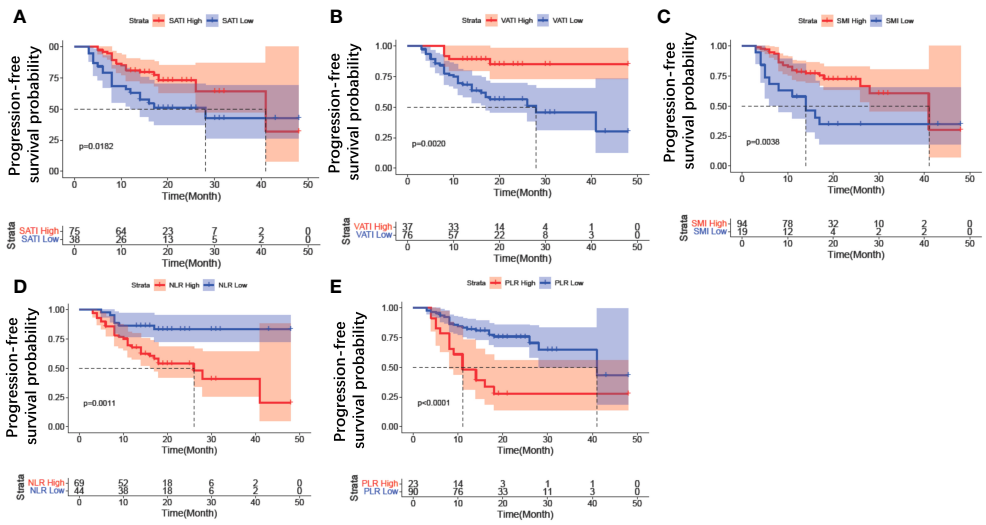

FIGURE 5
Kaplan–Meier curve analysis of clinical parameters for patients with serous adenocarcinoma. **(A)** SATI change; **(B)** VATI change; **(C)** SMI change; **(D)** NLR change; **(E)** PLR change. SATI, subcutaneous adipose tissue index; VATI, visceral adipose tissue index; SMI, skeletal muscle area index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Muscle mass and bone density are also reliable indicators of functional status and biomarkers of treatment outcomes (42). Lower skeletal muscle index has been shown to predict reduced overall survival in AEOC patients undergoing primary debulking surgery and in melanoma patients treated with immune checkpoint inhibitors (43). Additionally, a lower skeletal muscle index, as determined by CT scans, has been identified as a predictor of poor overall survival prognosis in small-cell lung cancer (44) and as a marker for shorter time to tumor progression in metastatic breast cancer (45). BMD, assessed before treatment, is independent prognostic factors for OS in patients with advanced cholangiocellular adenocarcinoma (46). The loss of BMD has been linked to shorter overall survival in AEOC patients undergoing primary debulking surgery and adjuvant chemotherapy, corroborating our study findings (20).

Additionally, the relationship between cancer-related inflammation response and alterations in muscle wastage and visceral adipose tissue is increasingly recognized. Inflammation markers, notably the NLR, have emerged as potential prognostic indicators for sarcopenia. The integration of NLR with other markers might enhance prognostic precision (47). Inflammation is now acknowledged as a pivotal factor in the development of various cancers and is recognized as a hallmark of cancer (48). For patients undergoing chemotherapy, normalization of elevated NLR levels early in treatment may correlate with improved outcomes (49, 50). A NLR exceeding the defined threshold has been linked with a higher hazard ratio for survival outcomes in colorectal carcinoma, gastroesophageal carcinoma, non–small cell lung cancer, and renal cell carcinoma (51). Moreover, a heightened NLR value correlates with an immunosuppressive profile (52) and portends a poorer overall survival rate in ovarian cancer patients. It is important to underscore that the malfunctioning of immune cells, particularly macrophages residing in adipose tissue, leading to chronic inflammation, has been intricately linked to the progression of cancer. Elevated baseline NLR has also been associated with poor survival in patients treated with immunotherapy, including those with cancer cachexia (53, 54). From this, one might deduce that high NLR concentrations can influence both muscle atrophy and visceral adipose tissue dynamics. The inhibition of PARP has demonstrated efficacy in moderating the inflammatory response, subsequently enhancing survival in sepsis scenarios (55). To a certain degree, Olaparib might mitigate inflammation, thus augmenting survival, although such a postulation warrants further empirical and foundational research validation. In our research, we discerned an association between NLR-a systemic inflammation-based prognostic marker-and the efficacy of Olaparib in EOC patients. Elevated NLR was pinpointed as an independent prognostic determinant of adverse PFS during Olaparib administration. Analogous observations have been noted in ovarian cancer studies, where inflammation markers such as elevated NLR and PLR correlate with advanced tumor staging, metastasis, and platinum resistance (25). Similarly, the elevated PLR is expected to have poor prognosis in non-small cell lung cancer (56) and hepatocellular cancer (57).

Limitations and future directions

Our study faces limitations. Firstly, its retrospective nature impedes acquiring dynamic CT evaluation and inflammatory index data, hindering understanding of temporal changes in body composition, and inflammatory markers during Olaparib maintenance therapy. Secondly, exclusively including Asian individuals limits generalizability due to potential genetic variations. Thirdly, small sample size, single-center design, and potential selection bias raise concerns about broader applicability. These underscore the need for cautious interpretation and emphasize future prospective, multi-center studies with diverse populations.

To validate findings and explore mechanisms, several future research directions are warranted. Firstly, prospective studies or trials with larger, diverse populations are essential to verify prognostic significance of body composition and inflammation variables in EOC patients treated with Olaparib. Incorporating longitudinal assessments to track changes in these markers and their correlation with treatment response is crucial.

Secondly, mechanistic studies are needed to elucidate biological pathways influencing treatment outcomes. Exploring the role of immune cells, particularly adipose tissue-resident macrophages, in modulating tumor microenvironment and response to PARP inhibition could offer insights into novel treatment strategies.

Conclusions

In conclusion, the early identification of patients displaying diminished VATI, SMI, and BMD, coupled with elevated NLR, provides preliminary evidence suggestive of an increased risk in disease progression and offers insights for guiding therapeutic interventions. These observations may hold significant clinical implications, particularly in tailoring personalized treatment approaches for EOC patients undergoing Olaparib maintenance. Our study serves as a preliminary step, highlighting the need for continued exploration and comprehensive investigations in this intricate clinical context.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Hunan Cancer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed

consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XG: Writing – original draft, Writing – review & editing, Project administration. JT: Data curation, Formal analysis, Writing – review & editing. HH: Data curation, Methodology, Writing – review & editing. LJ: Investigation, Resources, Software, Writing – review & editing. OQ: Investigation, Resources, Writing – review & editing. YX: Supervision, Validation, Writing – review & editing, Data curation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1359635/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

2. Xia C, Dong X, Li H, Cao M, Sun D, He S, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. *Chin Med J*. (2022) 135:584–90. doi: 10.1097/cm9.0000000000002108

3. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. *CA: Cancer J Clin*. (2016) 66:115–32. doi: 10.3322/caac.21338

4. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. *CA: Cancer J Clin*. (2018) 68:284–96. doi: 10.3322/caac.21456

5. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. *Int J Gynaecol Obstet*. (2021) 155 Suppl 1:61–85. doi: 10.1002/ijgo.13878

6. du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). *Cancer*. (2009) 115:1234–44. doi: 10.1002/cncr.24149

7. Louie KG, Ozols RF, Myers CE, Ostchega Y, Jenkins J, Howser D, et al. Long-term results of a cisplatin-containing combination chemotherapy regimen for the treatment of advanced ovarian carcinoma. *J Clin Oncol*. (1986) 4:1579–85. doi: 10.1200/jco.1986.4.11.1579

8. Banerjee S, Kaye SB, Ashworth A. Making the best of PARP inhibitors in ovarian cancer. *Nat Rev Clin Oncol*. (2010) 7:508–19. doi: 10.1038/nrclinonc.2010.116

9. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in patients with newly diagnosed advanced ovarian cancer. *New Engl J Med*. (2018) 379:2495–505. doi: 10.1056/NEJMoa1810858

10. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *New Engl J Med*. (2019) 381:2416–28. doi: 10.1056/NEJMoa1911361

11. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *New Engl J Med*. (2012) 366:1382–92. doi: 10.1056/NEJMoa1105535

12. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol*. (2014) 15:852–61. doi: 10.1016/s1470-2045(14)70228-1

13. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol*. (2017) 18:1274–84. doi: 10.1016/s1470-2045(17)30469-2

14. Lis CG, Gupta D, Lammersfeld CA, Markman M, Vashi PG. Role of nutritional status in predicting quality of life outcomes in cancer–a systematic review of the epidemiological literature. *Nutr J*. (2012) 11:27. doi: 10.1186/1475-2891-11-27

15. Zeng Q, Wang L, Dong S, Zha X, Ran L, Li Y, et al. CT-derived abdominal adiposity: Distributions and better predictive ability than BMI in a nationwide study of 59,429 adults in China. *Metabolism: Clin Exp*. (2021) 115:154456. doi: 10.1016/j.metabol.2020.154456

16. Parekh N, Chandran U, Bandera EV. Obesity in cancer survival. *Annu Rev Nutr*. (2012) 32:311–42. doi: 10.1146/annurev-nutr-071811-150713

17. Tao W, Lagergren J. Clinical management of obese patients with cancer. *Nat Rev Clin Oncol*. (2013) 10:519–33. doi: 10.1038/nrclinonc.2013.120

18. Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol*. (2008) 9:629–35. doi: 10.1016/s1470-2045(08)70153-0

19. Gao Q, Hu K, Gao J, Shang Y, Mei F, Zhao L, et al. Prevalence and prognostic value of sarcopenic obesity in patients with cancer: A systematic review and meta-analysis. *Nutr (Burbank Los Angeles County Calif)*. (2022) 101:111704. doi: 10.1016/j.nut.2022.111704

20. Wang X, Zhang C, Cao F, Wang CB, Dong JN, Wang ZH. Nomogram of combining CT-based body composition analyses and prognostic inflammation score: prediction of survival in advanced epithelial ovarian cancer patients. *Acad Radiol*. (2022) 29:1394–403. doi: 10.1016/j.acra.2021.11.011

21. Indini A, Rijavec E, Ghidini M, Tomasello G, Cattaneo M, Barbin F, et al. Impact of BMI on survival outcomes of immunotherapy in solid tumors: A systematic review. *Int J Mol Sci*. (2021) 22(5): 2628. doi: 10.3390/ijms22052628

22. Huang X, Xie C, Tang J, He W, Yang F, Tian W, et al. Adipose tissue area as a predictor for the efficacy of apatinib in platinum-resistant ovarian cancer: an exploratory imaging biomarker analysis of the AEROC trial. *BMC Med*. (2020) 18:267. doi: 10.1186/s12916-020-01733-4

23. Ose J, Schock H, Tjønneland A, Hansen L, Overvad K, Dossus L, et al. Inflammatory markers and risk of epithelial ovarian cancer by tumor subtypes: the

EPIC cohort. *Cancer epidemiology Biomarkers Prev*. (2015) 24:951–61. doi: 10.1158/1055-9965.EPI-14-1279-T

24. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol*. (2013) 88:218–30. doi: 10.1016/j.critrevonc.2013.03.010

25. Miao Y, Yan Q, Li S, Li B, Feng Y. Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio are predictive of chemotherapeutic response and prognosis in epithelial ovarian cancer patients treated with platinum-based chemotherapy. *Cancer Biomarkers section A Dis Markers*. (2016) 17:33–40. doi: 10.3233/cbm-160614

26. Wang YQ, Jin C, Zheng HM, Zhou K, Shi BB, Zhang Q, et al. A novel prognostic inflammation score predicts outcomes in patients with ovarian cancer. *Clinica chimica acta; Int J Clin Chem*. (2016) 456:163–9. doi: 10.1016/j.cca.2016.03.013

27. Duska LR, Kohn EC. The new classifications of ovarian, fallopian tube, and primary peritoneal cancer and their clinical implications. *Ann Oncol*. (2017) 28:viii8–viii12. doi: 10.1093/annonc/mdx445

28. von Haehling S, Ebner N, Anker SD. The journal of cachexia, sarcopenia and muscle in 2019. *J Cachexia Sarcopenia Muscle*. (2019) 10:715–20. doi: 10.1002/jcsm.12482

29. Ma M, Feng Z, Liu X, Jia G, Geng B, Xia Y. The saturation effect of body mass index on bone mineral density for people over 50 years old: A cross-sectional study of the US population. *Front Nutr*. (2021) 8:763677. doi: 10.3389/fnut.2021.763677

30. Zhong ME, Duan X, Ni-Jia-Ti MY, Qi H, Xu D, Cai D, et al. CT-based radiogenomic analysis dissects intratumor heterogeneity and predicts prognosis of colorectal cancer: a multi-institutional retrospective study. *J Trans Med*. (2022) 20:574. doi: 10.1186/s12967-022-03788-8

31. Pan T, Gao Y, Xu G, Zhou P, Li S, Guo J, et al. Pan-cancer analyses reveal the genetic and pharmacogenomic landscape of transient receptor potential channels. *NPJ Genomic Med*. (2022) 7:32. doi: 10.1038/s41525-022-00304-1

32. Bai P. Biology of poly(ADP-ribose) polymerases: the factotums of cell maintenance. *Mol Cell*. (2015) 58:947–58. doi: 10.1016/j.molcel.2015.01.034

33. Lee G, Kim YY, Jang H, Han JS, Nahmgoong H, Park YJ, et al. SREBP1c-PARP1 axis tunes anti-senescence activity of adipocytes and ameliorates metabolic imbalance in obesity. *Cell Metab*. (2022) 34:702–18.e5. doi: 10.1016/j.cmet.2022.03.010

34. Pirinen E, Cantó C, Jo YS, Morato L, Zhang H, Menzies KJ, et al. Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. *Cell Metab*. (2014) 19:1034–41. doi: 10.1016/j.cmet.2014.04.002

35. Nagy L, Rauch B, Balla N, Ujlaki G, Kis G, Abdul-Rahman O, et al. Olaparib induces browning of in *vitro* cultures of human primary white adipocytes. *Biochem Pharmacol*. (2019) 167:76–85. doi: 10.1016/j.bcp.2019.06.022

36. Watanabe T, Nanamiya H, Endo Y, Kojima M, Nomura S, Furukawa S, et al. Identification and clinical significance of somatic oncogenic mutations in epithelial ovarian cancer. *J Ovarian Res*. (2021) 14:129. doi: 10.1186/s13048-021-00876-z

37. Catalán V, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Silva C, Rotellar F, et al. Up-regulation of the novel proinflammatory adipokines lipocalin-2, chitinase-3 like-1 and osteopontin as well as angiogenic-related factors in visceral adipose tissue of patients with colon cancer. *J Nutr Biochem*. (2011) 22:634–41. doi: 10.1016/j.jnutbio.2010.04.015

38. Harada K, Baba Y, Ishimoto T, Kosumi K, Tokunaga R, Izumi D, et al. Low visceral fat content is associated with poor prognosis in a database of 507 upper gastrointestinal cancers. *Ann Surg Oncol*. (2015) 22:3946–53. doi: 10.1245/s10434-015-4432-4

39. Cheng W, Wang S, Zhang Z, Morgens DW, Hayes LR, Lee S, et al. CRISPR-Cas9 screens identify the RNA helicase DDX3X as a repressor of C9ORF72 (GGGGCC)n repeat-associated non-AUG translation. *Neuron*. (2019) 104:885–98.e8. doi: 10.1016/j.neuron.2019.09.003

40. Basile D, Bartoletti M, Polano M, Bortot L, Gerratana L, Di Nardo P, et al. Prognostic role of visceral fat for overall survival in metastatic colorectal cancer: A pilot study. *Clin Nutr (Edinburgh Scotland)*. (2021) 40:286–94. doi: 10.1016/j.clnu.2020.05.019

41. Slaughter KN, Thai T, Penaroza S, Benbrook DM, Thavathiru E, Ding K, et al. Measurements of adiposity as clinical biomarkers for first-line bevacizumab-based chemotherapy in epithelial ovarian cancer. *Gynecologic Oncol*. (2014) 133:11–5. doi: 10.1016/j.ygyno.2014.01.031

42. Ataseven B, Luengo TG, du Bois A, Waltering KU, Traut A, Heitz F, et al. Skeletal muscle attenuation (Sarcopenia) predicts reduced overall survival in patients with advanced epithelial ovarian cancer undergoing primary Debulking surgery. *Ann Surg Oncol*. (2018) 25:3372–9. doi: 10.1245/s10434-018-6683-3

43. Young AC, Quach HT, Song H, Davis EJ, Moslehi JJ, Ye F, et al. Impact of body composition on outcomes from anti-PD1 +/- anti-CTLA-4 treatment in melanoma. *J Immunother Cancer*. (2020) 8(2):e000821. doi: 10.1136/jitc-2020-000821

44. Kim EY, Kim YS, Park I, Ahn HK, Cho EK, Jeong YM. Prognostic significance of CT-determined Sarcopenia in patients with small-cell lung cancer. *J Thorac Oncol*. (2015) 10:1795–9. doi: 10.1097/jto.0000000000000690

45. Prado CM, Baracos VE, McCargar LJ, Reiman T, Mourtzakis M, Tonkin K, et al. Sarcopenia as a determinant of chemotherapy toxicity and time to tumor progression in metastatic breast cancer patients receiving capecitabine treatment. *Clin Cancer Res*. (2009) 15:2920–6. doi: 10.1158/1078-0432.Ccr-08-2242

46. Jördens MS, Wittig L, Loberg C, Heinrichs L, Keitel V, Schulze-Hagen M, et al. Bone mineral density is a predictor of mortality in female patients with Cholangiocellular carcinoma undergoing palliative treatment. *Biomedicines*. (2022) 10(7):1660. doi: 10.3390/biomedicines10071660

47. Feliciano EMC, Kroenke CH, Meyerhardt JA, Prado CM, Bradshaw PT, Kwan ML, et al. Association of systemic inflammation and Sarcopenia with survival in nonmetastatic colorectal cancer: results from the C SCANS study. *JAMA Oncol*. (2017) 3:e172319. doi: 10.1001/jamaoncol.2017.2319

48. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013

49. Kao SC, Pavlakis N, Harvie R, Vardy JL, Boyer MJ, van Zandwijk N, et al. High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in Malignant mesothelioma patients undergoing systemic therapy. *Clin Cancer Res*. (2010) 16:5805–13. doi: 10.1158/1078-0432.Ccr-10-2245

50. Chua W, Charles KA, Baracos VE, Clarke SJ. Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. *Br J Cancer*. (2011) 104:1288–95. doi: 10.1038/bjc.2011.100

51. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Institute*. (2014) 106:dju124. doi: 10.1093/jnci/dju124

52. Baert T, Van Camp J, Vanbrabant L, Busschaert P, Laenen A, Han S, et al. Influence of CA125, platelet count and neutrophil to lymphocyte ratio on the immune system of ovarian cancer patients. *Gynecologic Oncol*. (2018) 150:31–7. doi: 10.1016/j.ygyno.2018.05.004

53. Bai Y, Sun Q. Macrophage recruitment in obese adipose tissue. *Obes Rev*. (2015) 16:127–36. doi: 10.1111/obr.12242

54. Zhang Q, Song MM, Zhang X, Ding JS, Ruan GT, Zhang XW, et al. Association of systemic inflammation with survival in patients with cancer cachexia: results from a multicentre cohort study. *J Cachexia Sarcopenia Muscle*. (2021) 12:1466–76. doi: 10.1002/jcsm.12761

55. Wasyluk W, Zwolak A. PARP inhibitors: an innovative approach to the treatment of inflammation and metabolic disorders in sepsis. *J Inflammation Res*. (2021) 14:1827–44. doi: 10.2147/jir.S300679

56. Zhang H, Gao L, Zhang B, Zhang L, Wang C. Prognostic value of platelet to lymphocyte ratio in non-small cell lung cancer: a systematic review and meta-analysis. *Sci Rep*. (2016) 6:22618. doi: 10.1038/srep22618

57. Ma W, Zhang P, Qi J, Gu L, Zang M, Yao H, et al. Prognostic value of platelet to lymphocyte ratio in hepatocellular carcinoma: a meta-analysis. *Sci Rep*. (2016) 6:35378. doi: 10.1038/srep35378

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Enhanced amphiregulin exposure promotes modulation of the high grade serous ovarian cancer tumor immune microenvironment

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High grade serous ovarian cancer (HGSOC) is a lethal gynecologic malignancy in which chemoresistant recurrence rates remain high. Furthermore, HGSOC patients have demonstrated overall low response rates to clinically available immunotherapies. Amphiregulin (AREG), a low affinity epidermal growth factor receptor ligand is known to be significantly upregulated in HGSOC patient tumors following neoadjuvant chemotherapy exposure. While much is known about AREG's role in oncogenesis and classical immunity, it is function in tumor immunology has been comparatively understudied. Therefore, the objective of this present study was to elucidate how increased AREG exposure impacts the ovarian tumor immune microenvironment (OTIME). Using NanoString IO 360 and protein analysis, it was revealed that treatment with recombinant AREG led to prominent upregulation of genes associated with ovarian pathogenesis and immune evasion (*CXCL8*, *CXCL1*, *CXCL2*) along with increased STAT3 activation in HGSOC cells. *In vitro* co-culture assays consisting of HGSOC cells and peripheral blood mononuclear cells (PBMCs) stimulated with recombinant AREG (rAREG) led to significantly enhanced tumor cell viability. Moreover, PBMCs stimulated with rAREG exhibited significantly lower levels of *IFNy and IL-2*. *In vivo* rAREG treatment promoted significant reductions in circulating levels of IL-2 and IL-5. Intratumoral analysis of rAREG treated mice revealed a significant reduction in $CD8^+$ T cells coupled with an upregulation of PD-L1. Finally, combinatorial treatment with an AREG neutralizing antibody and carboplatin led to a synergistic reduction of cell viability in HGSOC cell lines OVCAR8 and PEA2. Overall, this study demonstrates AREG's ability to modulate cytotoxic responses within the OTIME and highlights its role as a novel HGSOC immune target.

KEYWORDS

amphiregulin (AREG), high-grade serous ovarian cancer, tumor immune microenvioronment, immunosupperssion, chemoresistance

Introduction

High grade serous ovarian cancer (HGSOC) is the most lethal of all gynecologic malignancies with a 5-year survival rate just below 50%, due to the fact that patients are frequently diagnosed at an advanced stage and possess high recurrence rates 12–18 months after initially achieving remission (Luvero et al., 2019; Macchia et al., 2023). Furthermore, recurrent HGSOC tumors are heavily chemoresistant and therefore do not always respond to traditional platinum-taxane based chemotherapies that are utilized in the frontline setting. In recent years, targeted approaches such as the anti-angiogenic therapy bevacizumab and the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib have been implemented in the maintenance setting as standard of care for HGSOC patients. However, with the exception of *BRCA1/2* mutated patients who significantly benefit from olaparib treatment, these targeted therapies have not had profound effects on overall HGSOC survival rates (OMalley et al., 2023). In addition, the majority of HGSOC patients derive no significant benefit from clinically available immunotherapies, as numerous clinical trials have demonstrated low response rates to programmed cell death protein 1 (PD-1) based therapies (James et al., 2020), despite the fact that intratumoral T cells are known to be highly prognostic in ovarian cancer (Zhang et al., 2003; Hwang et al., 2012). Hence, it has been theorized that the muted response to clinically available immunotherapies can be attributed to the uniquely immunosuppressive ovarian tumor immune microenvironment (OTIME), which is composed of high levels of T regulatory cells (Tregs), adipose tissue, and cancer associated fibroblasts (CAFs) that collectively contribute to tumor immune evasion and further drive ovarian pathogenesis (James et al., 2020).

In an effort to identify novel immune targets that are more representative of the unique OTIME, we previously performed a genomic analysis in matched diagnostic biopsy and interval debulking HGSOC patient tissue, obtained both prior to and following neoadjuvant chemotherapy (NACT) exposure to characterize OTIME adaptations (James et al., 2022). This analysis revealed that the gene amphiregulin (AREG) exhibited the highest fold-upregulation of out a panel of 770 of the most commonly studied immune oncology genes. AREG is a secreted glycoprotein and low-affinity epidermal growth factor receptor (EGFR) ligand and has an established role in promoting ovarian cell proliferation, metastasis, cancer stemness, and therapy resistance in ovarian cancer (Cheng et al., 2016; Tung et al., 2017). Furthermore, in classical immunity, AREG is thought to function as a Th2 cytokine that controls inflammation and downregulates adaptive immune responses (Zaiss et al., 2013; Zaiss et al., 2015; Singh et al., 2022). However, there are limited studies evaluating AREG's role in tumor immunology. Therefore, in this current investigation we sought to begin to elucidate the impact of AREG on multiple aspects of the OTIME.

Methods

Cell culture

HGSOC cell lines PEA1/PEA2 cells were obtained from Millipore Sigma and cultured in RPMI 1640 supplemented with 2 mM Glutamine, 2 mM Sodium Pyruvate, and 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin. OVCAR8 HGSOCs were obtained from American Type Culture Collection (ATCC) and ID8 $p53^{-/-}$ cells were generously gifted by the Freiman lab at Brown University that were originally generated by the McNeish lab at the University of Glasgow. Both OVCAR8 and ID8 $p53^{-/-}$ cells were cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin. All cells were kept in a 37°C/5% CO_2 humidified chamber. Cells were treated with 200 ng/mL human recombinant AREG (rAREG; R&D Systems, 262-AR-100) or with BSA control at various timepoints (15 min- 4 h). HGSOC cells were treated with 10 μM of ruxolitinib (Selleckchem, S1378) or DMSO control (Sigma Aldrich, D54879) for 48 h.

RNA isolation and NanoString nCounter® PanCancer IO360

OVCAR8 and PEA1 cells were stimulated with 200 ng/mL of rAREG or BSA control for 2 h. RNA isolation was performed using the Trizol extraction/LiCl high salt precipitation and NanoString nCounter® PanCancer IO360 was performed as previously described in detail (James et al., 2022). A total of three biological replicates per treatment in each cell line were submitted for analysis.

NanoString nCounter® PanCancer IO360 analysis

Data was analyzed in nSolver Advanced Analysis software and ROSALIND® (https://rosalind.bio/), with a HyperScale architecture developed by ROSALIND, Inc. (San Diego, CA). The QC step generated read distribution percentages, violin plots identify heatmaps, and sample MDS plots. Normalization, fold changes and *p*-values were calculated using criteria provided by NanoString® (https://nanostring.com). Control and rAREG samples were used to construct groups, respective to each cell line. ROSALIND® follows the nCounter® Advanced Analysis protocol of dividing counts within a lane by the geometric mean of the normalizer probes from the same lane. Housekeeping probes to be used for normalization are selected based on the geNorm algorithm as implemented in the NormqPCR R library (Perkins et al., 2012). Fold changes and *p*-values are calculated using the fast method as described in the nCounter® Advanced Analysis 2.0 User Manual Document Library (nanostring.com). The Benjamini-Hochberg method of estimating false discovery rates (FDR) was used to adjust *p*-values. The clustering of genes for the final heatmap of differentially expressed genes was performed using the Partitioning Around Medoids (PAM) method using the fpc R library (Hening, 2024) that takes into the account the direction and type of all signals on the pathway, the position, role and type of every gene, etc. Hypergeometric distribution was employed to analyze the enrichment of pathways, gene ontology domain structure, and other ontologies. The topGO R library (Alexa and Rahnenfuher, 2019) was employed to determine local similarities and dependencies between GO terms in order to perform Elim pruning correction. Interpro (Mitchell et al., 2019), NCB (Geer

et al., 2010), MSigDB (Subramanian et al., 2005; Liberzon et al., 2011), REACTOME (Fabregat et al., 2018), and WikiPathways (Slenter et al., 2018) databases were referenced for enrichment analysis. Enrichment was calculated relative to a set of background genes relevant to this experiment. RCC files were deposited in NCBI's Gene Expression Omnibus (GEO) (Edgar et al., 2002) and are accessible through GEO series accession number GSE252495.

RNA isolation and quantitative PCR

RNA isolation and quantitative PCR was performed as previously described (James et al., 2022). Validated human primers were purchased from Bio-Rad (*CXCL1*, *DUSP5*, *IL-11*, *CXCL2*, *IL6*, *IFNy*, *IL-2*, *GZMB*). Custom primer sequences (Invitrogen) are as follows:

18s rRNA-F-CCGCGGTTCTATTTTGTTGG
18s rRNA-R-GGCGCTCCCTCTTAATCATG

Phosphoproteomics

OVCAR8 and PEA1 cells were treated with 200 ng/mL of rAREG or BSA control for 15 min, and then protein was collected in lysis buffer supplied by the Proteome Profiler Human Phospho-Kinase Array Kit (R&D Systems, ARY003C). Manufacturer's instructions were followed and membranes were developed using the Bio-Rad ChemiDoc Imaging System. ImageJ was employed to perform background subtraction and measure spot density.

Western blot

Protein was extracted from cell pellets using Cell Lysis Buffer (Cell Signaling 9803) with 1 mM of a protease inhibitor cocktail (AbCam, ab65621). Concentrations for all extracted proteins were determined by the DC Protein Assay (Bio-Rad Laboratories, 5000116). Equal amounts of proteins were boiled at 70°C with Novex Sample Reducing Agent (Life Technologies, NP009) and NuPAGE LDS sample buffer (ThermoFisher Scientific, NP0007) into a 4%–12% gradient SurPAGE™ Bis-Tris Gel (GeneScript, M00652). The gel was transferred using a semi-dry method to methanol activated PVDF membrane using the Trans-Blot Turbo RTA Transfer Kit PVDF (Bio-Rad, 1704273), Trans-Blot Turbo 5x Transfer Buffer (Bio-Rad, 10026938), and the Bio-Rad Trans-Blot Turbo Transferring System (1.3A-25V) for 10 min. Membranes were then blocked in 5% milk in phosphate-buffered saline with 0.05% Tween 20 (PBS-T) for 30 min at room temperature, and primary antibodies were incubated overnight at 4°C diluted in 5% milk in PBS-T. Secondary antibodies were then diluted in 5% milk in PBS-T for 1 h at room temperature. Membranes were washed with PBS-T in between primary and secondary incubations and following the secondary incubation. Clarity™ Western ECL substrate (Biorad, 102030779 [peroxide solution], 102030787 [luminol/enhancer solution]) was used to detect HRP-tagged secondary antibodies. The Bio-Rad ChemiDoc Imaging System was used to image all blots and GAPDH was employed as a loading control. All uncropped blots can be seen in Supplementary Material S1. Antibodies and dilutions were as follows:

STAT3 (Cell Signaling, 4904S, 1:500) or (Proteintech, 60199-1-1g, 1:500)
Phospho-STAT3 (Cell Signaling, 9145S, 1:500)
PD-L1(Proteintech, 66248-1-1g, 1:500)
GAPDH (Santa Cruz Biotechnology, 47724, 1:1,000)
ERK (Cell Signaling, 9102S, 1:500) or (Proteintech, 11257-1-AP, 1:500)
Phospho-ERK (Cell Signaling, 4376SS, 1:500)
AKT (Proteintech, 60203-2-1g, 1:500)
Phospho-AKT (Proteintech, 28731-1-AP, 1:500)
AREG (Protientech, 16036-1-AP, 1:500)
Anti-Rabbit (Cell Signaling, 7074S, 1:1,000)
Anti-Mouse (Cell Signaling, 7076S, 1:1,000)

Enzyme-linked immunosorbent assay (ELISA)

OVCAR8 and PEA1 cells were treated with 200 ng/mL of rAREG or BSA control for 2 and 4 h. Their respective media was collected and secreted levels of IL-6 were examined using a commercially available IL-6 ELISA kit (ab178013). Media was diluted 4-fold using the kit provided Sample Diluent NS solution. Manufacturer's instructions were followed with the endpoint reading at 450 nm. All samples were run in duplicate, with three biological replicates of each sample.

Cell viability assays

HGSOC and peripheral blood mononuclear cell (PBMC) co-cultures

HGSOC cells were seeded in a 96-well plate (20,000 cells/well) and allowed to grow for 24-h. PBMCs (HumanCells Biosciences, PBMC-C10M) were co-cultured with HGSOC cells in a 5:1 ratio (James et al., 2019) and stimulated with 200 ng/mL of rAREG or BSA control. After 24 h, 10 µL/well of CellTiter 96® Aqueous One Solution Cell proliferation MTS Assay (Promega, G3580), incubated for 1 h at 37°C/5% CO_2, and finally read at 492 nm to assess cell viability.

AREG neutralizing antibody and chemotherapy treatments

PEA2 and OVCAR8 cells were seeded in a 96-well plate (20,000 cells/well) and allowed to grow for 24-h. Cells were pre-treated with carboplatin (400 µM for PEA2, 300 µM for OVCAR8; Santa Cruz Biotechnology, CAS 4157.5-94-4) or DMSO control (Sigma Aldrich, D54879) for 24-h, and with 30 µM of AREG neutralizing antibody (R&D Systems, MAB262-100) or corresponding IgG control (MAB002) for 48-h prior to cell viability assessment as described above.

Animals

C57BL/6 mice were purchased from Jackson Laboratories (strain#000664) All animal protocols were approved by the Brown University Animal Care and Use Committee (#22-09-

0002) and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. This protocol was reviewed and acknowledged by the Lifespan University Institutional Animal Care and Use Committee (#505422).

In vivo treatment and tissue collection

7-week-old C57BL/6 mice were inoculated with five million ID8p53$^{-/-}$ cells intraperitoneally (IP). 28-day post tumor inoculation, mice were treated with either rAREG (400 μg/kg; R&D Systems, 989-AR-CF) or saline, daily for a maximum of 6 days until large ascites formation, at which point mice were euthanized by carbon dioxide inhalation. Tissue was harvested and immediately fixed in a 1:10 formalin solution overnight and then placed in 30%, 50%, and 70% ethanol for 30 min each. Previously fixed tumors were then submitted to the Brown University Molecular Pathology Core for standard paraffin embedding and 5 μM serial sectioning.

Mouse ascites and serum multiplex assays

Ascites was collected from mice post-mortem and then spun at 5,000 g for 10 min at 4°C. Whole blood was collected via cardiac puncture post-mortem into serum separator tubes, allowed to clot for 30 min and then spun at 3,000 g for 15 min at 4°C. Both ascites supernatant and serum was collected and stored at −80°C. Ascites and serum from mice treated with rAREG ($n = 5$) and saline ($n = 5$) were analyzed using a Mouse Cytokine/Chemokine 44-Plex Discovery Assay® Array (MD44) by Eve Technologies (Calgary, Canada), to simultaneously determine the levels of the of the following immune factors: Eotaxin, Erythropoietin, 6Ckine, Fractalkine, G-CSF, GM-CSF, IFNB1, IFNy, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-20, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MDC, MIG, MIP-1α, MIP-1β, MIP-3α, MIP-3B, RANTES, TARC, TNFα, VEGF-A. Each analyte was bound to a differently colored/fluorescent bead to allow for simultaneous detection of all of the aforementioned immune factors in a single assay. A bead analyzer (Bio-Plex 200) first activates the fluorescent dye via laser, then excites the streptavidin-phycoerythrin fluorescent conjugate with a second laser, allowing for measurement of each specific analyte. Each sample was performed in duplicate.

Fluorescent immunohistochemistry

FFPE mouse tumors were baked for 2 h at 65°C and then washed in SafeClear xylene substitute, 100% ethanol, 95% ethanol, 70% ethanol, deoxygenated water, and FTA Hemagglutination buffer for 10 min at each wash on a shaker. Antigen retrieval was then performed via Antigen retrieval solution (1X; Vector Laboratories, H-3300) and heated at 95°C for 20 min. Slides were blocked in 5% horse serum diluted in FTA Hemagglutination buffer and incubated overnight in primary antibody at 4°C. Secondary antibody was then added for 1 h in the dark at room temperature. Between each step slides were washed with FTA Hemagglutination buffer. Lastly, slides were cover-slipped with DAPI containing mounting medium (Vector Laboratories, H-1200). Primary and secondary antibodies and respective dilutions were as follows:

CD8 (Proteintech, 29896-1-AP, 1:50)
PD-L1 (Proteintech, 66248-1-1g, 1:50)
CD4 (Proteintech, 677886-1-1g, 1:50)
CD45 (Proteintech, 20103-1-AP, 1:50)
CD45 (Proteintech, 67786-1-1g, 1:50)
Anti-Rabbit DyLight™488 (Vector Laboratories, DI-1488, 1:1,000)
Anti-Mouse DyLight™594 (Vector Laboratories, DI-2594, 1:1,000)

Image analysis

For PD-L1 intensity and CD8+/CD4+ T cell counts, three and five randomly selected fields per case were selected based on DAPI staining, respectively. Images were acquired via a spinning disk confocal Nikon Eclipse Ti microscope at a ×20 objective. Image processing and analysis was performed utilizing ImageJ. For PD-L1 staining analysis, images were thresholded for specific staining and mean intensity was calculated. For CD8$^+$ and CD4$^+$ T cells, the total number of positive cells co-stained with CD45 and DAPI were counted. Representative images were taken at ×20 or ×40.

Statistical analysis

Statistical analyses were performed in GraphPad Prism. Student t-tests were performed to determine differences in control and rAREG treated cell lines and mice. All *p*-values reported with the exception of ROSALIND NanoString Analysis were 2-tailed and unadjusted.

cBioPortal

cBioPortal (Cerami et al., 2012; Gao et al., 2013) was used to analyze TCGA ovarian serous cystadenocarcinoma cohorts from the Firehose Legacy ($n = 617$) or Nature 2011 ($n = 489$) studies. AREG's association with platinum status (Nature 2011), tumor mutational burden (TMB), mutation count, and Spearman's rank correlation analysis with genes of interest (Firehose Legacy) were determined.

Kaplan-Meier plotter analysis

The Kaplan-Meier Plotter ovarian cancer analysis (https://kmplot.com/analysis/index.php?p=service&cancer=ovar) (Lánczky and Győrffy, 2021) was used to examine the association of AREG with progression-free survival (PFS) and overall survival (OS) in stage III-IV, grade 3 serous ovarian cancer using either the lower or upper quartile as a cutoff.

Tumor immune dysfunction and exclusion

Tumor Immune Dysfunction and Exclusion (TIDE) (Jiang et al., 2018; Fu et al., 2020) query gene analysis was employed to examine AREG and cytotoxic T lymphocyte levels and T-cell dysfunction score/z-score of interaction between AREG and cytotoxic lymphocytes (CTLs) in a Cox proportional hazard model. TCGA ovarian cancer cohort was used by TIDE for these analyzes. As described detail in Jiang et al. (2018); briefly, an interaction test within the multivariate Cox-PH regression was applied to identify AREG genomic levels in association with the T cell dysfunction phenotype. Then the Cox-PH survival regression was employed to test how CTL levels interact with AREG in the tumor to affect overall survival outcomes. The linear model Hazard was solved (=*a*XCTL+*b*XV+*d*XCTLxV+C) using the Cox-PH regression, where the CTL level is estimated from the bulk-tumor expression average of cytotoxicity T cell markers (*CD8A*, *CD8B*, *GZMA*, *GZMB*, *PRF1*). The death hazard within the Cox-PH model was estimated via patient survival clinical outcome, the variable V is the expression

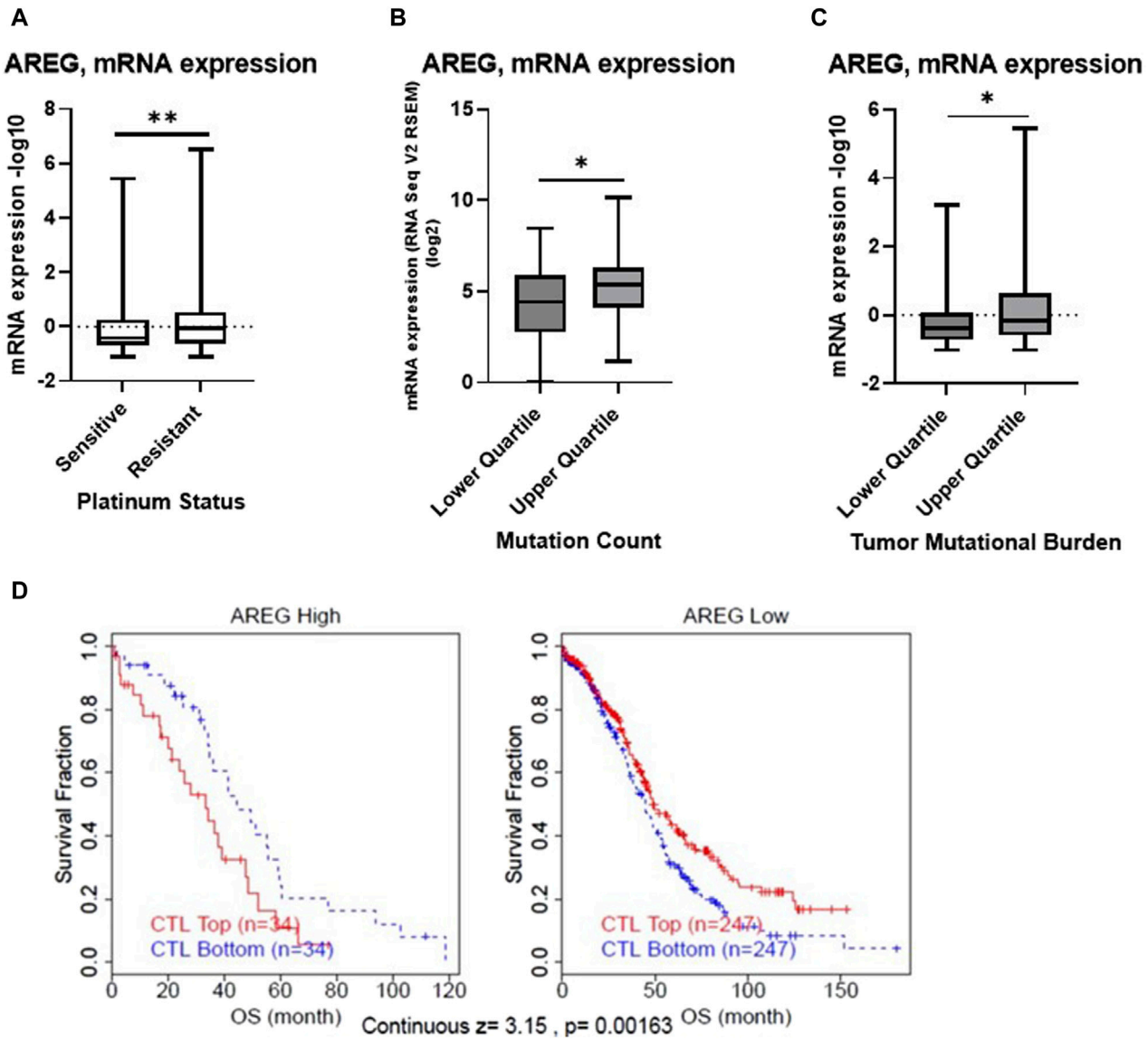


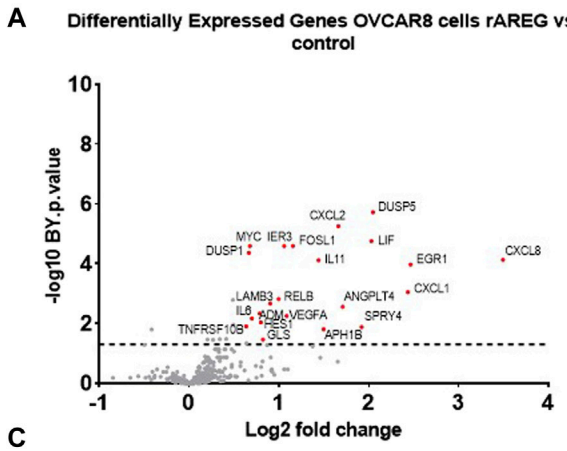
FIGURE 1
Bioinformatic analysis of AREG in ovarian cancer. TCGA Ovarian Cancer Nature 2011 cohort was employed to compare **(A)** mRNA expression of AREG in patients with platinum sensitive (n = 197) or resistant (n = 90) disease. The TCGA Ovarian Cancer Firehose Legacy was used to examine AREG mRNA expression stratified by upper and lower quartile **(B)** mutation count (n = 41, upper quartile, n = 52, lower quartile) and **(C)** tumor mutational burden (n = 79, for both upper and lower quartile). **(D)** TIDE cox proportional hazards model analysis of OS using TCGA ovarian cohort data demonstrating z-score of interaction effect between AREG and CTLs. *p < 0.05, **p < 0.005, as indicated. TCGA, The Cancer Genome Atlas; TIDE, Tumor Immune Dysfunction Exclusion; CTLs, Cytotoxic T lymphocytes; OS, overall survival.

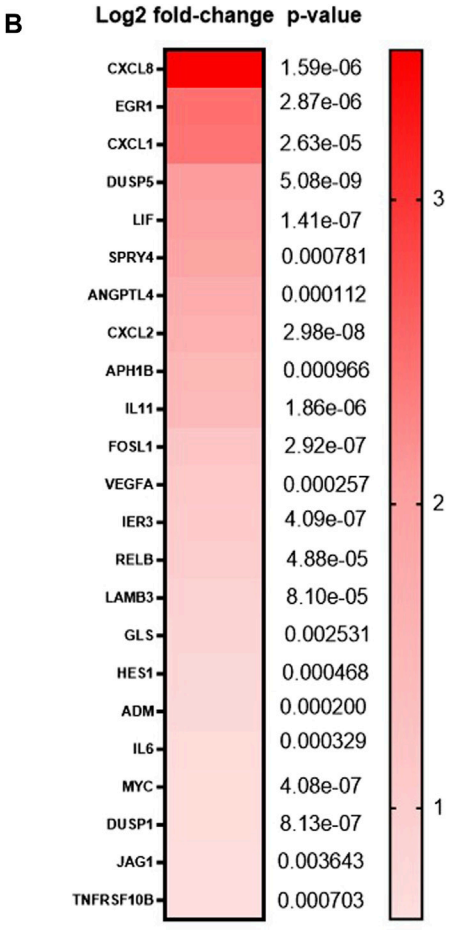
level of the candidate gene in the test (in this case *AREG*). The T cell dysfunction score listed is defined as the Wald test z score, which represents the coefficient d, dived by its standard error. The p-value listed was adjusted using the Benjamini-Hochberg method.

Results

Bioinformatic analysis of AREG in ovarian cancer

Our past study revealed that AREG was significantly upregulated in HGSOC patient tumors following NACT exposure compared to matched pre-treatment diagnostic biopsy specimens (James et al., 2022). Therefore, using publicly available datasets we first sought to uncover *AREG*'s relationship to clinical outcomes in HGSOC. TCGA ovarian cancer cohort analysis revealed that *AREG* mRNA levels were significantly (p = 0.007) upregulated in patients defined as having a chemoresistant versus sensitive platinum status (Figure 1A). As approximately 80% of patients are defined as platinum sensitive, this small population of patients defined as chemoresistant exhibits an exceptionally poor survival of 6 months or less (Luvero et al., 2019). Interestingly, Kaplan Meier curve analysis of publicly available GSE and TCGA databases found no significant association between *AREG* expression and progression-free survival (PFS) or overall survival (OS; Supplementary Figure S1).





C

Gene Set Analysis

Term	Genes	Significance Score
Myeloid Compartment	*CXCL2, IER3, LAMB3, CXCL1, LIF, FOSL1*	6.937
Wnt Signaling	*FOSL1, MYC*	4.943
MAPK	*DUSP5, VEGFA, RELB, MYC, DUSP1*	4.670
JAK-STAT Signaling	*LIF, IL11, IL6, MYC*	4.632
Notch Signaling	*JAG1, MYC, HES1, APH1B*	4.214
Cytokine and Chemokine Signaling	*CXCL8, CXCL1, CXCL2, IL11, IL6*	4.212
TGF-beta Signaling	*MYC*	3.837
Lymphoid Compartment	*EGR1, IL11*	3.294
Cytotoxicity	*LIF, SPRY4*	3.185
Metabolic Stress	*CXCL8, VEGFA, GLS, IL6, MYC*	2.783

FIGURE 2
rAREG drives tumor intrinsic immune changes that promote immune evasion and ovarian pathogenesis. **(A)** Volcano plot demonstrating differential gene expression in OVCAR8 cells stimulated with 200 ng/mL of rAREG relative to BSA control, measured by NanoString Human PanCancer IO360. **(B)** Top differentially expressed genes in OVCAR8 rAREG treated cells relative to BSA control, with Benjamini-Hochberg adjusted *p*-values listed. **(C)** Gene set analysis with top pathway changes and significance scores listed in rAREG treated AREG cells relative to BSA control.

Further bioinformatic analysis revealed that despite being associated with chemoresistant disease, *AREG* mRNA levels were significantly ($p < 0.05$) higher in patients with a higher mutation count and tumor mutational burden (TMB), when stratified by quartile (Figures 1B, C). Moreover, Tumor Immune Dysfunction and Exclusion (TIDE) analysis revealed that higher levels of *AREG* were significantly (continuous z-score, 3.15, $p = 0.00136$) associated with a cytotoxic lymphocyte (CTL) dysfunction phenotype (Figure 1D). Overall, these results demonstrate that despite higher *AREG* levels detected in patient tumors with a higher TMB count, *AREG* was also associated with chemoresistant disease and T cell dysfunction.

AREG exposure leads to tumor intrinsic immune changes that drive ovarian pathogenesis and immune evasion

Next, in order to recapitulate the high levels of AREG that are seen in post-NACT treated HGSOC tumors, we stimulated the HGSOC cell lines OVCAR8 and PEA1 with 200 ng/mL recombinant AREG (rAREG) and respective controls for 2 h. Extracted RNA was subjected to NanoString IO 360 analysis with the goal of broadly capturing tumor intrinsic changes resulting from increased AREG exposure. Unexpectedly, we found no significant differences in PEA1 treated cells. However, in OVCAR8 cells, several genes were significantly upregulated relative to control, including *CXCL8* (3.49-fold, $p = 1.59e-06$), *EGR1* (2.47-fold, $p = 2.87e-06$), *CXCL1* (2.43-fold, $p = 2.63e-05$), *DUSP5* (2.05-fold, $p = 5.08e-09$), *LIF* (2.03-fold, $p = 1.41e-07$), *CXCL2* (1.66-fold, $p = 2.98e-08$), and IL-11 (1.44-fold, $p = 1.86e-06$; Figure 2A, B). Furthermore, gene set analysis revealed prominent changes in Wnt, MAPK, Notch, TGF-beta, JAK-STAT, and cytokine and chemokine signaling, as well as changes in cytotoxicity, metabolic stress and myeloid and lymphoid compartment (Figure 2C), showcasing that increased AREG leads to significant tumor intrinsic immune changes that can contribute to cell proliferation, migration, and angiogenesis, while simultaneously promoting tumor immune suppression.

Following our NanoString analysis, we re-treated PEA1 cells with 200 ng/mL of rAREG and collected RNA at an earlier 1h

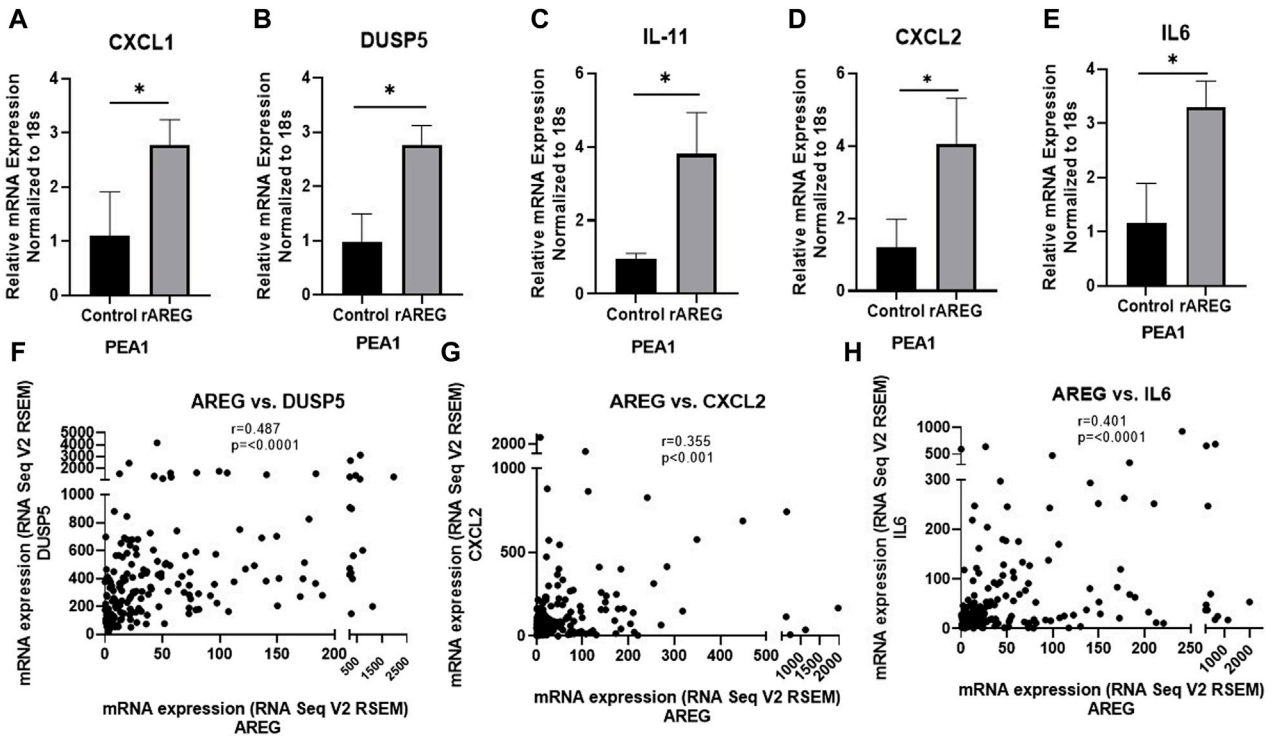


FIGURE 3
qPCR analysis of rAREG stimulated PEA1 cells. **(A)** *CXCL1*, **(B)** *DUSP5*, **(C)** *IL-11*, **(D)** *CXCL2*, and **(E)** *IL-6* mRNA levels in PEA1 cells stimulated with 200 ng/mL rAREG for 1 h and analyzed via qPCR. Spearman Rank Correlation analysis of mRNA expression (RNA Seq V2 RSEM) of *AREG* with **(F)** *DUSP5*, **(G)** *CXCL2*, and **(H)** *IL-6* using TCGA-OV Firehose Legacy cohort (n = 307). Error bars represent standard deviation of ≥ 3 biological replicates. *$p < 0.05$ as indicated. TCGA, The Cancer Genome Atlas.

timepoint. We performed quantitative PCR (qPCR) with the goal of examining levels of differentially expressed genes (DEGs) identified in OVCAR8 cells. We found that mRNA levels of *CXCL1* (2.53-fold, $p = 0.036$), *DUSP5* (2.82-fold, $p = 0.008$), *IL-11* (4.01-fold, $p = 0.012$), *CXCL2* (3.36-fold, $p = 0.029$), and *IL6* (2.84-fold, $p = 0.013$), were all significantly increased following 1h rAREG exposure (Figures 3A–E), with no significant changes at 2 h stimulation (Supplementary Figure S2), confirming what we previously observed in our NanoString analysis. The discrepancies in OVCAR8 and PEA1 could potentially be explained by the fact that it is known that OVCAR8 cells harbor *Erbb2* and *KRAS* mutations (Mei et al., 2021), which could lead to differential rAREG effects. Finally, we observed that the DEGs *DUSP5* (r = 0.487, $p < 0.0001$), *CXCL2* (r = 0.355, $p < 0.0001$), and *IL-6* (r = 0.401, $p < 0.0001$) were amongst some of the top correlative genes with *AREG* in the TCGA ovarian cancer cohort (Figures 3F–H), adding a further degree of clinical relevance to our NanoString analysis.

AREG promotes upregulation of downstream EGFR cell growth pathways

As the EGFR pathway is upstream of numerous cancer cell growth pathways (Wee and Wang, 2017), we employed a commercially available proteome profiler array to unbiasedly uncover notable signaling changes in HGSOC cells following rAREG exposure. Interestingly, we found that STAT3 expression was upregulated 2.93-fold in OVCAR8 and 1.63-fold in PEA1 cells after only 15 min of exposure (Figure 4A). Western blot analysis was employed to validate findings and compare phospho-STAT3 (p-STAT3) levels at multiple timepoints, which revealed the highest upregulation of p-STAT3 at 1 h and 4 h in OVCAR8 and PEA1 cells, respectively (Figure 4B). Moreover, we found that programmed-death ligand 1 (PD-L1), a major immune target downstream of the STAT3 pathway (Zerdes et al., 2019; Zou et al., 2020), was also increased strikingly starting at 30 min following rAREG treatment in both OVCAR8 and PEA1 cells (Figure 4B).

To further examine AREG's influence on the STAT3 pathway, we evaluated secreted levels of the pro-inflammatory and major STAT3-associated cytokine, IL-6, in media from rAREG stimulated HGSOC cells. At both 2 h and 4 h time points following rAREG exposure, IL-6 levels in conditioned media were 21.1-fold and 49.6-fold higher in OVCAR8 cells, respectively and 12.2-fold higher at both 2 h and 4 h post-rAREG exposure timepoints in PEA1 cells (IL-6 levels reached or exceeded the upper limit of detection at 500 pg/mL; Figures 4C, D). As IL-6 possesses the unique ability to induce STAT3 target genes, which in turn produce multifaceted downstream effects that drive tumor cell growth, angiogenesis, invasion, metastasis, and immunosuppression (Wang and Sun, 2014; Čokić et al., 2015; Johnson et al., 2018), our results highlight AREG's indirect pro-tumorigenic effects through IL-6 stimulation. In addition, we treated OVCAR8 and PEA1 cells

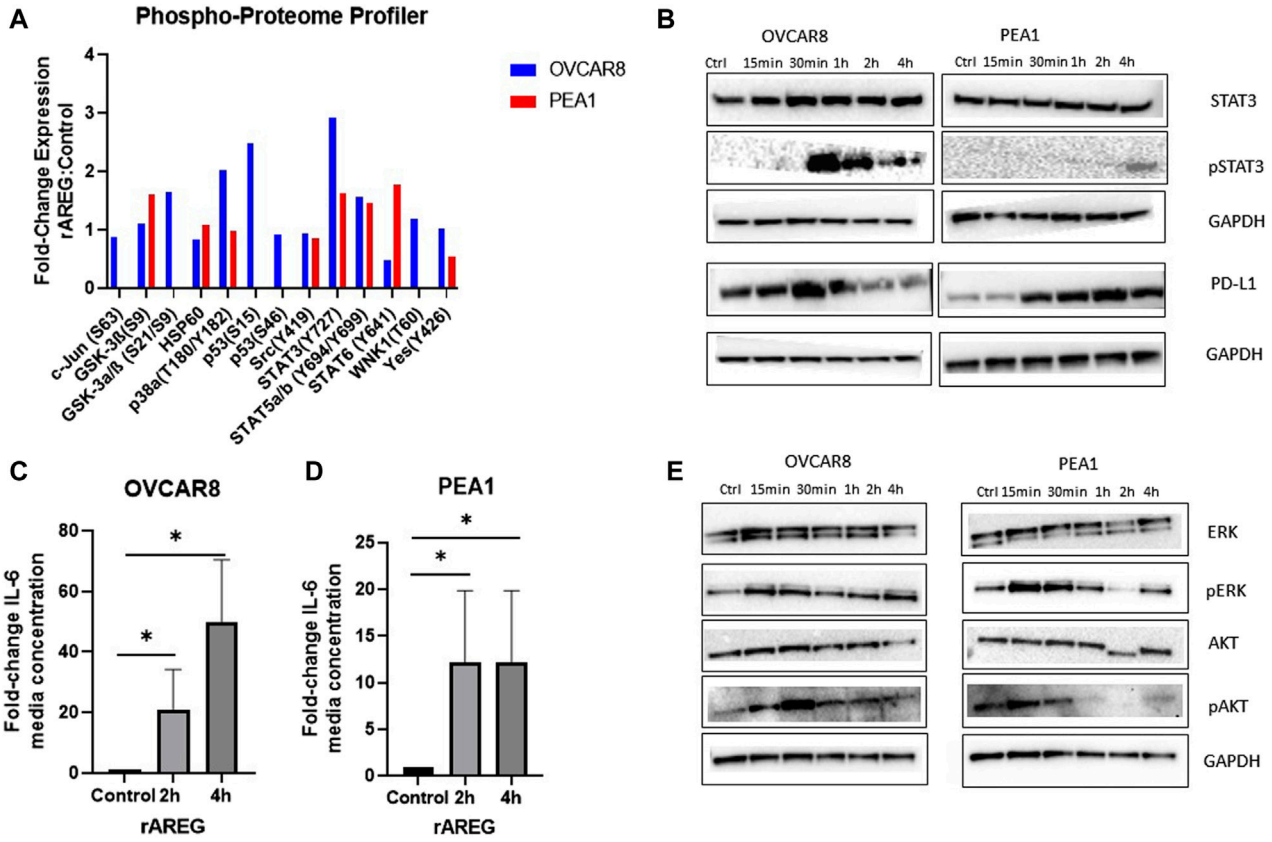


FIGURE 4
AREG exposure leads to prominent upregulation of STAT3 signaling in HGSOC cells. **(A)** Phospho-proteome profiler analysis of fold-change expression of phosphor-proteins in rAREG (200 ng/mL) treated OVCAR8 and PEA1 cells relative to BSA control. **(B)** Western blot analysis of rAREG (200 ng/mL) exposed OVCAR8WT and PEA1 cells of STAT3, pSTAT3, PD-L1, with respective GADPH loading controls at various indicated timepoints. ELISA levels of IL-6 in conditioned media of **(C)** OVCAR8 and **(D)** PEA1 cells following 200 ng/mL of rAREG exposure at 2 and 4 h or BSA control. **(E)** Western blot analysis of downstream EGFR cell growth pathways p-ERK/ERK and pAKT/AKT with respective GAPDH loading controls in OVCAR8 and PEA1 cells following rAREG treatment at various indicated timepoints.*$p < 0.05$, as indicated. ELISA, enzyme-linked immunosorbent assay.

with ruxolitinib, a small molecule JAK/STAT3 inhibitor (Han et al., 2018), which resulted in unaltered AREG levels (Supplementary Figure S3), suggesting that STAT3 does not have a bidirectional influence on AREG in HGSOC.

Finally, Western blot analysis revealed that rAREG exposure led to activation of additional tumor cell growth pathways downstream of EGFR, illustrated by increased p-ERK and p-AKT levels starting at 15 min of exposure in both OVCAR8 and PEA1 cells (Figure 4E). Taken together, these results showcase that AREG greatly contributes to the activation of numerous cell growth pathways in HGSOC, with predominant effects on STAT3 and its associated targets.

AREG reduces cytotoxic immune response *in vitro*

As we previously found that AREG leads to tumor intrinsic immune changes that drive ovarian pathogenesis and promote immune evasion, we sought to evaluate if increased AREG exposure affects cytotoxic immune responses. To investigate this phenomenon, we co-cultured OVCAR8 and PEA1 cells with peripheral blood mononuclear cells (PBMCs) that were stimulated with or without rAREG for 24-h. We observed significantly (p = 0.001) reduced viability of 38.8% in OVCAR8 cells stimulated with PBMCs + BSA control compared to a 24.2% reduction in viability in cells stimulated with PBMCs + rAREG (Figure 5A). Similarly, PEA1 cells co-cultured with PBMCs + BSA demonstrated a 22.6% reduction in viability, compared to 14.4% with PBMCs + rAREG (p = 0.007; Figure 5B). Furthermore, we stimulated PBMCs alone with rAREG and performed qPCR analysis, which revealed a significant ($p < 0.05$) decrease in both *IL-2* and *IFNγ* (Figures 5C, D), in addition to a trend toward reduced *GZMB* levels (Supplementary Figure S4). Collectively, these studies show that increased AREG dampens PBMCs' ability to promote tumor cell death potentially through the reduction of cytokines crucially responsible for carrying out cytotoxic immune responses.

In vivo AREG exposure predominantly drives immunosuppresive adaptations within the OTIME

In order to characterize the effect of AREG on the ovarian tumor immune microenvironment, we carried out an immunocompetent

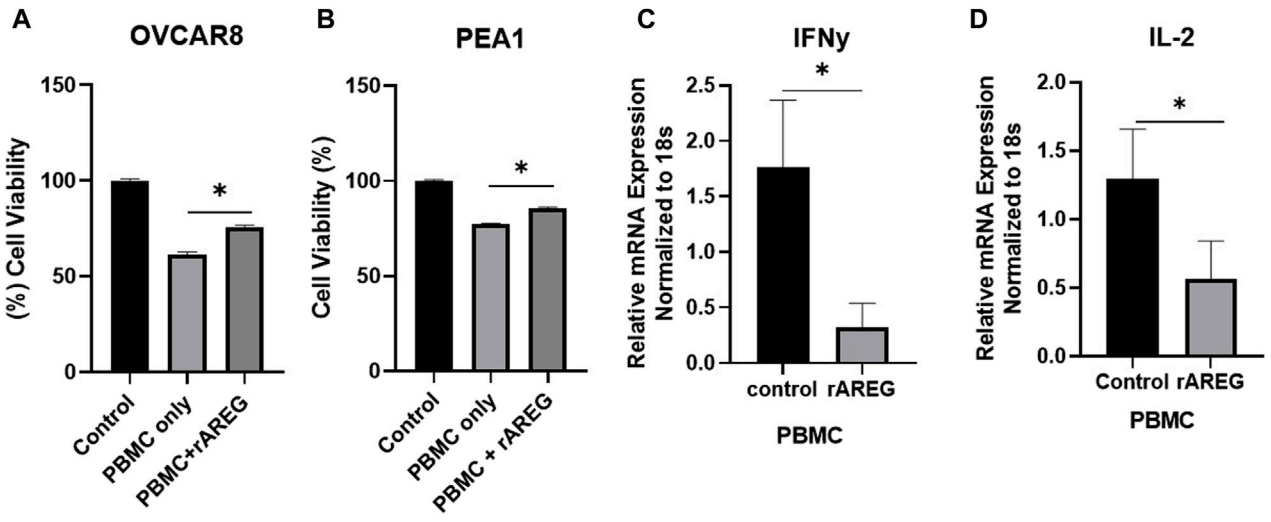


FIGURE 5
AREG compromises PBMC cytotoxicity. Cell viability analysis **(A)** OVCAR8 and **(B)** PEA1 cells following 24-h co-cultured with PBMCs+ BSA control or PBMCs+ 200 ng/mL of rAREG. qPCR analysis of **(C)** *IFNy* and **(D)** IL-2 in PBMCs treated with BSA control, or rAREG for 2 h. Error bars represent standard deviation of ≥ 3 biological replicates. *$p < 0.05$, as indicated, PBMCs, peripheral blood mononuclear cells.

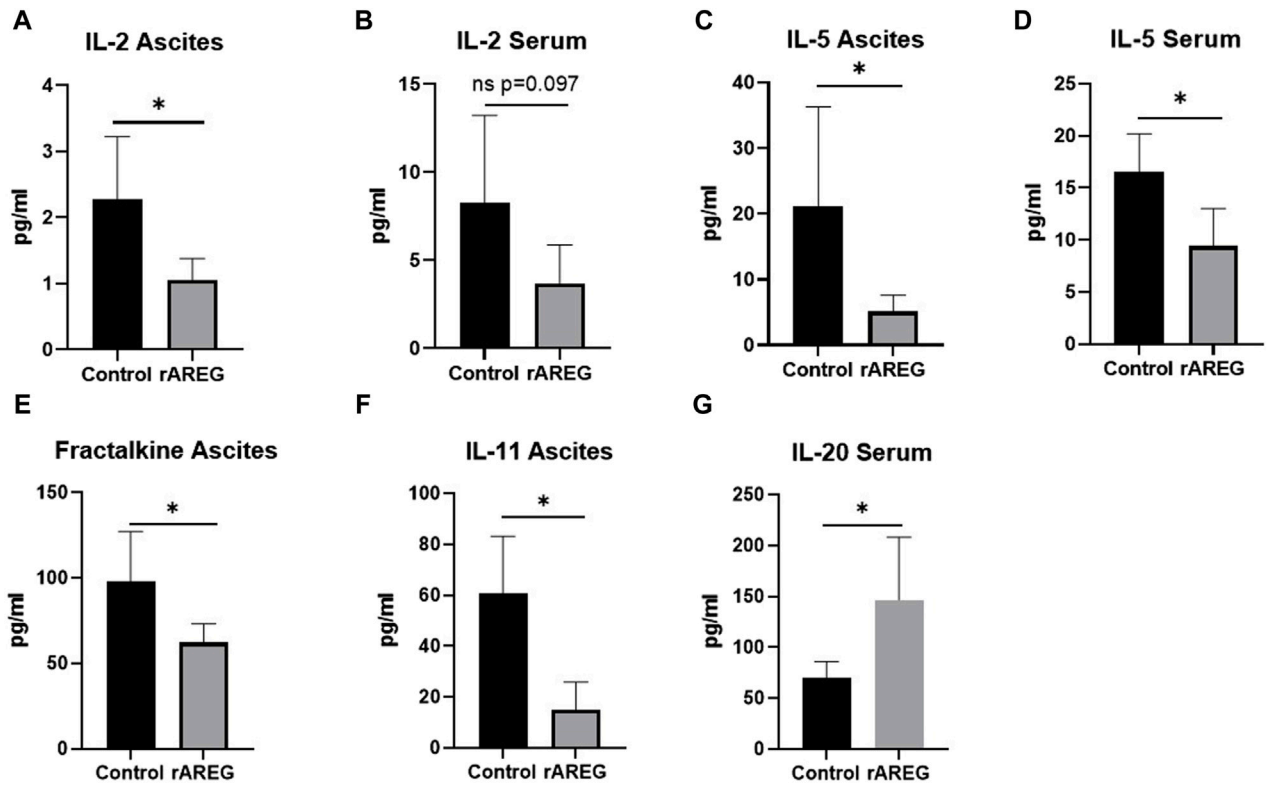


FIGURE 6
Multiplex cytokine and chemokine analysis of C57BL/6 ID8p53$^{-/-}$ mouse ascites and serum following *in vivo* rAREG exposure. Concentrations of IL-2 in **(A)** serum and **(B)** ascites of mice treated with saline control ($n = 5$) or 400 ug/kg of rAREG ($n = 5$). IL-5 levels in the ascites **(C,D)** serum in mice exposed to saline control or rAREG. **(E)** Fractalkine ascites, **(F)** IL-11 ascites, and **(G)** IL-20 serum levels in saline and rAREG treated mice. *$p < 0.05$ as indicated.

in vivo study using an ID8p53$^{-/-}$ C57BL/6 model in which mice were treated with 400 μg/kg of rAREG or a saline control. Ascites and serum obtained post-euthanasia were submitted for multiplex cytokine and chemokine analysis which revealed a significant ($p = 0.026$) reduction of IL-2 levels in ascites of mice treated with rAREG compared to saline control mice (Figure 6A). A

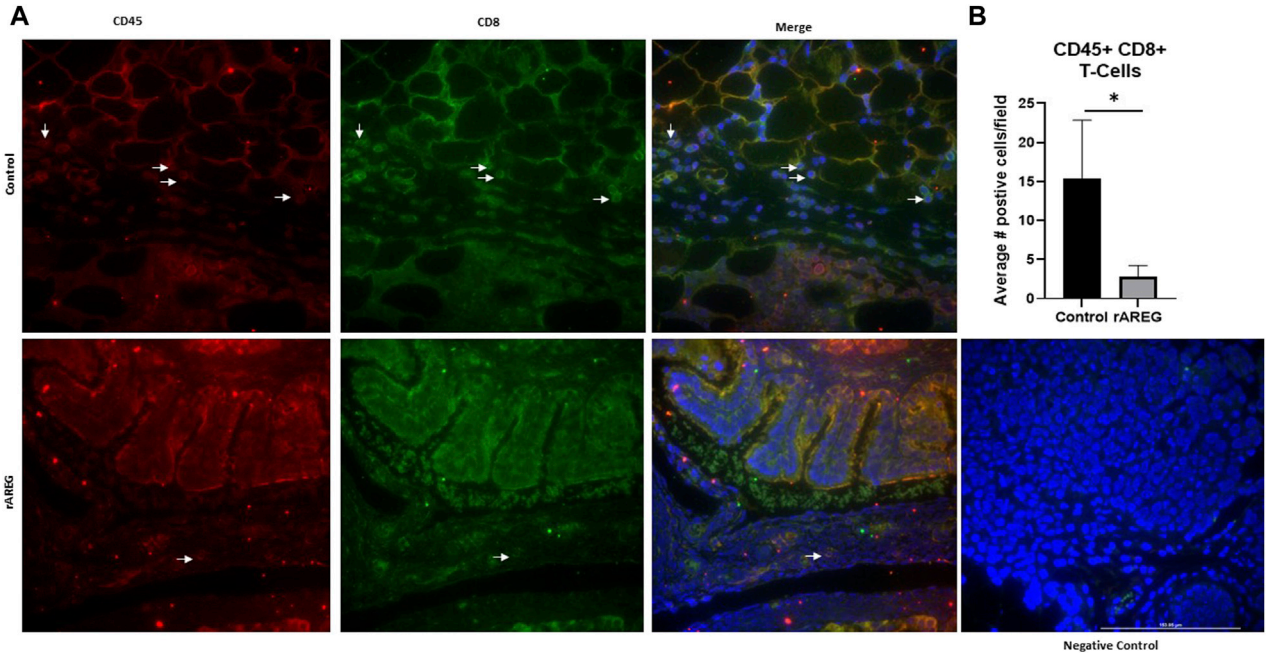


FIGURE 7
AREG exposure *in vivo* leads to a reduction in intratumoral cytotoxic CD8$^+$ T cells. **(A)** Representative images and associated **(B)** Fluorescent Immunohistochemistry analysis of intratumoral CD8$^+$ T cells levels denoted by the number of positive cells per field in saline control (n = 3) and 400 μg/kg rAREG exposed (n = 4) mice.*p < 0.05, as indicated.

similar reduction in IL-2 levels was seen in rAREG treated mouse serum, however this did not reach significance (p = 0.097) (Figure 6B). This result corroborates our *in vitro* findings that rAREG exposure leads to reduced *IL-2* mRNA levels in PBMCs. Interestingly, we also observed that mice treated with rAREG had significantly (p < 0.05) reduced ascites and serum levels of IL-5, a pro-inflammatory cytokine that is primarily responsible for eosinophil production (Han et al., 2011) (Figures 6C, D). Furthermore, a significant (p = 0.034) reduction of Fractalkine, also known as CX3CL1 was observed in rAREG treated mouse ascites, which has been found to be a key mediator in of cytotoxic T cell immunity and associated with improved prognosis in numerous cancer subtypes (Conroy and Lysaght, 2020) (Figure 6E). In addition, a significant (p = 0.004) reduction in IL-11, an IL-6 associated cytokine (Zhao et al., 2018) was also was also observed (Figure 6F), which we previously observed to be upregulated in a tumor-intrinsic setting (Figures 2A, 3C). Finally, there was a significant (p = 0.028) increase in IL-20 (Figure 6G), a potent inflammatory cytokine that is classically associated with psoriasis and rheumatoid arthritis but has also been shown to promote tumorigenesis through promoting cellular proliferation and migration (Hsu et al., 2012a; Hsu et al., 2012b; Chiu et al., 2017; Lu et al., 2020). A complete list of all changes in cytokines and chemokines profiled can be seen in Supplementary Tables S1, S2.

In addition to evaluating circulating changes within the OTIME, we further observed a significant (p = 0.0212) reduction in the average number of intratumoral CD8$^+$ T cells, with an average of five positive CD8$^+$ T cells per field in saline tumors compared to one positive CD8$^+$ T cell per field in mice exposed to rAREG (Figures 7A, B). Conversely, we observed no significant changes in CD4$^+$ T cell populations (Figure 8). Finally, these tumors were also stained for PD-L1, which revealed significantly (p = 0.009) higher mean intensity levels of PD-L1 in tumors treated with rAREG compared to saline control (Figures 9A, B), recapitulating our results in HGSOC cell lines.

Combinatorial AREG inhibition and carboplatin promotes synergistic HGSOC cell death

Finally, we have targeted AREG *in vitro* using an AREG neutralizing antibody (nab) in combination with carboplatin. HGSOC cell lines OVCAR8 and PEA2 (the chemoresistant counterpart to PEA1), were employed for this experiment. Both cell lines were pre-treated with carboplatin for 24 h and then treated with either an IgG control or AREG nab for 48 h. In both cell lines, it was observed that co-treatment with carboplatin and an AREG nab led to a significant (p < 0.005) reduction in cell viability compared to either carboplatin or AREG nab treatment alone, with the most striking reduction in chemoresistant PEA2 cells where combinatorial treatment produced a 73% reduction in viability compared to DMSO control (Figures 10A, B). While these cell viability assays were performed in an immune devoid context, it will be pertinent to validate these findings using an immunocompetent *in vivo* model.

Discussion

The goal of this study was to characterize how increased AREG levels that are detected clinically in post-frontline chemotherapy

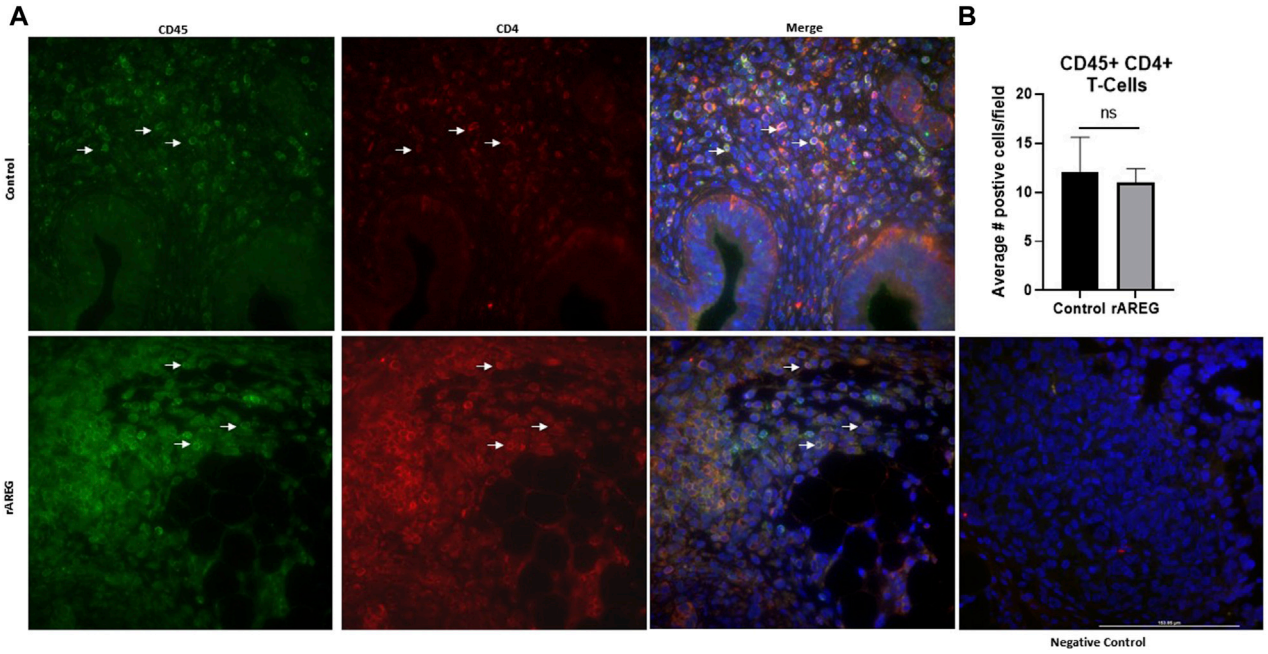


FIGURE 8
Intratumoral analysis of CD4⁺ T cells following *in vivo* rAREG exposure. **(A)** Representative images and associated **(B)** Fluorescent Immunohistochemistry analysis of intratumoral CD4⁺ T cells (number of positive cells per field) in saline control (n = 3) and 400 μg/kg rAREG exposed (n = 4) mice. ns, non-significant.

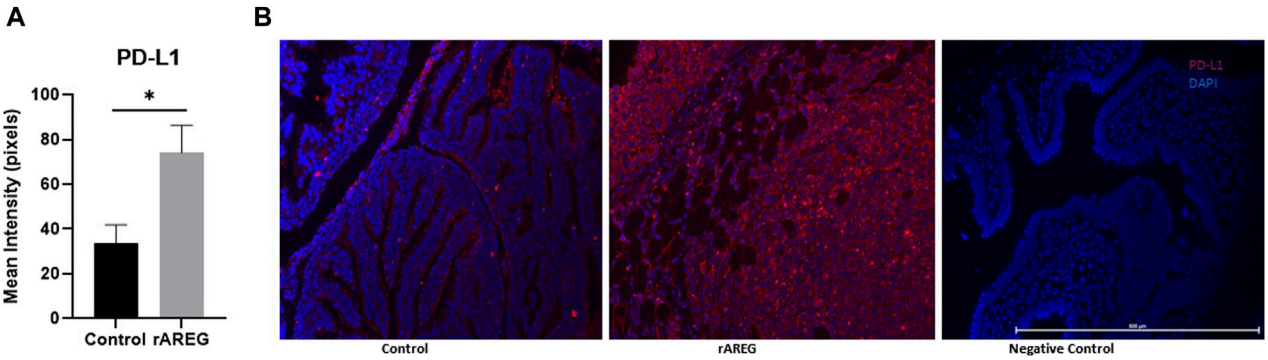


FIGURE 9
AREG exposure leads to an upregulation of intratumoral PD-L1 expression. Fluorescent Immunohistochemistry analysis of **(A)** of PD-L1 in saline control (n = 3) and 400 μg/kg rAREG exposed (n = 3) mice, demonstrated by mean intensity (pixels) with **(B)** representative images. *$p < 0.05$, as indicated.

exposed HGSOC patient tumors impacts the OTIME. This investigation revealed that enhanced AREG exposure produced multifaceted effects within the OTIME that collectively drive tumor immune evasion. While it has been previously reported that AREG is overexpressed in ovarian cancer (Lindzen et al., 2021) and is associated with advanced stage disease (Tung et al., 2017), using bioinformatic analyses we failed to observe an association between *AREG* expression and patient survival. Previously performed *in vitro* studies led by Tung et al. described AREG's role in ovarian cancer chemoresistance through the promotion of cancer stemness and drug resistance mediated by the EGFR/ERK pathway (Tung et al., 2017). Similarly, the role of AREG in chemoresistance has been described in other cancer subtypes (Yoshida et al., 2012; Hsieh et al., 2019; Xu et al., 2019; Huang et al., 2020). Our analysis of publicly available datasets revealed AREG's upregulation in chemoresistant ovarian cancer patients, further strengthening our previous observation of AREG's significant upregulation in HGSOC patient tumors following exposure to frontline carboplatin and paclitaxel (James et al., 2022).

In this present investigation we first sought to specifically uncover how elevated AREG expression impacts tumor intrinsic immune changes. Interestingly, we found through our NanoString analysis that exposure of HGSOC cells to AREG

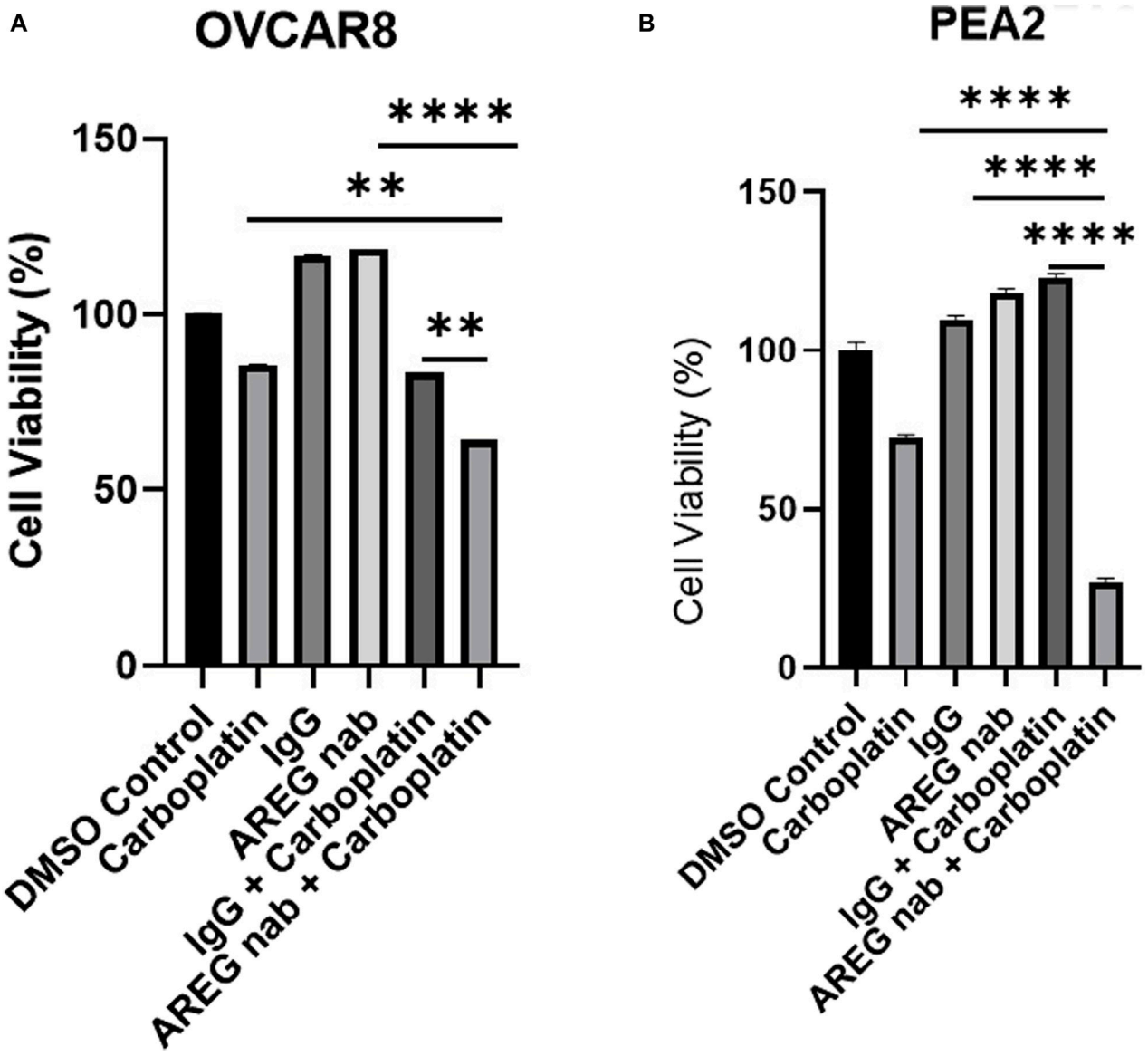


FIGURE 10
Targeting AREG in combination with carboplatin synergistically reduces HGSOC cell viability. Cell viability analysis of **(A)** OVCAR8 and **(B)** PEA2 cells pre-treated with carboplatin (300 μM for OVCAR8 cells, 400 μm for PEA2 cells) for 24 h and then stimulated with 30 μM of AREG nab or corresponding IgG control for an additional 48 h. Error bars represent standard deviation of ≥ 3 biological replicates. $^{**}p < 0.005$, $^{***}p < 0.0005$, $^{****}p < 0.00005$, as indicated. AREG nab, AREG neutralizing antibody.

led to an upregulation in genes related tumor cell growth, angiogenesis, and immune evasion. Most notably, we saw significant changes in angiogenic factors *CXCL8* and *VEGFA* (Marjon et al., 2004), as well as prominent changes in *CXCL1* and *CXCL2*, two chemokines known to contribute to chemoresistance via the recruitment of myeloid derived suppressor cells (MDSCs) (Ozga et al., 2021) and known to be associated with ovarian tumorigenesis (Zhang et al., 2021; Korbecki et al., 2023). Finally, pathway analysis revealed substantial upregulation of genes associated with STAT3 and MAPK/ERK signaling in HGSOC cells. Increased STAT3 and MAPK/ERK activation was confirmed via Western blot, while simultaneously detecting increased AKT pathway activation following rAREG exposure. Plausibly, it can be inferred that the observed increases in multiple cell growth pathways following rAREG treatment can be attributed to AREG's unique binding to its receptor EGFR. AREG's characterization as a low affinity EGFR ligand is due to a single amino acid mutation in its receptor binding domain which produces an unstable interaction with the EGFR receptor and consequential failure of EGFR internalization and enhanced downstream signaling. In contrast, when a high affinity ligand such as EGF binds to EGFR, this action promotes rapid internalization and associated negative feedback signaling loops from downstream cell growth pathways (Zaiss et al., 2015). While one limitation of this study is that we did not we did not confirm that AREG's mechanism of action is indeed through EGFR, this will be imperative to investigate in further studies.

Our finding that AREG robustly activates the STAT3 pathway is particularly noteworthy given STAT3's widespread effects on immunosuppression, cell proliferation, angiogenesis, and metastasis (Zou et al., 2020). Moreover, there has been vested clinical interest in targeting the STAT3 pathway with small molecule inhibitors (Song et al., 2023). Intriguingly, ruxolitinib, a JAK/STAT inhibitor, which is known to inhibit pSTAT3 in ovarian cancer cells (Han et al., 2018), was recently evaluated in a phase I/II clinical trial in combination with frontline carboplatin and paclitaxel chemotherapy (NRG-GY007, NCT02713386) (Landen et al., 2022). Despite the fact that the addition of ruxolitinib was narrowly insignificant, this study demonstrated feasibility and acceptable toxicity and has opened the door for additional combination approaches including ruxolitinib in ovarian cancer in the frontline setting. As this present study has shown that increased AREG levels promote STAT3 signaling activation, targeting AREG could conceivably lead to a reduction in STAT3 activation concomitantly with other growth signaling pathways, and potentially reduce immunosuppression within the OTIME. While our present investigation did not evaluate this hypothesis, it will be pertinent to examine how inhibiting AREG affects downstream cell growth pathways such as STAT3.

To the best of our knowledge, this study is the first to show that AREG promotes upregulation of intratumoral PD-L1 levels in HGSOC, as there been only one study in prostate cancer that previously demonstrated that paracrine AREG induces PD-L1 activity (Xu et al., 2019). Binding of programmed cell death 1 (PD-1) to PD-L1 has been established as one of the critical ways in which tumor cells become able to evade immune surveillance (Pardoll, 2012). Immunotherapies targeting the PD-1/PD-L1 axis have revolutionized the field of oncology, however, monotherapy response rates to PD-1/PD-L1 inhibitors have demonstrated low overall response rates (ORRs) in HGSOC. Despite this fact, it is well known that PD-L1 is highly expressed in ovarian tumors (Alwosaibai et al., 2023) and that anti-tumor immune responses are detected in ovarian tumors (Preston et al., 2011). Therefore, it has been suggested that due to the highly immunosuppressive nature of the OTIME (James et al., 2020), more than one immunotherapeutic approach may be necessary to effectively combat this immunosuppression. Hence, our finding that AREG directly contributes to HGSOC immunosuppression through upregulating PD-L1 expression indicates that targeting AREG in combination with PD-1/PD-L1 blockade could potential improve HGSOC response rates to clinically available PD-1 based immunotherapies. Future pre-clinical studies to evaluate this hypothesis are necessary.

Using *in vitro* and *in vivo* models, this investigation has established that AREG compromises cytotoxic immune responses in HGSOC. It has been widely reported that AREG has a role in promoting immunosuppression within the context of classical immunity. AREG is known to be expressed by Tregs and directly fosters Treg function through the secretion of exosomes that transfer immunosuppressive micro-RNAs to effector T cells (Zaiss et al., 2015). In addition, it is known that AREG possesses the ability to downregulate costimulatory B7 molecules, enhancing cytotoxic T cell death (Dreschers et al., 2023). While AREG's role in classical immunity has been well defined, its specific function in the context of tumor immunology has been comparatively understudied. We have shown for the first time in HGSOC that elevated AREG exposure *in vivo* leads to a reduction in intratumoral $CD8^+$ T cells. Interestingly, a study by Yuan et al. (2015) found that Tregs co-cultured with $CD8^+$ T cells in the presence of AREG led to a reduction in $CD8^+$ T cell activation markers such as IFNy. While a limitation of our study is that we did not specifically isolate these T cell subsets, we similarly observed a reduction in cytotoxic responses with significant reductions in IFNy and IL-2 expression in PBMCs cultured with rAREG. Yuan et al. further discovered that EGFR was not expressed by either intratumoral or splenic $CD8^+$ T cells and that blocking AREG inhibited Treg activation specifically, leading the group to postulate that AREG does not likely impact $CD8^+$ T cells directly, but through influencing Treg function (Yuan et al., 2015). Moreover, studies in melanoma, as well as gastric and lung cancer have similarly observed that AREG leads to immunosuppression through the regulation of Treg function (Wang et al., 2016; Green et al., 2017; Sun et al., 2023). While our present study only identified the reduction of cytotoxic responses, it will be pertinent to also examine how AREG affects Treg function as well as other pertinent immune cell subsets within the OTIME. These future studies will be critical in order to understand how AREG mechanistically compromises cytotoxic immune responses in HGSOC.

Out of an extensive panel of chemokines and cytokines, IL-5 was found to be significantly downregulated by *in vivo* rAREG exposure in both ascites and serum. IL-5 is an essential cytokine required for eosinophil development, and like AREG functions as a Th2 cytokine (Dent et al., 1990; Morimoto et al., 2018). Several studies have demonstrated that IL-5 and eosinophils are vital to the production of anti-tumor immune response (Ikutani et al., 2012; Blomberg et al., 2023; Jacenik et al., 2023). Hence, the ability of AREG to downregulate IL-5 may potentially contribute to the suppressive OTIME and the muted response to immunotherapies that is seen clinically in HGSOC. While this to the best of our knowledge is the first study to identify the relationship between AREG and IL-5 in the context of tumor immunology, it is known that AREG is expressed by human eosinophils in response to IL-5 exposure (Matsumoto et al., 2009). Moreover, connections between IL-5 and AREG have been reported in the severe asthma and lung fibrosis (Morimoto et al., 2018; Bagnasco et al., 2020). Future mechanistic examination examining how IL-5 and AREG interact in the context OTIME are warranted.

Similar to our *in vivo* analysis, which found a decrease of IL-2 expression in PBMCs with rAREG exposure, we also saw a marked reduction of IL-2 in ascites from mice exposed to rAREG. Interestingly, recombinant IL-2 has been a long-standing immunotherapy, with the goal of eliciting anti-tumoral immune responses. However, there have been major limitations associated with this therapy due to systemic toxicity, which has prevented many cancer patients from benefiting from IL-2 treatment (Briukhovetska et al., 2021). Recently, a Phase 1/2 trial was initiated to analyze the safety and efficacy of encapsulated IL-2 nanoparticles administered intraperitoneally (AVB-001; NCT05538624), specifically in a cohort of recurrent HGSOC patients, with the goal of maximizing cytotoxic immune

activation and decreasing toxicity through local peritoneal administration. Overall, our data shows that AREG treatment leads to the pronounced downregulation of a vital pro-inflammatory, clinically relevant HGSOC cytokine IL-2.

We have shown that even in an immune devoid context, targeting AREG in combination with HGSOC standard of care chemotherapy synergistically promotes HGSOC cell death. Two prior studies have targeted AREG in ovarian cancer mouse models. The first, a study by Lindzen et al. (2021) found that an AREG is significantly abundant in ovarian cancer patient ascites and that treatment with an AREG blocking antibody led to prolonged survival in an immunocompetent *in vivo* wildtype p53 HGSOC model. The authors theorized that this efficacy is attributed to the presumed binding of wildtype p53 to AREG's promoter which in turn leads to EGFR activation that can be blocked by an AREG monoclonal antibody (Lindzen et al., 2021). However, given that p53 is mutated in over 96% of all HGSOC (Oien et al., 2016), this finding is clinically relevant to a minute subset of HGSOC patients. The second study by Carvalho et al. (2016) found that an AREG neutralizing antibody as a single agent and in combination with cisplatin led to a synergistic reduction in tumor burden. Although promising, this study was performed in a nude xenograft model and therefore cannot inform consequences of AREG inhibition on the OTIME. In a prostate cancer model, Xu et al. (2019) tested combinatorial AREG blockade with chemotherapy, which demonstrated superior anti-tumor efficacy, even compared to co-treatment with the EGFR mab cetuximab and chemotherapy. Fascinatingly, this finding suggests that EGFR may not be AREG's sole surface receptor within the tumor immune microenvironment (Xu et al., 2019). In the future, it will be necessary to examine the combinatorial efficacy of AREG and chemotherapy regimens in an immunocompetent HGSOC *in vivo* model, in order to determine if this strategy leads to reduced tumor burden and rescues the rAREG-induced diminished cytotoxic immune responses that we have seen in this present study. Furthermore, as was evaluated by Xu et al. (2019), it would be worthwhile to compare the efficacy of AREG neutralization with EGFR blockade in order to further elucidate AREG's mechanism of action within the OTIME.

In conclusion, this study demonstrates that AREG promotes immunomodulation within the OTIME and leads to the reduction of cytotoxic responses, indicating its putative role as a novel HGSOC immune target. In addition, AREG's function in promoting chemoresistance and PD-L1 immune dysfunction provides strong rationale for combinatorial approaches with HGSOC standard of care chemotherapy and PD-1 based immunotherapy. Future pre-clinical studies testing these new immune modulating regimens will be informative to a patient population that has yet to respond meaningfully to clinically available immunotherapies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/, GSE252495.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. The animal study was approved by the Brown University Animal Care and Use Committee (#22-09-0002). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JE: Data curation, Formal Analysis, Validation, Writing–original draft, Writing–review and editing. JM: Data curation, Formal Analysis, Validation, Writing–review and editing. CK: Data curation, Writing–review and editing. CJ: Data curation, Writing–review and editing. MW: Data curation, Writing–review and editing. PD: Data curation, Writing–review and editing. CS: Data curation, Methodology, Writing–review and editing. JR: Conceptualization, Data curation, Writing–review and editing. NJ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1375421/full#supplementary-material

SUPPLEMENTARY FIGURE S1
AREG is not associated with HGSOC survival outcomes. Kaplan-Meier Plotter analysis using TCGA analysis and Gene Expression Omnibus Series data in patients with stage 3 and 4 ovarian cancer, demonstrating AREG's association with PFS stratified by **(A)** lower quartile and **(B)** upper quartile AREG levels. AREG's association with OS stratified by **(C)** lower quartile and **(D)** upper quartile AREG levels. TCGA, The Cancer Genome Atlas; PFS, progression-free survival; OS, overall-survival.

SUPPLEMENTARY FIGURE S2
PEA1 qPCR analysis at 2 h rAREG exposure timepoint. qPCR analysis CXCL1 **(B)** DUSP5 **(C)** IL-11 **(D)** CXCL2 **(E)** IL-6 of PEA1 cells stimulated with 200 ng/ml of rAREG or BSA control for 1 h. ns, non-significant.

SUPPLEMENTARY FIGURE S3
Small molecule JAK/STAT inhibition does not affect AREG levels in HGSOC cells. Western blot analysis of AREG levels and corresponding GAPDH loading control in PEA1 and OVCAR8 cells following 10 μm of ruxolitinib treatment for 48 h.

SUPPLEMENTARY FIGURE S4
qPCR analysis of GRZB in PBMCs. ns, non-significant; PBMC, peripheral blood mononuclear cell.

References

Alexa, A., and Rahnenfuher, J. (2019). topGO: enrichment analysis for gene ontology. *R. package version* 1.38.1.

Alwosaibai, K., Aalmri, S., Mashhour, M., Ghandorah, S., Alshangiti, A., Azam, F., et al. (2023). PD-L1 is highly expressed in ovarian cancer and associated with cancer stem cells populations expressing CD44 and other stem cell markers. *BMC Cancer* 23 (1), 13. doi:10.1186/s12885-022-10404-x

Bagnasco, D., Riccio, A. M., De Ferrari, L., Massolo, A., Caminati, M., Senna, G., et al. (2020). Serum amphiregulin expression in severe asthma patients treated with anti-IL-5 therapy. *Eur. Respir. J.* 56, 2262. doi:10.1183/13993003.congress-2020.2262

Blomberg, O. S., Spagnuolo, L., Garner, H., Voorwerk, L., Isaeva, O. I., van Dyk, E., et al. (2023). IL-5-producing CD4+ T cells and eosinophils cooperate to enhance response to immune checkpoint blockade in breast cancer. *Cancer Cell* 41 (1), 106–123.e10. doi:10.1016/j.ccell.2022.11.014

Briukhovetska, D., Dörr, J., Endres, S., Libby, P., Dinarello, C. A., and Kobold, S. (2021). Interleukins in cancer: from biology to therapy. *Nat. Rev. Cancer* 21 (8), 481–499. doi:10.1038/s41568-021-00363-z

Carvalho, S., Lindzen, M., Lauriola, M., Shirazi, N., Sinha, S., Abdul-Hai, A., et al. (2016). An antibody to amphiregulin, an abundant growth factor in patients' fluids, inhibits ovarian tumors. *Oncogene* 35 (4), 438–447. doi:10.1038/onc.2015.93

Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., et al. (2012). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2 (5), 401–404. doi:10.1158/2159-8290.CD-12-0095

Cheng, J.-C., Chang, H.-M., Xiong, S., So, W.-K., and Leung, P. C. K. (2016). Sprouty2 inhibits amphiregulin-induced down-regulation of E-cadherin and cell invasion in human ovarian cancer cells. *Oncotarget* 7 (49), 81645–81660. doi:10.18632/oncotarget.13162

Chiu, Y.-S., Hsing, C.-H., Li, C.-F., Lee, C.-Y., Hsu, Y.-H., and Chang, M.-S. (2017). Anti-IL-20 monoclonal antibody inhibited tumor growth in hepatocellular carcinoma. *Sci. Rep.* 7 (1), 17609. doi:10.1038/s41598-017-17054-1

Čokić, V. P., Mitrović-Ajtić, O., Beleslin-Čokić, B. B., Marković, D., Buač, M., Diklić, M., et al. (2015). Proinflammatory cytokine IL-6 and JAK-STAT signaling pathway in myeloproliferative neoplasms. *Mediat. Inflamm.* 29, 453020. doi:10.1155/2015/453020

Conroy, M. J., and Lysaght, J. (2020). CX3CL1 signaling in the tumor microenvironment. *Adv. Exp. Med. Biol.* 1231, 1–12. doi:10.1007/978-3-030-36667-4_1

Dent, L. A., Strath, M., Mellor, A. L., and Sanderson, C. J. (1990). Eosinophilia in transgenic mice expressing interleukin 5. *J. Exp. Med.* 172 (5), 1425–1431. doi:10.1084/jem.172.5.1425

Dreschers, S., Platen, C., Oppermann, L., Doughty, C., Ludwig, A., Babendreyer, A., et al. (2023). EGF-receptor against amphiregulin (AREG) influences costimulatory molecules on monocytes and T cells and modulates T-cell responses. *J. Immunol. Res.* 2023, 8883045. doi:10.1155/2023/8883045

Edgar, R., Domrachev, M., and Lash, A. E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 30 (1), 207–210. doi:10.1093/nar/30.1.207

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P., et al. (2018). The reactome pathway knowledgebase. *Nucleic Acids Res.* 46 (D1), D649-D655–55. doi:10.1093/nar/gkx1132

Fu, J., Li, K., Zhang, W., Wan, C., Zhang, J., Jiang, P., et al. (2020). Large-scale public data reuse to model immunotherapy response and resistance. *Genome Med.* 12 (1), 21. doi:10.1186/s13073-020-0721-z

Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., et al. (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal* 6 (269), pl1. doi:10.1126/scisignal.2004088

Geer, L. Y., Marchler-Bauer, A., Geer, R. C., Han, L., He, J., He, S., et al. (2010). The NCBI BioSystems database. *Nucleic Acids Res.* 38, D492–D496. doi:10.1093/nar/gkp858

Green, J. A., Arpaia, N., Schizas, M., Dobrin, A., and Rudensky, A. Y. (2017). A nonimmune function of T cells in promoting lung tumor progression. *J. Exp. Med.* 214 (12), 3565–3575. doi:10.1084/jem.20170356

Han, E. S., Wen, W., Dellinger, T. H., Wu, J., Lu, S. A., Jove, R., et al. (2018). Ruxolitinib synergistically enhances the anti-tumor activity of paclitaxel in human ovarian cancer. *Oncotarget* 9 (36), 24304–24319. doi:10.18632/oncotarget.24368

Han, X., Zhang, P., Wang, J., and He, B. (2011). Eosinophilic infiltration of lung cancer tissue and its correlation with the expression of interleukin-5. *Zhonghua Yi Xue Za Zhi* 91 (14), 956–960.

Hening, C. (2024). Cran-package fpc. Available at: https://cran.r-project.org/web/packages/fpc/index.html.

Hsieh, M.-J., Chen, Y.-H., Lee, I.-N., Huang, C., Ku, Y.-J., and Chen, J.-C. (2019). Secreted amphiregulin promotes vincristine resistance in oral squamous cell carcinoma. *Int. J. Oncol.* 55 (4), 949–959. doi:10.3892/ijo.2019.4866

Hsu, Y.-H., Hsing, C.-H., Li, C.-F., Chan, C.-H., Chang, M.-C., Yan, J.-J., et al. (2012b). Anti-IL-20 monoclonal antibody suppresses breast cancer progression and bone osteolysis in murine models. *J. Immunol.* 188 (4), 1981–1991. doi:10.4049/jimmunol.1102843

Hsu, Y.-H., Wei, C.-C., Shieh, D.-B., Chan, C.-H., and Chang, M.-S. (2012a). Anti-IL-20 monoclonal antibody alleviates inflammation in oral cancer and suppresses tumor growth. *Mol. Cancer Res.* 10 (11), 1430–1439. doi:10.1158/1541-7786.MCR-12-0276

Huang, Y.-W., Lin, C.-Y., Tsai, H.-C., Fong, Y.-C., Han, C.-K., Huang, Y.-L., et al. (2020). Amphiregulin promotes cisplatin chemoresistance by upregulating ABCB1 expression in human chondrosarcoma. *Aging (Albany NY)* 12 (10), 9475–9488. doi:10.18632/aging.103220

Hwang, W.-T., Adams, S. F., Tahirovic, E., Hagemann, I. S., and Coukos, G. (2012). Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. *Gynecol. Oncol.* 124 (2), 192–198. doi:10.1016/j.ygyno.2011.09.039

Ikutani, M., Yanagibashi, T., Ogasawara, M., Tsuneyama, K., Yamamoto, S., Hattori, Y., et al. (2012). Identification of innate IL-5-producing cells and their role in lung eosinophil regulation and antitumor immunity. *J. Immunol.* 188 (2), 703–713. doi:10.4049/jimmunol.1101270

Jacenik, D., Karagiannidis, I., and Beswick, E. J. (2023). Th2 cells inhibit growth of colon and pancreas cancers by promoting anti-tumorigenic responses from macrophages and eosinophils. *Br. J. Cancer* 128 (2), 387–397. doi:10.1038/s41416-022-02056-2

James, N. E., Oliver, M. T., Ribeiro, J. R., Cantillo, E., Rowswell-Turner, R. B., Kim, K.-K., et al. (2019). Human epididymis secretory protein 4 (HE4) compromises cytotoxic mononuclear cells via inducing dual specificity phosphatase 6. *Front. Pharmacol.* 10, 216. doi:10.3389/fphar.2019.00216

James, N. E., Woodman, M., De La Cruz, P., Eurich, K., Ozsoy, M. A., Schorl, C., et al. (2022). Adaptive transcriptomic and immune infiltrate responses in the tumor immune microenvironment following neoadjuvant chemotherapy in high grade serous ovarian cancer reveal novel prognostic associations and activation of pro-tumorigenic pathways. *Front. Immunol.* 13, 965331. doi:10.3389/fimmu.2022.965331

James, N. E., Woodman, M., DiSilvestro, P. A., and Ribeiro, J. R. (2020). The perfect combination: enhancing patient response to PD-1-based therapies in epithelial ovarian cancer. *Cancers (Basel)* 12 (8), 2150. doi:10.3390/cancers12082150

Jiang, P., Gu, S., Pan, D., Fu, J., Sahu, A., Hu, X., et al. (2018). Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat. Med.* 24 (10), 1550–1558. doi:10.1038/s41591-018-0136-1

Johnson, D. E., O'Keefe, R. A., and Grandis, J. R. (2018). Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat. Rev. Clin. Oncol.* 15 (4), 234–248. doi:10.1038/nrclinonc.2018.8

Korbecki, J., Bosiacki, M., Barczak, K., Łagocka, R., Brodowska, A., Chlubek, D., et al. (2023). Involvement in tumorigenesis and clinical significance of CXCL1 in reproductive cancers: breast cancer, cervical cancer, endometrial cancer, ovarian cancer and prostate cancer. *Int. J. Mol. Sci.* 24 (8), 7262. doi:10.3390/ijms24087262

Lánczky, A., and Győrffy, B. (2021). Web-based survival analysis tool tailored for medical research (KMplot): development and implementation. *J. Med. Internet Res.* 23 (7), e27633. doi:10.2196/27633

Landen, C. N., Buckanovich, R. J., Sill, M., Mannel, R. S., Walker, J. L., Disilvestro, P., et al. (2022). A phase I/II study of ruxolitinib with frontline neoadjuvant and post-surgical therapy in patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer. *J. Clin. Oncol.* 40 (Suppl. 16), 5501. doi:10.1200/jco.2022.40.16_suppl.5501

Liberzon, A., Subramanian, A., Pinchback, R., Thorvaldsdóttir, H., Tamayo, P., and Mesirov, J. P. (2011). Molecular signatures database (MSigDB) 3.0. *Bioinformatics* 27 (12), 1739–1740. doi:10.1093/bioinformatics/btr260

Lindzen, M., Ghosh, S., Noronha, A., Drago, D., Nataraj, N. B., Leitner, O., et al. (2021). Targeting autocrine amphiregulin robustly and reproducibly inhibits ovarian cancer in a syngeneic model: roles for wildtype p53. *Oncogene* 40 (21), 3665–3679. doi:10.1038/s41388-021-01784-8

Lu, S.-W., Pan, H.-C., Hsu, Y.-H., Chang, K.-C., Wu, L.-W., Chen, W.-Y., et al. (2020). IL-20 antagonist suppresses PD-L1 expression and prolongs survival in pancreatic cancer models. *Nat. Commun.* 11 (1), 4611. doi:10.1038/s41467-020-18244-8

Luvero, D., Plotti, F., Aloisia, A., Montera, R., Terranova, C., Nardone, C.De C., et al. (2019). Ovarian cancer relapse: from the latest scientific evidence to the best practice. *Crit. Rev. Oncol. Hematol.* 140, 28–38. doi:10.1016/j.critrevonc.2019.05.014

Macchia, G., Titone, F., Restaino, S., Arcieri, M., Pellecchia, G., Andreetta, C., et al. (2023). Is it time to reassess the role of radiotherapy treatment in ovarian cancer? *Healthc. (Basel)* 11 (17), 2413. doi:10.3390/healthcare11172413

Marjon, P. L., Bobrovnikova-Marjon, E. V., and Abcouwer, S. F. (2004). Expression of the pro-angiogenic factors vascular endothelial growth factor and interleukin-8/CXCL8 by human breast carcinomas is responsive to nutrient deprivation and endoplasmic reticulum stress. *Mol. Cancer* 3, 4. doi:10.1186/1476-4598-3-4

Matsumoto, K., Fukuda, S., Nakamura, Y., and Saito, H. (2009). Amphiregulin production by human eosinophils. *Int. Arch. Allergy Immunol.* 149 (Suppl. 1), 39–44. doi:10.1159/000210652

Mei, J., Tian, H., Huang, H. S., Hsu, C. F., Liu, Y. M., Wu, N., et al. (2021). Cellular models of development of ovarian high-grade serous carcinoma: a review of cell of origin and mechanisms of carcinogenesis. *Cell Prolif.* 54 (5), e13029. doi:10.1111/cpr.13029

Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., et al. (2019). InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Res.* 47 (D1), D351-D360–60. doi:10.1093/nar/gky1100

Morimoto, Y., Hirahara, K., Kiuchi, M., Wada, T., Ichikawa, T., Kanno, T., et al. (2018). Amphiregulin-Producing pathogenic memory T helper 2 cells instruct eosinophils to secrete osteopontin and facilitate airway fibrosis. *Immunity* 49 (1), 134–150. doi:10.1016/j.immuni.2018.04.023

Oien, D., Chien, J., and Cheng, N. (2016). *TP53* mutations as a biomarker for high-grade serous ovarian cancer: are we there yet? *Transl. Cancer Res.* 5 (5), S514–S519. doi:10.21037/tcr.2016.09.32

OMalley, D. M., Krivak, T. C., Kabil, N., Munley, J., and Moore, K. N. (2023). PARP inhibitors in ovarian cancer: a review. *Target Oncol.* 18 (4), 471–503. doi:10.1007/s11523-023-00970-w

Ozga, A. J., Chow, M. T., and Luster, A. D. (2021). Chemokines and the immune response to cancer. *Immunity* 54 (5), 859–874. doi:10.1016/j.immuni.2021.01.012

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12 (4), 252–264. doi:10.1038/nrc3239

Perkins, J. R., Dawes, J. M., McMahon, S. B., Bennett, D. L. H., Orengo, C., and Kohl, M. (2012). ReadqPCR and NormqPCR: R packages for the reading, quality checking and normalisation of RT-qPCR quantification cycle (Cq) data. *BMC Genomics* 13, 296. doi:10.1186/1471-2164-13-296

Preston, C. C., Goode, E. L., Hartmann, L. C., Kalli, K. R., and Knutson, K. L. (2011). Immunity and immune suppression in human ovarian cancer. *Immunotherapy* 3 (4), 539–556. doi:10.2217/imt.11.20

Singh, S. S., Chauhan, S. B., Kumar, A., Kumar, S., Engwerda, C. R., Sundar, S., et al. (2022). Amphiregulin in cellular physiology, health, and disease: potential use as a biomarker and therapeutic target. *J. Cell Physiol.* 237 (2), 1143–1156. doi:10.1002/jcp.30615

Slenter, D. N., Kutmon, M., Hanspers, K., Riutta, A., Windsor, J., Nunes, N., et al. (2018). WikiPathways: a multifaceted pathway database bridging metabolomics to other omics research. *Nucleic Acids Res.* 46 (D1), D661-D667–7. doi:10.1093/nar/gkx1064

Song, J., Wang, J., Tian, S., and Li, H. (2023). Discovery of STAT3 inhibitors: recent advances and future perspectives. *Curr. Med. Chem.* 30 (16), 1824–1847. doi:10.2174/0929867329666220819093117

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* 102 (43), 15545–15550. doi:10.1073/pnas.0506580102

Sun, R., Zhao, H., Gao, D. S., Ni, A., Li, H., Chen, L., et al. (2023). Amphiregulin couples IL1RL1+ regulatory T cells and cancer-associated fibroblasts to impede antitumor immunity. *Sci. Adv.* 9 (34), eadd7399. doi:10.1126/sciadv.add7399

Tung, S. L., Huang, W. C., Hsu, F. C., Yang, Z. P., Jang, T. H., Chang, J. W., et al. (2017). miRNA-34c-5p inhibits amphiregulin-induced ovarian cancer stemness and drug resistance via downregulation of the AREG-EGFR-ERK pathway. *Oncogenesis* 6 (5), e326. doi:10.1038/oncsis.2017.25

Wang, S., Zhang, Y., Wang, Y., Ye, P., Li, J., Li, H., et al. (2016). Amphiregulin confers regulatory T cell suppressive function and tumor invasion via the EGFR/GSK-3β/Foxp3 Axis. *J. Biol. Chem.* 291 (40), 21085–21095. doi:10.1074/jbc.M116.717892

Wang, S.-W., and Sun, Y.-M. (2014). The IL-6/JAK/STAT3 pathway: potential therapeutic strategies in treating colorectal cancer (Review). *Int. J. Oncol.* 44 (4), 1032–1040. doi:10.3892/ijo.2014.2259

Wee, P., and Wang, Z. (2017). Epidermal growth factor receptor cell proliferation signaling pathways. *Cancers (Basel)* 9 (5), 52. doi:10.3390/cancers9050052

Xu, Q., Long, Q., Zhu, D., Fu, D., Zhang, B., Han, L., et al. (2019). Targeting amphiregulin (AREG) derived from senescent stromal cells diminishes cancer resistance and averts programmed cell death 1 ligand (PD-L1)-mediated immunosuppression. *Aging Cell* 18 (6), e13027. doi:10.1111/acel.13027

Yoshida, M., Shimura, T., Fukuda, S., Mizoshita, T., Tanida, S., Kataoka, H., et al. (2012). Nuclear translocation of pro-amphiregulin induces chemoresistance in gastric cancer. *Cancer Sci.* 103 (4), 708–715. doi:10.1111/j.1349-7006.2012.02204.x

Yuan, C.-H., Sun, X.-M., Zhu, C.-L., Liu, S.-P., Wu, L., Chen, H., et al. (2015). Amphiregulin activates regulatory T lymphocytes and suppresses CD8+ T cell-mediated anti-tumor response in hepatocellular carcinoma cells. *Oncotarget* 6 (31), 32138–32153. doi:10.18632/oncotarget.5171

Zaiss, D. M. W., Gause, W. C., Osborne, L. C., and Artis, D. (2015). Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. *Immunity* 42 (2), 216–226. doi:10.1016/j.immuni.2015.01.020

Zaiss, D. M. W., van Loosdregt, J., Gorlani, A., Bekker, C. P. J., Gröne, A., Sibilia, M., et al. (2013). Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity* 38 (2), 275–284. doi:10.1016/j.immuni.2012.09.023

Zerdes, I., Wallerius, M., Sifakis, E. G., Wallmann, T., Betts, S., Bartish, M., et al. (2019). STAT3 activity promotes programmed-death ligand 1 expression and suppresses immune responses in breast cancer. *Cancers (Basel)* 11 (10), 1479. doi:10.3390/cancers11101479

Zhang, F., Jiang, J., Xu, B., Xu, Y., and Wu, C. (2021). Over-expression of CXCL2 is associated with poor prognosis in patients with ovarian cancer. *Med. Baltim.* 100 (4), e24125. doi:10.1097/MD.0000000000024125

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., et al. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N. Engl. J. Med.* 348 (3), 203–213. doi:10.1056/NEJMoa020177

Zhao, M., Liu, Y., Liu, R., Qi, J., Hou, Y., Chang, J., et al. (2018). Upregulation of IL-11, an IL-6 family cytokine, promotes tumor progression and correlates with poor prognosis in non-small cell lung cancer. *Cell Physiol. Biochem.* 45 (6), 2213–2224. doi:10.1159/000488166

Zou, S., Tong, Q., Liu, B., Huang, W., Tian, Y., and Fu, X. (2020). Targeting STAT3 in cancer immunotherapy. *Mol. Cancer* 19 (1), 145. doi:10.1186/s12943-020-01258-7

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Efficacy and safety of anti-angiogenic drug monotherapy and combination therapy for ovarian cancer: a meta-analysis and trial sequential analysis of randomized controlled trials

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Background: As the development of novel anti-angiogenic drugs and the continuous evolution of guideline recommendations, the efficacy and safety of anti-angiogenic agents in ovarian cancer (OC) remains unclear. Consequently, a meta-analysis was carried out to assess the efficacy and safety of anti-angiogenic drug monotherapy and combination therapy for OC.

Methods: An exhaustive literature review was performed across multiple databases, including PubMed, Embase, Web of Science, and Cochrane, encompassing all relevant randomized controlled trials (RCTs) up until 6 April 2024. The evaluation of efficacy outcomes incorporated progression-free survival (PFS), overall survival (OS), and objective response rate (ORR). Safety was assessed through the occurrence of any grade adverse events (AEs) and grade ≥3 AEs. Synthesis of the data involved the calculation of hazard ratios (HRs), relative risks (RRs), and their corresponding 95% confidence intervals (CIs) and prediction intervals (PIs). Trial sequential analysis was executed employing TSA v0.9.5.10 Beta software, STATA 12.0, and R software 4.3.1.

Results: In this meta-analysis, 35 RCTs were included, encompassing 16,199 subjects in total. The overall analysis indicated that anti-angiogenic drug combination therapy significantly improved PFS (HR [95% CI] = 0.678 [0.606–0.759], 95% PI: 0.415–1.108), OS (HR [95% CI] = 0.917 [0.870–0.966], 95% PI: 0.851–0.984), and ORR (RR [95% CI] = 1.441 [1.287–1.614], 95% PI: 1.032–2.014), but also increased the incidence of grade ≥3 AEs (RR [95% CI] = 1.137 [1.099–1.177], 95% PI: 1.011–1.252). The analysis did not corroborate any benefit of anti-angiogenic monotherapy over placebo concerning PFS (HR [95% CI] = 0.956 [0.709–1.288], 95% PI: 0.345–2.645) and OS (HR [95% CI] = 1.039 [0.921–1.173], 95% PI: 0.824–1.331). However, it was observed that monotherapy with anti-angiogenic drugs did increase the incidence of any grade AEs (RR [95% CI] = 1.072 [1.036–1.109], 95% PI: 0.709–1.592).

Conclusion: Our study confirmed the PFS, OS, and ORR benefits of anti-angiogenic drug combination therapy for OC patients. The efficacy results of

anti-angiogenic monotherapy necessitates further evaluation as more RCTs become available. Clinicians should be vigilant of AEs when administering anti-angiogenic agents in a clinical setting.

KEYWORDS

anti-angiogenic drugs, VEGF, bevacizumab, ovarian cancer, monotherapy, combination therapy, meta-analysis

1 Introduction

Ovarian cancer (OC) stands as the primary cause of death related to gynecologic cancer and the fifth most prevalent malignancy, thereby posing a substantial global health risk to women (Siegel et al., 2021). The difficulty in early-stage detection of OC often leads to diagnoses at advanced stages, contributing to a reduced 5-year relative survival rate (Wang et al., 2021). The current standard of care for newly diagnosed patients typically encompasses cytoreductive surgery and platinum-based systemic chemotherapy, with the optional inclusion of bevacizumab. Even with optimal treatment leading to complete remission, about 70% of patients experience a recurrence within 5 years (Hope et al., 2010; McGee et al., 2017). Notably, recurrence rates are nearly 25% for those in early stages and exceed 80% in advanced stages (Garzon et al., 2020). Despite the availability of multiple active therapies for recurrent OC, such as targeted therapy (e.g., poly ADP-ribose polymerase [PARP] inhibitors), chemotherapy, and immunotherapy, the median survival post-recurrence remains less than 3 years, underscoring the critical need to explore new therapeutic options for this patient group (Richardson et al., 2018).

New therapeutic agents, particularly those inhibiting angiogenesis, have shown considerable potential for the treatment of OC. Aberrant angiogenesis, a defining characteristic of solid tumors, is instrumental in tumor advancement (Jászai et al., 2019). By interfering with the formation of blood vessels, anti-angiogenic medications impede the nutrient supply to tumor cells, both by causing damage to the established tumor vasculature and by blocking the creation of new blood vessels (Abdalla et al., 2018). Additionally, these treatments may induce normalization of the tumor vasculature, reversing tumor microenvironment hypoxia, reducing the tumor's aggressive nature, and augmenting the effectiveness of traditional therapies (Teleanu et al., 2019). The efficacy of angiogenesis inhibitors results from intricate interactions among various pathways, including numerous angiogenic factors like angiopoietin, vascular endothelial growth factor (VEGF), and VEGF receptor (VEGFR) (Saman et al., 2020). Recently, a variety of anti-VEGF strategies, including monoclonal antibodies against VEGF (for instance, bevacizumab) and VEGFR inhibitors (such as cediranib, pazopanib, sorafenib, and apatinib), have undergone evaluation in OC patients (Monk et al., 2016a). The AURELIA trial, a phase III randomized trial, revealed that OC patients experienced a notable extension in progression-free survival (PFS) when treated with a regimen of bevacizumab in combination with chemotherapy *versus* chemotherapy alone. The trial also recorded an enhancement in the objective response rate (ORR) by 15.5% over chemotherapy exclusively. Nonetheless, the addition of bevacizumab to chemotherapy did not yield a statistically significant increase in overall survival (OS) (Pujade-Lauraine et al., 2014).

In the comparison of combined therapy involving angiogenesis inhibitors and standard chemotherapy *versus* conventional chemotherapy alone, a number of randomized controlled trials (RCTs) have illustrated an enhancement in PFS. Nevertheless, debates persist regarding the OS advantage and the safety profile of these combined treatments (Chekerov et al., 2018; Ray-Coquard et al., 2020; Pignata et al., 2021). Prior meta-analyses have explored the efficacy and toxicity of anti-angiogenic drugs in different subtypes of OC (Yi et al., 2017; Helali et al., 2022; Zhang et al., 2022). However, there is a lack of a comprehensive meta-analysis to evaluate the effects of monotherapy or combination therapy with anti-angiogenic drugs on OC. Moreover, multiple RCTs have published the latest relevant clinical results in recent years (Liu et al., 2022; Roque et al., 2022; Ferron et al., 2023; Nicum et al., 2024). Therefore, we conducted a meta-analysis to systematically assess the efficacy and safety of anti-angiogenic drug monotherapy or combined with chemotherapy or PARP inhibitors in the treatment of OC.

2 Materials and methods

2.1 Study design

The methodology and reporting of our study were aligned with the guidelines delineated in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021). Furthermore, our study protocol was registered within the PROSPERO database (registration number: CRD42024534864). Given the nature of this research as a meta-analysis synthesizing findings from existing literature, it does not necessitate ethical approval and informed consent, as it neither engages with ethics nor patient privacy.

2.2 Search strategy

Our comprehensive search encompassed the databases of PubMed, Web of Science, Embase, and the Cochrane Library of clinical trials, aiming to identify all relevant articles published in English until 6 April 2024. The key search terms employed were: ("anti-angiogenic", "angiogenesis inhibitor", "antiangiogenetic", "anti-angiogenesis", "vascular endothelial growth factor", "VEGF", "VEGFR", "anti-VEGF") OR ("bevacizumab", "cediranib", "pazopanib", "afibercept", "nintedanib", "sorafenib", "trebananib", "avastin", "recentin", "votrient", "perifosine") AND ("ovar*" AND "cancer*", "tumor*", "tumour*", "carcinoma*", "neoplasm*", "malignan*"). A thorough description of the search strategy can be found in Supplementary Files S1. We also manually

scrutinized references cited in pertinent review articles to uncover additional studies that may meet the eligibility criteria.

2.3 Study selection

Eligibility for study selection was determined by the following criteria: 1) RCTs; 2) the participants are adult women (aged 18 and above) diagnosed with OC at any stage through histological examination; 3) intervention: monotherapy with anti-angiogenic medication or its combination with chemotherapy or PARP inhibitors; 4) comparison: treatment with placebo alone or chemotherapy (alone or plus placebo) or PARP inhibitors (alone or plus placebo); 5) outcomes: PFS, OS, ORR, adverse events (AEs) of any grade, or grade ≥3 AEs. Studies were excluded based on the following: 1) retrospective studies and non-interventional, non-comparative or single-arm trials; 2) studies lacking pertinent outcomes or presenting duplicated data; 3) trial design involving both the intervention and control groups receiving anti-angiogenic drugs; 4) literature reviews, case reports, conference abstracts, commentaries, and study protocols.

2.4 Data extraction and quality assessment

Two independent reviewers conducted the study screening, selection, exclusion, and extraction of data. From each RCT, we collated details such as the name of the lead author, year of publication, trial name and phase, patient condition, variety of anti-angiogenic medication used, number of participants and their median age, the doses and cycles of drugs used in the anti-angiogenic agent treatment group and the control group, duration of follow-up, and the outcomes in meta-analysis. PFS and OS were designated as the primary endpoints for this meta-analysis, with ORR, AEs of any grade, and grade ≥3 AEs serving as secondary endpoints. When encountering multiple reports from a single trial, preference was given to the most updated or complete report offering the necessary details. If PFS or OS outcomes were not available directly, the Engauge Digitizer Version 10.8 tool (available at http://markummitchell.github.io/engauge-digitizer/) and Tierney et al.'s proposed methodology (Tierney et al., 2007) were employed to derive data from Kaplan-Meier curves (Xie et al., 2022).

The quality of the RCTs was evaluated utilizing the modified Jadad scale (Jadad et al., 1996), which includes criteria such as the process of randomization, concealment of randomization, implementation of double-blinding, and the tracking of withdrawals and dropouts. Trials were categorized based on their quality with scores ranging from 0 to 3 indicating low quality, while scores from 4 to 7 signified high-quality research.

2.5 Statistical analysis

Statistical analyses were carried out using R software Version 4.3.1 and STATA Version 12.0. We calculated the combined hazard ratios (HRs) along with their 95% confidence intervals (CIs) for both PFS and OS. Dichotomous data outcomes were synthesized by computing relative risks (RRs) and delineating these with 95% CIs. We employed I^2 statistics, Cochran's Q test, and the 95% prediction interval (PI) to assess heterogeneity across studies (Bowden et al., 2011; IntHout et al., 2016). Findings with I^2 exceeding 50% or a p-value less than 0.10 were deemed to show significant heterogeneity, prompting the use of a random-effects model; if not, we used the fixed-effects model (Higgins et al., 2002). We performed subgroup analysis considering OC subtypes or the types of anti-angiogenic agents. To identify potential sources of heterogeneity, we conducted a sensitivity analysis. Furthermore, the trim-and-fill method was employed to detect and adjust for any publication bias (Duval et al., 2000). A two-sided $p < 0.05$ was considered statistically significant.

2.6 Trial sequential analysis

We conducted a trial sequential analysis (TSA) to determine whether the compiled data met the required information size (RIS) for a conclusive finding (Wetterslev et al., 2017). This methodological approach, applied to dichotomous outcomes, utilized TSA software v0.9.5.10 Beta (accessible at www.ctu.dk/tsa). The RIS was calculated, and O'Brien-Fleming α-spending boundaries were established, based on a 5% type I error and a 20% type II error, both set for two-side tests. We engaged STATA Version 12.0, employing the *metacumbounds* and *rsource* function, and R software Version 4.3.1, using the *foreign* and *ldbounds* packages, to execute TSA on the PFS and OS data, adopting the *a priori* information size (APIS) approach. The crossing of the cumulative Z-curve over the trial sequential monitoring boundary or the RIS (or APIS) threshold was interpreted as an indication that no additional trials are necessary, and the evidence could be considered conclusive.

3 Results

3.1 Literature search

The preliminary search of the database yielded 3,847 entries. Following the removal of 1,590 duplicate entries, a set of 2,257 records persisted for further scrutiny. Out of these, 2,171 were discarded due to irrelevance indicated by their titles or abstracts, leaving 86 articles for full-text review regarding their eligibility. Upon detailed examination, 51 studies were deemed unfit for inclusion: 5 were single-arm clinical trials; 2 were non-comparative clinical studies; 3 trials included duplicate patient data; 23 trials exhibited intervention and control designs that did not align with the inclusion criteria; and 18 articles failed to report the necessary outcome data. Finally, 35 RCTs were selected for inclusion in the meta-analysis (Aghajanian et al., 2012; Aghajanian et al., 2015; Burger et al., 2011; Chekerov et al., 2018; Coleman et al., 2017; du Bois et al., 2014; du Bois et al., 2016; Duska et al., 2020; Ferron et al., 2023; Gore et al., 2019; Gotlieb et al., 2012; Hall et al., 2020; Herzog et al., 2013; Karlan et al., 2012; Kim et al., 2018; Ledermann et al., 2021; Ledermann et al., 2016; Ledermann et al., 2011; Liu et al., 2019; Liu et al., 2022; Marth et al., 2017; Monk et al., 2016b; Nicum et al., 2024; Oza et al., 2015; Pignata et al., 2021; Pignata et al., 2015; Pujade-Lauraine et al., 2014; Ray-Coquard et al.,

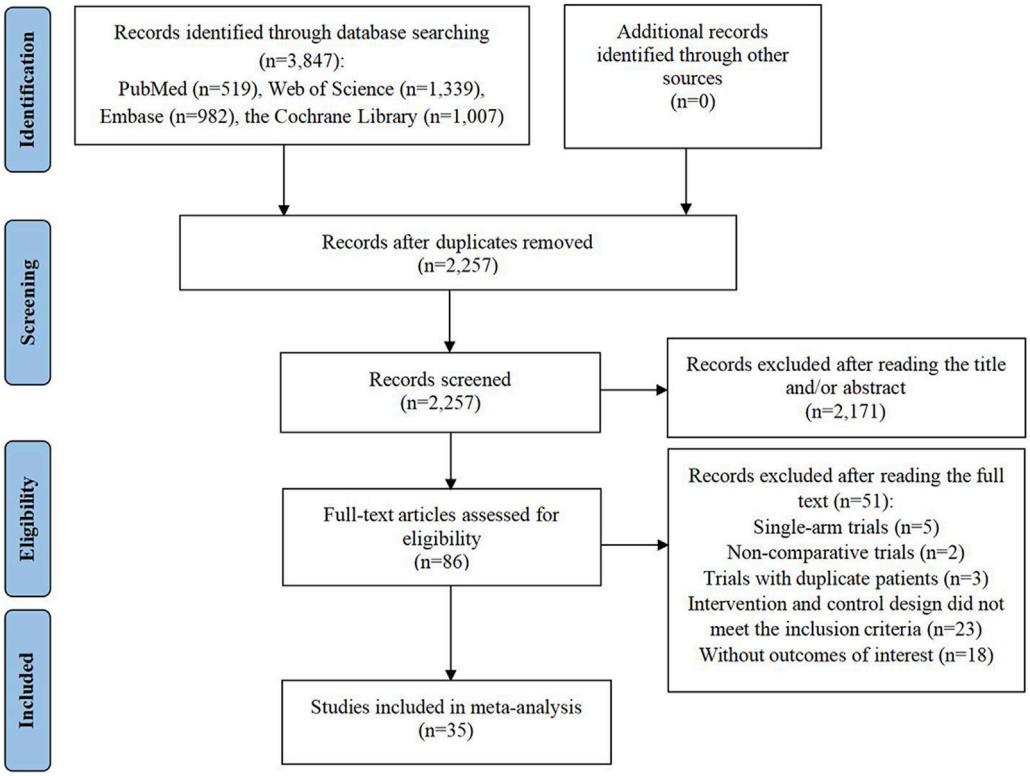

FIGURE 1
Flow diagram of the process of study selection.

2020; Richardson et al., 2018; Roque et al., 2022; Shoji et al., 2022; Tewari et al., 2019; Vergote et al., 2019a; Vergote et al., 2019b; Wang et al., 2022) (Figure 1).

3.2 Study characteristics and quality assessment

Table 1 provided a detailed overview of the characteristics of the RCTs and the participants that were incorporated into the study. This research encompassed a total of 35 RCTs, which included 15 phase 2 trials and 20 phase 3 trials, all of which were published in English between the years 2011 and 2024. The study population consisted of 8,839 OC patients who were assigned to the anti-angiogenic agent treatment group, while 7,360 patients were administered either a placebo alone or underwent drug therapy that did not involve anti-angiogenic agents. The anti-angiogenic drugs utilized were categorized into VEGF inhibitors (specifically bevacizumab), VEGFR inhibitors (which included pazopanib, cediranib, apatinib, sorafenib, and nintedanib), and angiopoietin inhibitors (solely trebananib). The design of anti-angiogenic therapy was bifurcated into monotherapy with anti-angiogenic drugs and combination therapy with chemotherapy or PARP inhibitors. The corresponding control design was either placebo alone, chemotherapy (alone or plus placebo), or PARP inhibitors only. Notably, the only PARP inhibitor used in the trials was olaparib. Out of the 35 RCTs, 31 were assessed as high quality, whereas 4 were deemed low quality. A notable methodological limitation observed was the lack of double-blinding in the trial design among multiple RCTs (Supplementary Files S2).

3.3 Overall analysis of anti-angiogenic drug monotherapy

5 RCTs were conducted to evaluate the PFS benefit of anti-angiogenic drug monotherapy in OC patients. Owing to substantial heterogeneity observed across these trials, a random-effects model was employed for analysis (I^2 = 72.1%, Tau^2 = 0.0791). The combined estimate indicated that anti-angiogenic monotherapy did not provide a significant PFS advantage over placebo (HR [95% CI] = 0.956 [0.709–1.288], 95% PI: 0.345–2.645). Similarly, the consolidated results from a fixed-effects model (I^2 = 8.6%, Tau^2 = 0.0027), derived from 6 RCTs, revealed that anti-angiogenic drug monotherapy did not significantly enhance OS (HR [95% CI] = 1.039 [0.921–1.173], 95% PI: 0.824–1.331). A single study reported on the ORR associated with monotherapy (Ferron et al., 2023), revealing a lower ORR with the use of anti-angiogenic monotherapy (specifically nintedanib) as compared to placebo (RR [95% CI] = 0.628 [0.447–0.882]). Concerning AEs, pooled results from 3 trials suggested that the incidence of any grade AEs was significantly higher with anti-angiogenic monotherapy compared to placebo (RR [95% CI] = 1.072 [1.036–1.109], 95% PI: 0.709–1.592; I^2 = 40.1%, Tau^2 = 0.0006). However, there was no significant difference in the risk of grade ≥3 AEs between the monotherapy group and the control group (RR [95% CI] = 1.905 [0.766–4.736]; I^2 = 95.0%, Tau^2 = 0.6005) (Table 2; Figure 2).

TABLE 1 The basic characteristics of the included RCTs.

Study ID (trial name/ Phase)act	Patients' status	Drug type	Sample size (E/C)	Median age (E/C, years)	Anti-angiogenic agent treatment group	Control group treatment	Median follow-up duration (E/C, months)	Outcomes in meta-analysis
Coleman 2017 (GOG-0213/ Phase 3)	Recurrent, platinum-sensitive, epithelial ovarian, primary peritoneal, or fallopian tube cancer; GOG PS of 0–2	VEGF inhibitor	337/337	59.5/60.6	Pac (175 mg/m^2) + Carbo (AUC 5) + Bev (15 mg/kg), q3w	Pac (175 mg/m^2) + Carbo (AUC 5), q3w	49.6	PFS, OS, ORR, Grade ≥3 AEs
Pignata 2021 (MITO16b/ MANGO–OV2/ ENGOT–ov17/ Phase 3)	Platinum-sensitive, FIGO stage IIIB-IV ovarian cancer, fallopian tube carcinoma, or peritoneal carcinoma; ECOG PS of 0–2	VEGF inhibitor	203/203	61/60	Carbo-based doublet + Bev (10 mg/kg intravenous every 14 days)	Carbo-based doublet, i.v	20.1	PFS, OS, ORR, Grade ≥3 AEs
Richardson 2018 (NCT01468909/ Phase 2)	Recurrent or persistent epithelial ovarian, fallopian tube, or primary peritoneal cancer; GOG PS of 0–1	VEGFR inhibitor	54/52	61/61	Pac (80 mg/m^2 on days 1, 8 and 15 every 28 days) + Pazo 800 mg daily	Pac (80 mg/m^2 on days 1, 8 and 15 every 28 days) + Placebo 800 mg daily	17.7	PFS, OS, ORR
Monk 2016b (TRINOVA-1/ Phase 3)	Recurrent partially platinum-sensitive or resistant epithelial ovarian, primary peritoneal or fallopian tube cancers; GOG PS of 0–1	Angiopoietin inhibitor	461/458	60/59	Pac (80 mg/m^2, days 1, 8, 15, q4w) + Tre (15 mg/kg, qw)	Pac (80 mg/m2, days 1, 8, 15, q4w) + Placebo (15 mg/kg, qw)	18/17.5	PFS, OS, AEs of any grade, Grade ≥3 AEs
Aghajanian 2015 (OCEANS/ Phase 3)	Platinum-sensitive, recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma; ECOG PS of 0–1	VEGF inhibitor	242/242	60/61	Cycles 1–6: Gem (1,000 mg/m^2, days 1 and 8) + Carbo (AUC 4, day 1) + Bev (15 mg/kg on day 1, 6–10 cycles of 21 days); Cycles 10+: Bev (15 mg/kg)	Cycles 1–6: Gem (1,000 mg/m^2, days 1 and 8) and Carbo (AUC 4, day 1) + Placebo (15 mg/kg on day 1,6–10 cycles of 21 days); Cycles 10+: Placebo (15 mg/kg)	9.6/8.4	OS, AEs of any grade, Grade ≥3 AEs
Karlan 2012 (NCT00479817/ Phase 2)	FIGO stage II-IV, recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer; ECOG PS of 0–1	Angiopoietin inhibitor	53 (Tre 3 mg/kg)/53 (Tre 10 mg/kg)/ 55	60 (Tre 3 mg/kg)/59 (Tre 10 mg/kg)/ 62	Pac (80 mg/m^2, days 1, 8, 15, q4w) + Tre (3 mg/kg or 10 mg/kg, qw)	Pac (80 mg/m^2, days 1, 8, 15, q4w) + Placebo (3 mg/kg or 10 mg/kg, qw)	15.2 (Tre 3 mg/kg)/15.4 (Tre 10 mg/kg)/14.9	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs
Nicum 2024 (OCTOVA/ Phase 2)	Platinum-resistant, relapsed, ovarian, fallopian tube, or primary peritoneal cancer; ECOG PS of ≤2	VEGFR inhibitor	47/46	66/65	Ola (300 mg twice daily) + Ced (20 mg once daily)	Ola (300 mg twice daily)	18	PFS, OS, ORR

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TABLE 1 (*Continued*) The basic characteristics of the included RCTs.

Study ID (trial name/ Phase)act	Patients' status	Drug type	Sample size (E/C)	Median age (E/C, years)	Anti-angiogenic agent treatment group	Control group treatment	Median follow-up duration (E/C, months)	Outcomes in meta-analysis
Ledermann 2016 (ICON6/Phase 3)	Platinum-sensitive, relapsed, epithelial ovarian cancer, primary peritoneal carcinomatosis or fallopian tube cancer after first-line platinum-based chemotherapy; ECOG PS of 0–1	VEGFR inhibitor	164/118	62/62	Platinum-based chemotherapy + Ced (20 mg, qd) then maintenance Ced (20 mg, qd) alone	Platinum-based chemotherapy + Placebo (20 mg, qd) then maintenance Placebo (20 mg, qd) alone	19.5	PFS
Wang 2022 (APPROVE/ Phase 2)	Platinum-resistant, recurrent epithelial ovarian cancer, primary peritoneal cancer, or fallopian tube cancer; ECOG PS of 0–1	VEGFR inhibitor	78/74	54/56	PLD (i.v., 40 mg/m^2, q4w, up to 6 cycles) + Apa (orally, 250 mg, qd, up to 6 cycles)	PLD (i.v., 40 mg/m^2, q4w, up to 6 cycles)	8.7	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs
Shoji 2022 (JGOG3023/ Phase 2)	Platinum-resistant, epithelial ovarian, fallopian tube, or primary peritoneal carcinoma; ECOG PS of 0–2	VEGF inhibitor	52/51	60.3 (mean age)/60.7 (mean age)	Chemotherapy (PLD/Topo/Pac/Gem) + Bev (i.v., 15 mg/kg)	Chemotherapy (PLD/Topo/Pac/Gem)	NA	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs
Gotlieb 2012 (EFC6125/ Phase 2)	Platinum-resistant, and Topo-resistant and/or PLD-resistant disease; advanced ovarian cancer patients with recurrent symptomatic malignant ascites; ECOG PS of 0–2	VEGF inhibitor	29/26	60.0/53.5	Afli (i.v., 4.0 mg/kg, q2w)	Placebo (i.v., 4.0 mg/kg, q2w)	NA	OS, AEs of any grade
Marth 2017 (ENGOT-ov-6/ TRINOVA-2/ Phase 3)	Platinum-resistant epithelial ovarian, peritoneal or fallopian tube cancer; ECOG PS of 0–2	Angiopoietin inhibitor	114/109	61/60	PLD (50 mg/m^2, q4w) + Tre (15 mg/kg, qw)	PLD (50 mg/m^2, q4w) + Placebo (15 mg/kg, qw)	12.4	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs
Pignata 2015 (MITO 11/ Phase 2)	Platinum-resistant or refractory ovarian cancer; ECOG PS of 0–1	VEGFR inhibitor	37/36	56/58	Pac (80 mg/m^2 on days 1, 8 and 15 in every 28 days) + Pazo (800 mg daily)	Pac (80 mg/m^2 on days 1, 8 and 15 every 28 days)	16.3/16.1	PFS, OS, ORR, Grade ≥3 AEs
Chekerov 2018 (TRIAS/Phase 2)	Platinum-resistant ovarian, peritoneal, or fallopian tube cancers; ECOG PS of 0–2	VEGFR inhibitor	83/89	59/58	Cycles 1–6: Topo (1–25 mg/m^2 on days 1–5) + Sor (400 mg oral bid on days 6–15, every 21 days); Cycles 6+:	Cycles 1–6: Topo (1–25 mg/m^2 on days 1–5) + Placebo (bid on days 6–15, every 21 days); Cycles 6+: Daily	11.3/8.7	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs

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TABLE 1 (*Continued*) The basic characteristics of the included RCTs.

Study ID (trial name/ Phase)act	Patients' status	Drug type	Sample size (E/C)	Median age (E/C, years)	Anti-angiogenic agent treatment group	Control group treatment	Median follow-up duration (E/C, months)	Outcomes in meta-analysis
					Daily maintenance Sor for up to 1 year	maintenance Placebo for up to 1 year		
Liu 2019 (NCT01116648/ Phase 2)	Relapsed platinum-sensitive ovarian cancer of high-grade serous or endometrioid histology or had a deleterious germline BRCA1/ 2 mutation	VEGFR inhibitor	44/46	58.1/57.8	Ola (200 mg, bid) + Ced (30 mg daily)	Ola (400 mg, bid)	46	PFS, OS
Pujade-Lauraine 2014 (AURELIA/ Phase 3)	Platinum-resistant, recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer; ECOG PS of 0–2	VEGF inhibitor	179/182	62/61	Chemotherapy (PLD/Pac/Topo) + Bev (15 mg/kg, q3w or 10 mg/kg, q2w)	Chemotherapy (PLD/Pac/Topo)	13.0/13.9	PFS, OS, ORR
Liu 2022 (NRG-GY004/Phase 3)	Platinum-sensitive, relapsed high-grade serous or high-grade endometrioid ovarian, primary peritoneal, or fallopian tube cancer	VEGFR inhibitor	189/189	NA	Ola (200 mg tablets, bid) + Ced (30 mg tablet, qd)	Ola (300 mg tablets, bid)	24 (mean duration)	PFS, ORR
Ledermann 2021 (ICON6/Phase 3)	Platinum-sensitive, relapsed, epithelial ovarian cancer, primary peritoneal carcinomatosis or fallopian tube cancer after first-line platinum-based chemotherapy; ECOG PS of 0–1	VEGFR inhibitor	164/118	62/62	Platinum-based chemotherapy + Ced (20 mg, qd) then maintenance Ced (20 mg, qd) alone	Platinum-based chemotherapy + Placebo (20 mg, qd) then maintenance Placebo (20 mg, qd) alone	25.6	OS
Ferron 2023 (GINECO/ Phase 2)	Newly diagnosed epithelial ovarian, fallopian tube, or primary peritoneal cancer; FIGO stage IIIC/ IV, and ECOG PS of ≤2	VEGFR inhibitor	124/64	64/63.5	Nin (200 mg, bid, on days 2–21, q3w, for up to 2 years)	Placebo (bid, on days 2–21, q3w, for up to 2 years)	42.6	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs
Burger 2011 (GOG-0218/ Phase 3)	Newly diagnosed, FIGO stage III or IV epithelial ovarian, primary peritoneal or fallopian tube cancer; GOG PS of 0–2	VEGF inhibitor	623/625	60/60	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 6) + Bev (15 mg/kg), q3w; Cycles 7–22: Bev (15 mg/kg), q3w	Cycles1-6: Pac (175 mg/m^2) + Carbo (AUC 6) + Placebo, q3w; Cycles 7–22: Placebo,q3w	17.4	PFS

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TABLE 1 (*Continued*) The basic characteristics of the included RCTs.

Study ID (trial name/ Phase)act	Patients' status	Drug type	Sample size (E/C)	Median age (E/C, years)	Anti-angiogenic agent treatment group	Control group treatment	Median follow-up duration (E/C, months)	Outcomes in meta-analysis
Aghajanian 2012 (OCEANS/ Phase 3)	Platinum-sensitive, recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma; ECOG PS of 0–1	VEGF inhibitor	242/242	60.5/61.6	Cycles 1–10: Gem (1,000 mg/m^2 on days 1 and 8) + Carbo (AUC 4 on day 1) + Bev (15 mg/kg on day 1), q3w	Cycles 1–10: Gem (1,000 mg/m^2, days 1 and 8) + Carbo (AUC 4, day 1) + Placebo (15 mg/kg, day 1), q3w	24	PFS, ORR
Oza 2015 (ICON7/ Phase 3)	FIGO stage I-IIA newly diagnosed ovarian cancer or more FIGO stage IIB-IV advanced disease	VEGF inhibitor	764/764	57	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 5 or 6), q3w + Bev (7.5 mg/kg, q3w); Cycles 7–18: Bev (7.5 mg/kg, q3w)	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 5 or 6), q3w	48.8/48.6	PFS, OS
du Bois 2016 (AGO-OVAR 12/ Phase 3)	Chemotherapy-naive, FIGO stage IIB-IV, epithelial ovarian cancer, fallopian tube or primary peritoneal cancer; ECOG PS of 0–2	VEGFR inhibitor	911/455	58/58	Cycles1-6: Pac (175 mg/m^2) + Carbo (AUC 5 or 6) + Nin (200 mg, bid, days 2–21, q3w) followed by Nin maintenance	Cycles1-6: Pac (175 mg/m^2) + Carbo (AUC 5 or 6) + Placebo (200 mg, bid, days 2–21, q3w) followed by Placebo maintenance	18	AEs of any grade, Grade ≥3 AEs
Ledermann 2011 (NCT00710762/ Phase 2)	Advanced ovarian carcinoma, fallopian tube carcinoma or primary peritoneal cancer of serous type with recurrent disease and who responded to second-, third-, or fourth-line chemotherapy; ECOG PS of 0–1	VEGFR inhibitor	43/40	60/63	Cycles 1–9: Nin (250 mg, bid, q4w)	Cycles 1–9: Placebo (250 mg, bid, q4w)	36 weeks (follow-up endpoint)	PFS, OS, Grade ≥3 AEs
du Bois 2014 (NCT00866697/ Phase 3)	FIGO stage II-IV, epithelial ovarian, fallopian tube or primary peritoneal carcinoma who have not progressed after first-line chemotherapy; ECOG PS of 0–1	VEGFR inhibitor	472/468	56/57	Pazo (800 mg, orally, qd, for up to 24 months)	Placebo (800 mg, orally, qd, for up to 24 months)	24.3	PFS, OS
Herzog 2013 (NCT00791778/ Phase 2)	FIGO stage III-IV ovarian epithelial cancer or primary peritoneal cancer who have achieved a response after standard platinum/taxane containing	VEGFR inhibitor	123/123	56.9/54.4	Sor (400 mg, orally, bid, every 12 h)	Placebo (400 mg, orally, bid, every 12 h)	NA	PFS, OS

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TABLE 1 (*Continued*) The basic characteristics of the included RCTs.

Study ID (trial name/ Phase)act	Patients' status	Drug type	Sample size (E/C)	Median age (E/C, years)	Anti-angiogenic agent treatment group	Control group treatment	Median follow-up duration (E/C, months)	Outcomes in meta-analysis
	chemotherapy (first-line therapy); ECOG PS of 0–1							
Tewari 2019 (GOG-0218/ Phase 3)	Newly diagnosed ovarian, fallopian tube, or primary peritoneal carcinoma	VEGF inhibitor	623/625	60/60	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 6) + Bev (15 mg/kg, cycle 2 +) every 21 days; Cycles 7–22: Bev maintenance (15 mg/kg) every 21 days	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 6) + Placebo (cycle 2+) every 21 days; Cycles 7–22: Placebo every 21 days	101.9/103.4	OS
Vergote 2019a (AGO-OVAR16/ Phase 3)	FIGO stage II-IV epithelial ovarian, fallopian tube, or primary peritoneal carcinoma	VEGFR inhibitor	472/468	56/57	Pazo (800 mg, qd, for up to 24 months)	Placebo (800 mg, qd, for up to 24 months)	NA	OS
Kim 2018 (East Asian study/ Phase 3)	Advanced ovarian, fallopian tube or primary peritoneal carcinoma	VEGFR inhibitor	73/72	51.7/54.1	Pazo (800 mg, qd, for up to 24 months)	Placebo (800 mg, qd, for up to 24 months)	NA	PFS, AEs of any grade, Grade ≥3 AEs
Ray-Coquard 2020 (AGO-OVAR 12/ Phase 3)	FIGO stage IIB-IV newly diagnosed advanced epithelial ovarian, fallopian tube or primary peritoneal cancer	VEGFR inhibitor	911/455	58/58	Nin (200 mg, bid, on days 2–21, every 21 days) + Pac (175 mg/m^2) + Carbo (AUC 5 or 6, day 1, every 21 days for six cycles)	Placebo (200 mg, bid, on days 2–21, every 21 days) + Pac (175 mg/m^2) + Carbo (AUC 5 or 6, day 1, every 21 days for six cycles)	60.9	PFS, OS
Vergote 2019b (TRINOVA-3/ Phase 3)	FIGO stage III-IV epithelial ovarian, primary peritoneal, or fallopian tube cancer; ECOG PS of 0–1	Angiopoietin inhibitor	678/337	59/59	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 5 or 6, every 3 weeks) + Tre (15 mg/kg); Cycles 6+: Tre for up to 18 additional months	Cycles 1–6: Pac (175 mg/m^2) + Carbo (AUC 5 or 6, every 3 weeks) + Placebo (15 mg/kg); Cycles 6+: Placebo for up to 18 additional months	27.4	PFS, OS, AEs of any grade, Grade ≥3 AEs
Duska 2020 (NCT01610206/ Phase 2)	Persistent or recurrent epithelial ovarian, fallopian tube or primary peritoneal carcinoma	VEGFR inhibitor	75/73	63	Gem (1,000 mg/m^2, weekly on days 1 and 8, every 21 days + Pazo (800 mg, orally, daily)	Gem (1,000 mg/m^2, weekly on days 1 and 8, every 21 days	13	PFS, ORR
Roque 2022 (NCT03093155/ Phase 2)	Platinum-resistant or refractory epithelial (non-mucinous) ovarian, fallopian tube, or primary peritoneal carcinoma; ECOG PS of 0–2	VEGF inhibitor	39/37	67/67	Ixa (20 mg/m^2, i.v., days 1, 8, and 15 of a 28-day cycle) + Bev (10 mg/kg, i.v., days 1, 15 every 28 days)	Ixa (20 mg/m^2, i.v., days 1, 8, and 15 of a 28-day cycle)	NA	PFS, OS, ORR

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TABLE 1 (*Continued*) The basic characteristics of the included RCTs.

Study ID (trial name/ Phase)act	Patients' status	Drug type	Sample size (E/C)	Median age (E/C, years)	Anti-angiogenic agent treatment group	Control group treatment	Median follow-up duration (E/C, months)	Outcomes in meta-analysis
Hall 2020 (NCT01610869/ Phase 2)	Platinum resistant or intolerant ovarian, fallopian tube or primary peritoneal carcinoma	VEGFR inhibitor	59/55	62.4/65.7	Cyc (orally, 100 mg, qd, in cycles of 6 weeks) + Nin (200 mg, bid)	Cyc (orally, 100 mg, qd, in cycles of 6 weeks)	19.2	PFS, OS, ORR, AEs of any grade, Grade ≥3 AEs
Gore 2019 (mEOC/GOG 0241/Phase 3)	FIGO stage II-IV primary mucinous epithelial ovarian cancer or recurrence after stage I disease	VEGF inhibitor	24/26	47; 51/55; 56	Pac (175 mg/m^2) + Carbo (AUC5 or 6) + Bev (15 mg/kg, 3-weekly maintenance, 12cycles); Oxa (130 mg/m^2) + Cap (850 mg/m^2, bid, days 1–14) + Bev (15 mg/kg, 3-weekly maintenance, 12 cycles)	Pac (175 mg/m^2) + Carbo (AUC 5 or 6); Oxa (130 mg/m^2) + Cap (850 mg/m^2, bid, days 1–14)	59	PFS, OS, ORR, Grade ≥3 AEs

E, experimental group; C, control group; GOG, the Gynecologic Oncology Group; PS, performance status; VEGF, vascular endothelial growth factor; Pac, paclitaxel; Carbo, carboplatin; AUC, area under curve; Bev, bevacizumab; q3w, every 3 weeks; PFS, progression-free survival; OS, overall survival; ORR, objective response rate; AEs, adverse events; FIGO, international federation of gynecology and obstetrics; ECOG, eastern cooperative oncology group; VEGFR, vascular endothelial growth factor receptor; Pazo, pazopanib; Tre, trebananib; Gem, gemcitabine; Ola, olaparib; Ced, cediranib; qd, once daily; PLD, pegylated liposomal doxorubicin; i.v., intravenously; Apa, Apatinib; NA, not available; Afli, aflibercept; Topo, topotecan; Sor, sorafenib; bid, twice daily; Nin, nintedanib; Ixa, ixabepilone; Cyc, cyclophosphamide; Oxa, oxaliplatin; Cap, capecitabine.

TABLE 2 Pooled effect of the efficacy and safety of monotherapy or combination therapy with anti-angiogenic drugs in the treatment of ovarian cancer.

Outcomes	Number of studies	Meta-analysis				Heterogeneity	
		HR/RR	95% CI	*p*-value	95% PI	I^2, Tau^2	*p*-value
Antiangiogenic agent monotherapy vs Placebo							
PFS	5	0.956	0.709–1.288	0.766	0.345–2.645	72.1%, 0.0791	0.006
OS	6	1.039	0.921–1.173	0.532	0.824–1.331	8.6%, 0.0027	0.361
ORR	1	0.628	0.447–0.882	0.007			
AEs of any grade	3	1.072	1.036–1.109	<0.001	0.709–1.592	40.1%, 0.0006	0.188
Grade ≥3 AEs	3	1.905	0.766–4.736	0.166	-	95.0%, 0.6005	<0.001
Antiangiogenic agents + Other drugs vs Other drugs (alone or + Placebo)							
PFS	24	0.678	0.606–0.759	<0.001	0.415–1.108	79.5%, 0.0529	<0.001
OS	23	0.917	0.870–0.966	0.001	0.851–0.984	2.6%, 0.0005	0.425
ORR	18	1.441	1.287–1.614	<0.001	1.032–2.014	52.1%, 0.0216	0.005
AEs of any grade	11	1.011	0.999–1.022	0.069	0.980–1.043	56.6%, 0.0002	0.011
Grade ≥3 AEs	15	1.137	1.099–1.177	<0.001	1.011–1.252	33.4%, 0.0019	0.101

PFS, progression-free survival; OS, overall survival; ORR, objective response rate; AEs, adverse events.

3.4 Overall analysis of anti-angiogenic drug combination therapy

A total of 24 RCTs evaluated PFS advantage of anti-angiogenic drug combination therapy in patients with OC. Given the notable heterogeneity in the studies regarding PFS, a random-effects model was utilized for the pooled PFS analysis (I^2 = 79.5%, Tau^2 = 0.0529). The overall analysis revealed that the combination therapy of anti-angiogenic drugs led to a 32.2% decrease in the risk of disease progression or death when contrasted with regimens excluding anti-angiogenic drugs (HR [95% CI] = 0.678 [0.606–0.759], 95% PI: 0.415–1.108). Likewise, the pooled findings from a fixed-effects

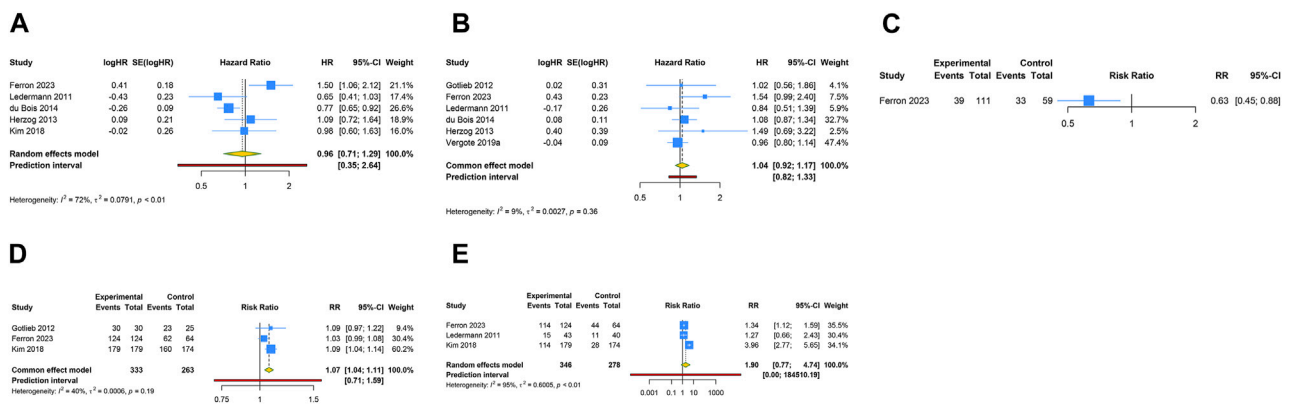

FIGURE 2
Forest plot of the efficacy and safety outcomes after anti-angiogenic agent monotherapy for ovarian cancer. **(A)** Progression-free survival; **(B)** Overall survival; **(C)** Objective response rate; **(D)** Adverse events of any grade; **(E)** Grade ≥3 adverse events.

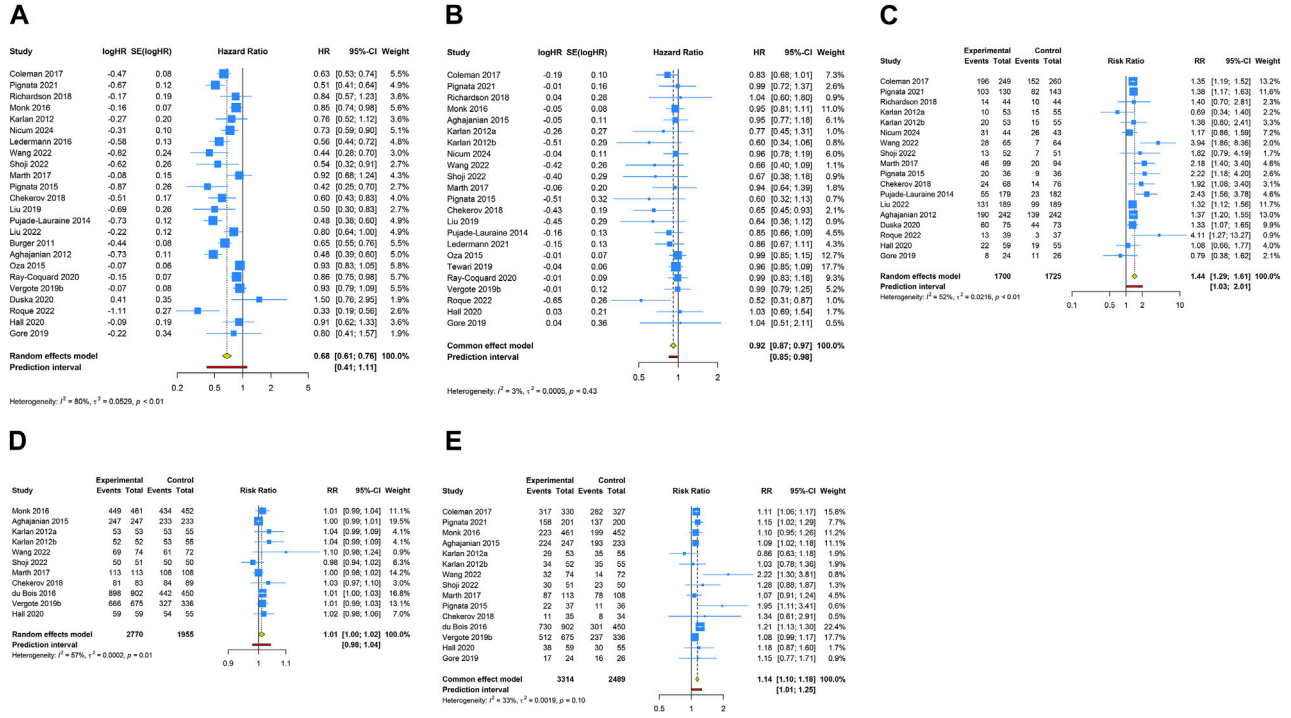

FIGURE 3
Forest plot of the efficacy and safety outcomes after anti-angiogenic drug combination therapy for ovarian cancer. **(A)** Progression-free survival; **(B)** Overall survival; **(C)** Objective response rate; **(D)** Adverse events of any grade; **(E)** Grade ≥3 adverse events.

model (I^2 = 2.6%, Tau^2 = 0.0005), based on 23 RCTs, demonstrated a significant improvement in OS when anti-angiogenic drugs were used in combination therapy compared with the control (HR [95% CI] = 0.917 [0.870–0.966], 95% PI: 0.851–0.984). Furthermore, 18 studies reported the ORR outcome of combination therapy, and the results showed that the ORR of anti-angiogenic agents combined with other drugs was significantly higher than that of other drugs alone (RR [95% CI] = 1.441 [1.287–1.614], 95% PI: 1.032–2.014; I^2 = 52.1%, Tau^2 = 0.0216). Regarding AEs, the consolidated results from 11 trials indicated no significant difference in the risk of any grade AEs between the combination therapy group and the control group (RR [95% CI] = 1.011 [0.999–1.022], 95% PI: 0.980–1.043; I^2 = 56.6%, Tau^2 = 0.0002). However, the occurrence of grade ≥3 AEs was significantly increased in the combination therapy group compared to the control group (RR [95% CI] = 1.137 [1.099–1.177], 95% PI: 1.011–1.252; I^2 = 33.4%, Tau^2 = 0.0019) (Table 2; Figure 3).

TABLE 3 Subgroup analysis of the efficacy and safety of anti-angiogenic agent monotherapy for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis			95% PI	Heterogeneity	
		HR/RR	95% CI	*p*-value		I^2, Tau^2	*p*-value
PFS							
Subgrouped by ovarian cancer subtypes							
Advanced ovarian cancer	3	1.003	0.603–1.671	0.99	0.003–396.633	76.2%, 0.1538	0.015
Subgrouped by types of anti-angiogenic drugs							
VEGFR inhibitors vs Placebo	5	0.956	0.709–1.288	0.766	0.345–2.645	72.1%, 0.0791	0.006
Nintedanib vs Placebo	2	1.001	0.441–2.271	0.998	-	87.9%, 0.3072	0.004
Pazopanib vs Placebo	2	0.791	0.670–0.934	0.006	-	0%, 0	0.366
OS							
Subgrouped by ovarian cancer subtypes							
Advanced ovarian cancer	4	1.179	0.899–1.548	0.234	0.474–2.911	19.6%, 0.0197	0.292
Subgrouped by types of anti-angiogenic drugs							
VEGFR inhibitors vs Placebo	5	1.04	0.919–1.177	0.532	0.707–1.597	26.8%, 0.0093	0.243
Nintedanib vs Placebo	2	1.151	0.636–2.084	0.643	-	68.3%, 0.1255	0.076
Pazopanib vs Placebo	2	1.007	0.880–1.154	0.917	-	0%, 0	0.403
AEs of any grade							
Subgrouped by ovarian cancer subtypes							
Advanced ovarian cancer	3	1.072	1.036–1.109	<0.001	0.709–1.592	40.1%, 0.0006	0.188
Subgrouped by types of anti-angiogenic drugs							
VEGFR inhibitors vs Placebo	2	1.059	1.003–1.119	0.04	-	68.2%, 0.0011	0.076
Grade ≥3 AEs							
Subgrouped by ovarian cancer subtypes							
Advanced ovarian cancer	3	1.905	0.766–4.736	0.166	-	95.0%, 0.6005	<0.001
Subgrouped by types of anti-angiogenic drugs							
VEGFR inhibitors vs Placebo	3	1.905	0.766–4.736	0.166	-	95.0%, 0.6005	<0.001
Nintedanib vs Placebo	2	1.326	1.109–1.586	0.002	-	0%, 0	0.869

PFS, progression-free survival; OS, overall survival; AEs, adverse events.

3.5 Subgroup analysis of anti-angiogenic drug monotherapy

Subgroup analyses were conducted only for categories comprising two or more studies. When stratified by OC subtype, it was observed that anti-angiogenic drug monotherapy escalated the risk of any grade AEs in patients with advanced OC, relative to placebo (RR [95% CI] = 1.072 [1.036–1.109], 95% PI: 0.709–1.592; I^2 = 40.1%, Tau^2 = 0.0006). Yet, in the context of advanced OC, no significant impact on PFS, OS, or the occurrence of grade ≥ 3AEs was observed with anti-angiogenic drug monotherapy (all $p > 0.05$). Further, stratified analyses predicated on the classification of anti-angiogenic drugs revealed an increased incidence of any grade AEs with VEGFR inhibitors compared to placebo (RR [95% CI] = 1.059 [1.003–1.119]; I^2 = 68.2%, Tau^2 = 0.0011). Subsequent analysis grouped by specific anti-angiogenic agents suggested that pazopanib significantly improved PFS (HR [95% CI] = 0.791 [0.670–0.934]; I^2 = 0%, Tau^2 = 0), while nintedanib was associated with a higher incidence of grade ≥3 AEs (RR [95% CI] = 1.326 [1.109–1.586]; I^2 = 0%, Tau^2 = 0). The complete results of the subgroup analysis were detailed in Table 3 and Supplementary Figure S1–S4.

3.6 Subgroup analysis of anti-angiogenic drug combination therapy

Subgroup analyses were carried out solely for groups that included two or more studies. Categorized by OC subtypes, it was observed that anti-angiogenic drug combination therapy significantly improved PFS compared with drug therapy without anti-angiogenic agents in patients with platinum-sensitive and recurrent OC (HR [95% CI] = 0.612 [0.519–0.722], 95% PI: 0.355–1.055; I^2 = 78.5%, Tau^2 = 0.0460), platinum-resistant OC (HR [95% CI] = 0.691 [0.494–0.966], 95% PI: 0.019–25.494; I^2 = 59.4%, Tau^2 = 0.0514), newly diagnosed OC (HR [95% CI] = 0.807 [0.657–0.990],

TABLE 4 Subgroup analysis of the efficacy and safety of anti-angiogenic agent combination therapy for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis			95% PI	Heterogeneity	
		HR/RR	95% CI	*p*-value		I^2, Tau^2	*p*-value
PFS							
Subgrouped by ovarian cancer subtypes							
Platinum-sensitive and recurrent ovarian cancer	9	0.612	0.519–0.722	<0.001	0.355–1.055	78.5%, 0.0460	<0.001
Platinum-resistant ovarian cancer	3	0.691	0.494–0.966	0.031	0.019–25.494	59.4%, 0.0514	0.085
Newly diagnosed ovarian cancer	3	0.807	0.657–0.990	0.039	0.066–9.808	85.3%, 0.0278	0.001
Recurrent or persistent ovarian cancer	3	0.872	0.678–1.120	0.283	0.048–16.920	33.4%, 0.0269	0.223
Platinum-resistant or refractory ovarian cancer	2	0.374	0.259–0.540	<0.001	-	0%, 0	0.52
Advanced ovarian cancer	3	0.752	0.493–1.146	0.185	0.004–135.898	89.1%, 0.1210	<0.001
Subgrouped by types of anti-angiogenic drugs							
VEGF inhibitors + CT vs CT (alone or + PL)	9	0.580	0.470–0.715	<0.001	0.286–1.175	86.7%, 0.0779	<0.001
Bevacizumab + CT vs CT (alone or + PL)	9	0.580	0.470–0.715	<0.001	0.286–1.175	86.7%, 0.0779	<0.001
VEGFR inhibitors + Other drugs vs Other drugs (alone or + PL)	11	0.697	0.595–0.818	<0.001	0.426–1.143	66.0%, 0.0410	0.001
Pazopanib + CT vs CT (alone or + PL)	3	0.786	0.415–1.490	0.461	-	78.7%, 0.2475	0.009
Cediranib + Other drugs vs Other drugs (alone or + PL)	4	0.669	0.552–0.810	<0.001	0.324–1.381	51.5%, 0.0189	0.103
Nintedanib + CT vs CT (alone or + PL)	2	0.865	0.763–0.982	0.025	-	0%, 0	0.782
Angiopoietin inhibitors + CT vs PL + CT	4	0.879	0.798–0.968	0.009	0.711–1.087	0%, 0	0.722
Trebananib + CT vs PL + CT	4	0.879	0.798–0.968	0.009	0.711–1.087	0%, 0	0.722
OS							
Subgrouped by ovarian cancer subtypes							
Platinum-sensitive and recurrent ovarian cancer	8	0.892	0.822–0.968	0.006	0.806–0.988	0%, 0	0.668
Platinum-resistant ovarian cancer	3	0.753	0.592–0.956	0.02	0.146–3.886	2.6%, 0.0013	0.358
Newly diagnosed ovarian cancer	3	0.977	0.899–1.061	0.575	0.570–1.673	0%, 0	0.937
Recurrent or persistent ovarian cancer	3	0.788	0.574–1.083	0.142	0.101–6.159	0%, 0	0.391
Platinum-resistant or refractory ovarian cancer	2	0.551	0.369–0.821	0.003	-	0%, 0	0.731
Advanced ovarian cancer	3	0.997	0.841–1.181	0.972	0.332–2.994	0%, 0	0.985
Subgrouped by types of anti-angiogenic drugs							
VEGF inhibitors + CT vs CT (alone or + PL)	9	0.923	0.859–0.991	0.028	0.791–1.061	13.5%, 0.0021	0.322
Bevacizumab + CT vs CT (alone or + PL)	9	0.923	0.859–0.991	0.028	0.791–1.061	13.5%, 0.0021	0.322
VEGFR inhibitors + Other drugs vs Other drugs (alone or + PL)	9	0.895	0.809–0.990	0.031	0.690–1.112	19.2%, 0.0064	0.272
Pazopanib + Paclitaxel vs Paclitaxel (alone or + PL)	2	0.822	0.544–1.242	0.351	-	40.1%, 0.0606	0.197
Cediranib + Other drugs vs Other drugs (alone or + PL)	3	0.892	0.763–1.043	0.152	0.323–2.461	0%, 0	0.392
Nintedanib + CT vs CT (alone or + PL)	2	0.996	0.850–1.167	0.960	-	0%, 0	0.860
Angiopoietin inhibitors + CT vs PL + CT	5	0.931	0.828–1.047	0.235	0.770–1.127	0%, 0	0.538
Trebananib + CT vs PL + CT	5	0.931	0.828–1.047	0.235	0.770–1.127	0%, 0	0.538
ORR							
Subgrouped by ovarian cancer subtypes							
Platinum-sensitive and recurrent ovarian cancer	6	1.454	1.237–1.710	<0.001	0.898–2.356	70.0%, 0.0234	0.005
Platinum-resistant ovarian cancer	3	2.034	1.473–2.809	<0.001	0.252–16.525	0%, 0	0.901
Recurrent or persistent ovarian cancer	4	1.235	1.008–1.512	0.042	0.698–2.288	9.1%, 0.0065	0.348
Platinum-resistant or refractory ovarian cancer	2	2.704	1.535–4.763	0.001	-	0%, 0	0.355
Advanced ovarian cancer	2	1.321	1.122–1.554	0.001	-	0%, 0	0.335

(Continued on following page)

TABLE 4 (*Continued*) Subgroup analysis of the efficacy and safety of anti-angiogenic agent combination therapy for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis			95% PI	Heterogeneity	
		HR/RR	95% CI	*p*-value		I^2, Tau^2	*p*-value
Subgrouped by types of anti-angiogenic drugs							
VEGF inhibitors + CT vs CT (alone or + PL)	7	1.441	1.241–1.674	<0.001	0.985–2.109	55.2%, 0.0161	0.037
Bevacizumab + CT vs CT (alone or + PL)	7	1.441	1.241–1.674	<0.001	0.985–2.109	55.2%, 0.0161	0.037
VEGFR inhibitors + Other drugs vs Other drugs (alone or + PL)	8	1.444	1.191–1.752	<0.001	0.874–2.389	50.8%, 0.0325	0.047
Pazopanib + CT vs CT (alone or + PL)	3	1.465	1.186–1.811	<0.001	0.139–15.216	18.4%, 0.0150	0.294
Cediranib + Olaparib vs Olaparib	2	1.290	1.115–1.493	<0.001	-	0%, 0	0.476
Angiopoietin inhibitors + CT vs PL + CT	3	1.342	0.719–2.505	0.355	0.001–1810.780	73.2%, 0.2204	0.024
Trebananib + CT vs PL + CT	3	1.342	0.719–2.505	0.355	0.001–1810.780	73.2%, 0.2204	0.024
AEs of any grade							
Subgrouped by ovarian cancer subtypes							
Platinum-sensitive and recurrent ovarian cancer	3	1.018	0.971–1.068	0.463	0.586–1.768	88.9%, 0.0013	<0.001
Platinum-resistant ovarian cancer	3	1.007	0.983–1.033	0.56	0.780–1.280	38.7%, 0.0002	0.196
Recurrent ovarian cancer	2	1.037	0.992–1.084	0.107	-	0%, 0	0.982
Advanced ovarian cancer	3	1.014	1.003–1.025	0.014	0.948–1.085	0%, 0	0.971
Subgrouped by types of anti-angiogenic drugs							
VEGF inhibitors + CT vs CT (alone or + PL)	2	0.997	0.985–1.008	0.58	-	23.7%, <0.0001	0.252
Bevacizumab + CT vs CT (alone or + PL)	2	0.997	0.985–1.008	0.58	-	23.7%, <0.0001	0.252
VEGFR inhibitors + CT vs CT (alone or + PL)	4	1.023	1.007–1.039	0.004	0.965–1.075	15.9%, <0.0001	0.312
Nintedanib + CT vs CT (alone or + PL)	2	1.014	1.001–1.027	0.032	-	0%, 0	0.809
Angiopoietin inhibitors + CT vs PL + CT	5	1.015	1.002–1.029	0.03	0.991–1.031	2.7%, <0.0001	0.391
Trebananib + CT vs PL + CT	5	1.015	1.002–1.029	0.03	0.991–1.031	2.7%, <0.0001	0.391
Grade ≥3 AEs							
Subgrouped by ovarian cancer subtypes							
Platinum-sensitive and recurrent ovarian cancer	4	1.12	1.036–1.210	0.004	0.836–1.500	57.8%, 0.0031	0.069
Platinum-resistant ovarian cancer	3	1.13	0.973–1.313	0.11	0.443–2.740	0%, 0	0.566
Recurrent ovarian cancer	2	0.943	0.764–1.164	0.586	-	0%, 0	0.408
Advanced ovarian cancer	4	1.151	1.097–1.209	<0.001	0.921–1.428	34.2%, 0.0015	0.207
Subgrouped by types of anti-angiogenic drugs							
VEGF inhibitors + CT vs CT (alone or + PL)	5	1.122	1.075–1.170	<0.001	1.048–1.184	0%, 0	0.885
Bevacizumab + CT vs CT (alone or + PL)	5	1.122	1.075–1.170	<0.001	1.048–1.184	0%, 0	0.885
VEGFR inhibitors + CT vs CT (alone or + PL)	5	1.259	1.172–1.352	<0.001	0.720–2.667	49.8%, 0.0291	0.093
Nintedanib + CT vs CT (alone or + PL)	2	1.208	1.126–1.296	<0.001	-	0%, 0	0.879
Angiopoietin inhibitors + CT vs PL + CT	5	1.068	1.002–1.138	0.045	0.966–1.178	0%, 0	0.724
Trebananib + CT vs PL + CT	5	1.068	1.002–1.138	0.045	0.966–1.178	0%, 0	0.724

PFS, progression-free survival; CT, chemotherapy; PL, placebo; OS, overall survival; ORR, objective response rate; AEs, adverse events.

95% PI: 0.066–9.808; I^2 = 85.3%, Tau^2 = 0.0278), and platinum-resistant or refractory OC (HR [95% CI] = 0.374 [0.259–0.540]; I^2 = 0%, Tau^2 = 0). Similarly, it was noted that combination therapy with anti-angiogenic drugs was associated with a significant improvement in OS among patients with platinum-sensitive and recurrent OC (HR [95% CI] = 0.892 [0.822–0.968], 95% PI: 0.806–0.988; I^2 = 0%, Tau^2 = 0), platinum-resistant OC (HR [95% CI] = 0.753 [0.592–0.956], 95% PI: 0.146–3.886; I^2 = 2.6%, Tau^2 = 0.0013), and platinum-resistant or refractory OC (HR [95% CI] = 0.551 [0.369–0.821]; I^2 = 0%, Tau^2 = 0). Moreover, the combined therapeutic approach of anti-angiogenic drugs exhibited a comparatively high ORR for patients with platinum-sensitive and recurrent OC, platinum-resistant OC, recurrent or persistent OC, platinum-resistant or refractory OC, and advanced OC (all $p < 0.05$). However, it is important to note that for individuals with advanced OC, combination therapy with anti-angiogenic drugs can also lead to a higher incidence of any grade AEs (RR [95% CI] = 1.014 [1.003–1.025], 95% PI: 0.948–1.085; I^2 = 0%, Tau^2 = 0) and grade ≥3 AEs (RR [95% CI] = 1.151 [1.097–1.209], 95% PI: 0.921–1.428; I^2 = 34.2%, Tau^2 = 0.0015). Particularly, patients with platinum-sensitive and recurrent OC receiving combination therapy experienced an elevated frequency of grade ≥3 AEs (RR [95% CI] = 1.120 [1.036–1.210], 95% PI: 0.836–1.500; I^2 = 57.8%, Tau^2 = 0.0031) (Table 4; Supplementary Figure S5–S9).

Subgroup analysis according to the types of anti-angiogenic drugs indicated that VEGF inhibitors combined with chemotherapy

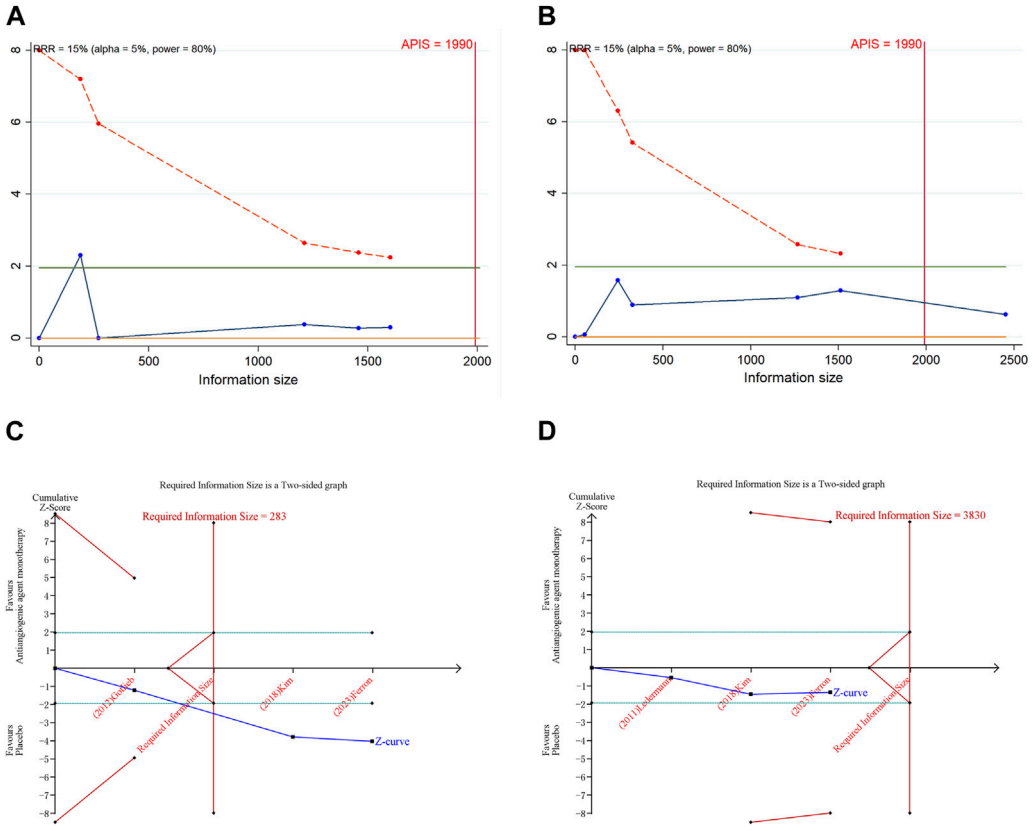

FIGURE 4
Trial sequential analysis of anti-angiogenic agent monotherapy for ovarian cancer. **(A)** Progression-free survival; **(B)** Overall survival; **(C)** Adverse events of any grade; **(D)** Grade ≥3 adverse events. Uppermost and lowermost red curves represent trial sequential monitoring boundary lines for benefit and harm, respectively. Inner red lines represent the futility boundary. Blue line represents evolution of cumulative Z-score. Horizontal green lines represent the conventional boundaries for statistical significance. Cumulative Z-curve crossing the trial sequential monitoring boundary or the RIS boundary provides firm evidence of effect.

significantly improved PFS (HR [95% CI] = 0.580 [0.470–0.715], 95% PI: 0.286–1.175; I^2 = 86.7%, Tau^2 = 0.0779) and OS (HR [95% CI] = 0.923 [0.859–0.991], 95% PI: 0.791–1.061; I^2 = 13.5%, Tau^2 = 0.0021), and also increased the ORR (RR [95% CI] = 1.441 [1.241–1.674], 95% PI: 0.985–2.109; I^2 = 55.2%, Tau^2 = 0.0161) and the risk of grade ≥3 AEs (RR [95% CI] = 1.122 [1.075–1.170], 95% PI: 1.048–1.184; I^2 = 0%, Tau^2 = 0) compared with chemotherapy alone or with placebo. These results were replicated in the combination therapy with bevacizumab. In addition, combination therapy with VEGFR inhibitors was found to be associated with improvements in PFS (HR [95% CI] = 0.697 [0.595–0.818], 95% PI: 0.426–1.143; I^2 = 66.0%, Tau^2 = 0.0410) and OS (HR [95% CI] = 0.895 [0.809–0.990], 95% PI: 0.690–1.112; I^2 = 19.2%, Tau^2 = 0.0064), along with an increase in the ORR (RR [95% CI] = 1.444 [1.191–1.752], 95% PI: 0.874–2.389; I^2 = 50.8%, Tau^2 = 0.0325). Yet, VEGFR inhibitor combination therapy also increased the incidence of any grade AEs (RR [95% CI] = 1.023 [1.007–1.039], 95% PI: 0.965–1.075; I^2 = 15.9%, Tau^2 < 0.0001) and grade ≥3 AEs (RR [95% CI] = 1.259 [1.172–1.352], 95% PI: 0.720–2.667; I^2 = 49.8%, Tau^2 = 0.0291). Further analysis grouped by specific anti-angiogenic agents suggested that combination therapy with cediranib significantly improved PFS and ORR. A similar enhancement in ORR was observed with pazopanib combination therapy. The nintedanib combination therapy, while improving PFS, also escalated the risk of any grade AEs and grade ≥3 AEs (all p < 0.05). With regard to angiopoietin inhibitors, the combined therapeutic strategy significantly improved PFS (HR [95% CI] = 0.879 [0.798–0.968], 95% PI: 0.711–1.087; I^2 = 0%, Tau^2 = 0), but it also led to an increase in the occurrence of any grade AEs (RR [95% CI] = 1.015 [1.002–1.029], 95% PI: 0.991–1.031; I^2 = 2.7%, Tau^2 < 0.0001) and grade ≥3 AEs (RR [95% CI] = 1.068 [1.002–1.138], 95% PI: 0.966–1.178; I^2 = 0%, Tau^2 = 0). The identical results were also observed in the combination therapy with trebananib (Table 4; Supplementary Figure S10–S14).

3.7 Trial sequential analysis results

In the execution of TSA for both PFS and OS, the analysis necessitated an APIS of 1,990. It was noted that in the monotherapy analysis with anti-angiogenic drugs, only the cumulative Z-curve for OS and any grade AEs breached the RIS threshold, albeit without breaching the trial sequential monitoring boundary. Theses results indicated the possibility of deriving a relatively solid conclusion. However, the cumulative Z-curve for PFS and grade ≥3 AEs in the

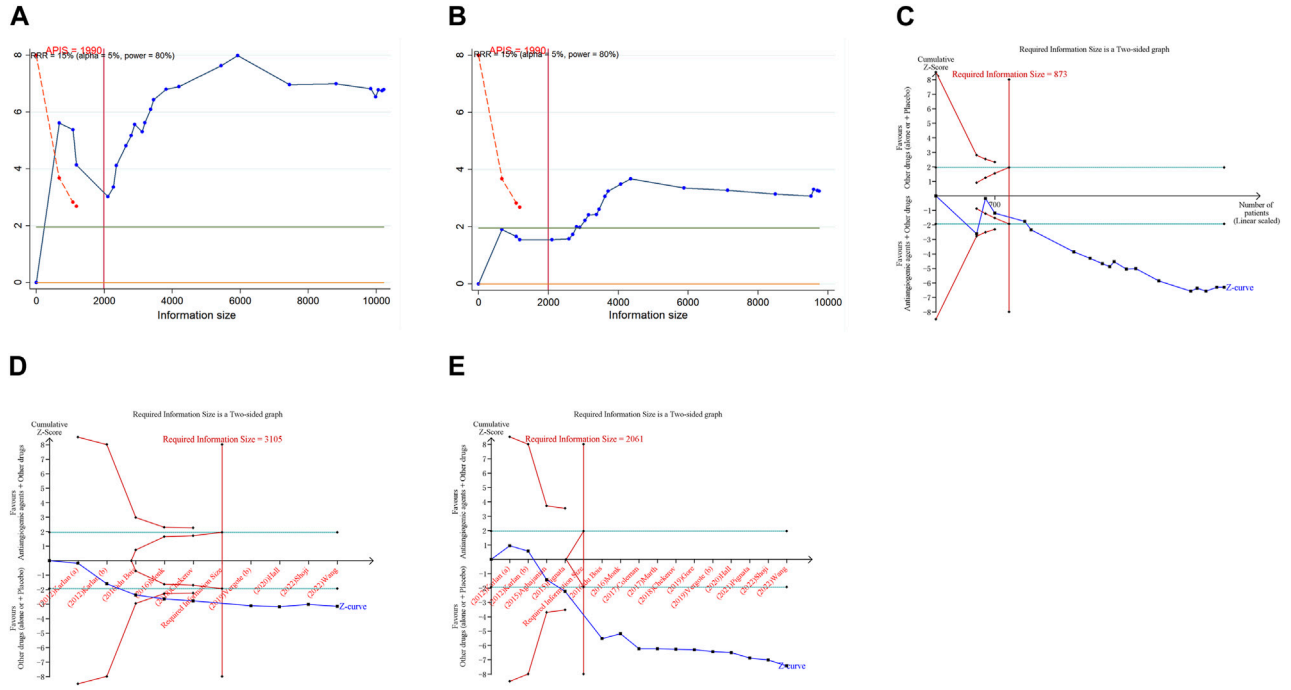

FIGURE 5
Trial sequential analysis of anti-angiogenic drug combination therapy for ovarian cancer. **(A)** Progression-free survival; **(B)** Overall survival; **(C)** Objective response rate; **(D)** Adverse events of any grade; **(E)** Grade ≥3 adverse events. Uppermost and lowermost red curves represent trial sequential monitoring boundary lines for benefit and harm, respectively. Inner red lines represent the futility boundary. Blue line represents evolution of cumulative Z-score. Horizontal green lines represent the conventional boundaries for statistical significance. Cumulative Z-curve crossing the trial sequential monitoring boundary or the RIS boundary provides firm evidence of effect.

same monotherapy analysis neither crossed the trial sequential monitoring boundary nor the RIS threshold, implying that the results are inconclusive and may include false positives (Figure 4). In the scenario of combination therapy with anti-angiogenic drugs, every cumulative Z-curve successfully crossed either the trial sequential monitoring boundary or the RIS threshold, suggesting that additional research is not necessary for a conclusive result (Figure 5).

3.8 Sensitivity analysis and publication bias

We performed sensitivity analyses and publication bias tests on the combined results that included more than 10 studies. The sensitivity analysis entailed the computation of pooled HRs or RRs along with their respective 95% CIs, excluding individual studies to ascertain if a single study significantly influenced the combined results. The sensitivity analysis demonstrated that the exclusion of any single study did not significantly impact the quantitative findings, which implies that the combined results from the anti-angiogenic drug combination therapy are both robust and dependable (Supplementary Figure S15). We also conducted a trim-and-fill analysis, yielding funnel plots with imputed studies for the outcomes of ORR, any grade AEs, and grade ≥3 AEs, indicating the potential for publication bias (Supplementary Figure S16). However, the trim-and-fill analysis correction for possible publication bias did not change the results for ORR, AEs of any grade, and grade ≥3 AEs, suggesting that the presence of publication bias did not significantly affect the final results.

4 Discussion

The progression of OC and the standard physiological processes of the ovary are both substantially reliant on angiogenesis. The growth and advancement of malignancies necessitate angiogenesis, as tumors cannot exceed 1–2 mm in size without adequate neovascularization. Consequently, anti-angiogenic drugs have been incorporated into OC treatment regimens. The VEGF pathway is the most extensively studied in the process of neovascularization. VEGF initiates the formation of new blood vessels, which is then sustained by platelet-derived growth factor, fibroblast growth factor, and angiopoietin-1 and -2 (Fernando et al., 2008; Timke et al., 2008; Ionescu et al., 2011). Overexpression of VEGF is associated with the tumor's prognosis and stage (Nusrat et al., 2016). A number of angiogenesis inhibitors targeting this pathway, including bevacizumab, cediranib, sorafenib, pazopanib, aflibercept, nintedanib, trebananib, and sunitinib, are currently under investigation (Singh et al., 2020). This study conducted a meta-analysis of previous RCTs and concluded that compared to drug therapy without anti-angiogenic agents, combination therapy with anti-angiogenic drugs significantly improved PFS and OS, while also elevating the ORR. Further subgroup analysis revealed that combination therapy with VEGF or VEGFR inhibitors can bring benefits in terms of PFS and OS, as well as an improvement in ORR.

Bevacizumab is the main VEGF inhibitor of interest in the trials included in this study. This agent, a humanized monoclonal antibody targeting VEGF, received approval in 2014 as the treatment for platinum-resistant OC, to be used in conjunction with chemotherapy (Monk et al., 2016a). Our findings revealed that bevacizumab in combination with chemotherapy not only significantly improved PFS and OS but also increased ORR compared with chemotherapy alone or plus placebo in patients with OC. Bevacizumab achieves its therapeutic effect by preventing VEGF-A from engaging with VEGFR, resulting in the destruction of established vessels, interference with new vessel formation, and the reduction of intratumoral pressure (Reinthaller, 2016). Research indicated that inhibiting VEGF signaling not only diminishes tumor vascularization but also aids in the morphological and functional normalization of the remaining vessels (Mei et al., 2023). In addition, trebananib stands out as the sole angiopoietin inhibitor in our comprehensive analysis. This peptide, which obstructs the action of angiopoietin-1 and angiopoietin-2-key players in angiogenesis-acts by preventing ANGPT from interacting with its receptor, Tie2 (Mullen et al., 2019). Utilizing photoacoustic tomography, one study observed notable changes in tumor vascularization following trebananib treatment, including significant vessel regression and a decrease in vessel density. Notably, while trebananib therapy did not halt angiogenesis entirely, it encouraged the formation of more stable and less permeable residual vessels (Bohndiek et al., 2015). The TRINOVA-1 trial, assessing patients with recurrent OC less than 12 months after previous platinum-based therapy, allocated participants to either a combination of weekly paclitaxel and trebananib or weekly paclitaxel with placebo. The trebananib cohort experienced prolonged PFS (HR = 0.66, $p < 0.001$) (Monk et al., 2016b). Our analysis confirmed the benefit of trebananib combined with chemotherapy in improving PFS. Regrettably, this study did not demonstrate any significant improvement in OS and ORR when comparing trebananib plus chemotherapy to placebo plus chemotherapy.

Currently, VEGFR inhibitors attracting substantial clinical attention include cediranib, nintedanib, and pazopanib. Cediranib, an orally administered tyrosine kinase inhibitor (TKI), acts on VEGFR-1, -2, and -3, and c-kit. Preclinical OC models have demonstrated that cediranib therapy leads to a significant reduction in tumor vascular density and vessel regression (Ruscito et al., 2016). When combined with standard chemotherapy as a maintenance therapy, cediranib has demonstrated an extension in PFS and OS compared to chemotherapy alone (Mahner et al., 2015). When paired with the PARP inhibitor olaparib in patients with platinum-sensitive relapse OC, cediranib has exhibited a remarkable 80% response rate and an increase in PFS from 9 to 17.7 months (Liu et al., 2014). However, our combined analysis did not corroborate that cediranib combination therapy could enhance OS compared to treatments devoid of cediranib. Our research did affirm the benefit of cediranib combination therapy in extending PFS. Notably, the cediranib and olaparib combination therapy demonstrated a higher ORR compared to olaparib monotherapy in our study. Additional RCTs are needed to further probe the effectiveness of pairing anti-angiogenic drugs with PARP inhibitors in OC treatment. Nintedanib, a multi-targeted antiangiogenic agent available orally, has been shown through dynamic magnetic resonance imaging assessments to significantly reduce blood flow in approximately 55% of OC patients. It also fosters vascular normalization and tumor regression in pre-clinical models (Khalique et al., 2017). Nintedanib, when combined with carboplatin and paclitaxel, has been proven to improve PFS, although it has no effect on OS (Ray-Coquard et al., 2020). Pazopanib, an oral multi-target TKI, inhibits platelet-derived growth factor receptors (PDGFR) alpha/beta, VEGFR, c-Kit, and fibroblast growth factor receptor (FGFR)-1 and −3. In mouse orthotopic OC models, pazopanib treatment significantly curtailed tumor microvessel density and pericyte coverage (Merritt et al., 2010). While not yet approved for OC, numerous phase 2 and 3 clinical trials have explored the potential role of pazopanib in OC therapy (Davidson et al., 2014; du Bois et al., 2012; Plummer et al., 2013). Our research indicated that the combination of nintedanib and chemotherapy can improve PFS compared with chemotherapy alone or plus placebo. The combination of pazopanib and chemotherapy has been shown to provide higher ORR, which aligns with a previous meta-analysis (Zhang et al., 2023).

In addition to examining the impacts of various VEGF, VEGFR, and angiopoietin inhibitors on OC by classifying specific anti-angiogenic medications, our analytical approach distinguished itself from prior meta-analyses by performing subgroup analyses based on multiple OC subtypes (Wang et al., 2018; Guo et al., 2021). The results from our subgroup analysis suggested that compared to drug therapy without anti-angiogenic agents, combination therapy with anti-angiogenic drugs notably improved PFS, OS, and ORR in platinum-sensitive and recurrent OC patients. Traditionally, OC has been classified as “platinum sensitive” if relapse occurs 6 months or more after the final dose of platinum-based chemotherapy, and “platinum resistant” if relapse happens earlier (Ledermann et al., 2013). For platinum-resistant OC, our research also concluded that anti-angiogenic drug combination therapy yielded benefits in terms of PFS and OS, along with a higher ORR. Bevacizumab is the sole anti-VEGF treatment for platinum-sensitive and recurrent OC approved by the US Food and Drug Administration (FDA). Its FDA approved indication is for combination with carboplatin/gemcitabine or carboplatin/paclitaxel, followed by single-agent maintenance (Arend et al., 2020). Bevacizumab is also available in the United States as a second-line and third-line treatment for platinum-resistant OC and frontline therapy for stage III/IV disease (Arend et al., 2020). The majority of the participants in the RCTs included in our study were OC patients of various subtypes. Grouping the subdivided subtypes of OC into a single category in a general manner could lead to some degree of bias and confusion. Furthermore, the fifth Ovarian Cancer Consensus Conference of the Gynecologic Cancer InterGroup recommended that tumors should be defined by a multitude of factors, including surgical outcomes, mutation status, platinum sensitivity, histology, and response to non-platinum treatments. Consequently, more RCTs need to be incorporated to bolster future meta-analysis targeting a specific and clearly defined subtype of OC.

The results of our monotherapy analysis indicated that anti-angiogenic monotherapy did not provide substantial improvements in PFS and OS compared with placebo. This monotherapy, however, was associated with an elevated risk of any grade AEs. Despite the pooled analysis revealing a greater ORR with the use of anti-angiogenic monotherapy (Ferron et al., 2023), the inference made

from a single trial could not be broadly applied. From a therapeutic efficacy standpoint, the combination of anti-angiogenic drugs with chemotherapy or PARP inhibitors seems to be a more effective alternative to monotherapy with anti-angiogenic drugs, as combination therapy brings benefits in terms of PFS, OS, and ORR. RCTs needs to be designed to directly compare the effectiveness of anti-angiogenic drug monotherapy and combination therapy to verify this hypothesis. Moreover, the increased incidence of AEs caused by combination therapy warrants attention. Our combined and subgroup analyses revealed that anti-angiogenic drug combination resulted in a higher incidence of grade ≥3 AEs. Additionally, the combination of VEGFR or angiopoietin inhibitor was linked to an increased risk of any grade AEs. These findings underscore the importance for vigilant monitoring and management of AEs during anti-angiogenic therapy to mitigate potential risks.

There are several limitations in our meta-analysis. First, the heterogeneity in PFS results could be attributed to the variations in the trial design, patient baseline characteristics, anti-angiogenic therapies utilized, chemotherapy protocols, OC stages, and duration of follow-up across the RCTs. The existence of considerable heterogeneity may compromise the dependability of pooled estimates. Second, it is noteworthy that despite the majority of the incorporated studies being featured in high-impact journals, certain inherent aspects like pharmaceutical industry sponsorship and an open-label design could potentially introduce elements of bias, such as publication bias, which might have an impact on the overall findings. Third, despite the participation of independent assessors and meticulous data extraction and quality assessment using the modified Jadad scale, subjective biases may still be present in the process of evaluating study quality and extracting data. Fourth, diversity in OC types across the original RCTs could make the subgroup analyses based on OC subtypes potentially biased and confusing. These subgroup analyses could potentially introduce the possibility of false positives and inflated type I error. Finally, TSA findings point out the need for future meta-analysis with larger sample sizes and more RCTs to validate the results related to PFS and grade ≥3 AEs in the context of anti-angiogenic drug monotherapy.

5 Conclusion

In summary, our meta-analysis of RCTs demonstrated that the combination of anti-angiogenic drugs with chemotherapy or PARP inhibitors significantly improved PFS, OS, and ORR in OC patients compared with chemotherapy or PARP inhibitors alone. Although the efficacy superiority of anti-angiogenic drug monotherapy over placebo has not been observed, the increased risk of AEs associated with anti-angiogenic drug monotherapy and combination therapy warrants attention. Clinicians should meticulously detect and manage AEs to mitigate the potential treatment-related risks while employing anti-angiogenic therapies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

YX: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing–original draft. FZ: Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1423891/full#supplementary-material

References

Abdalla, A. M. E., Xiao, L., Ullah, M. W., Yu, M., Ouyang, C., and Yang, G. (2018). Current challenges of cancer anti-angiogenic therapy and the promise of nanotherapeutics. *Theranostics* 8 (2), 533–548. doi:10.7150/thno.21674

Aghajanian, C., Blank, S. V., Goff, B. A., Judson, P. L., Teneriello, M. G., Husain, A., et al. (2012). OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J. Clin. Oncol.* 30 (17), 2039–2045. doi:10.1200/jco.2012.42.0505

Aghajanian, C., Goff, B., Nycum, L. R., Wang, Y. V., Husain, A., and Blank, S. V. (2015). Final overall survival and safety analysis of OCEANS, a phase 3 trial of

chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent ovarian cancer. *Gynecol. Oncol.* 139 (1), 10–16. doi:10.1016/j.ygyno.2015.08.004

Arend, R., Westin, S. N., and Coleman, R. L. (2020). Decision analysis for secondline maintenance treatment of platinum sensitive recurrent ovarian cancer: a review. *Int. J. Gynecol. Cancer* 30 (5), 684–694. doi:10.1136/ijgc-2019-001041

Bohndiek, S. E., Sasportas, L. S., Machtaler, S., Jokerst, J. V., Hori, S., and Gambhir, S. S. (2015). Photoacoustic tomography detects early vessel regression and normalization during ovarian tumor response to the antiangiogenic therapy trebananib. *J. Nucl. Med.* 56 (12), 1942–1947. doi:10.2967/jnumed.115.160002

Bowden, J., Tierney, J. F., Copas, A. J., and Burdett, S. (2011). Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Med. Res. Methodol.* 11, 41. doi:10.1186/1471-2288-11-41

Burger, R. A., Brady, M. F., Bookman, M. A., Fleming, G. F., Monk, B. J., Huang, H., et al. (2011). Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N. Engl. J. Med.* 365 (26), 2473–2483. doi:10.1056/NEJMoa1104390

Chekerov, R., Hilpert, F., Mahner, S., El-Balat, A., Harter, P., De Gregorio, N., et al. (2018). Sorafenib plus topotecan versus placebo plus topotecan for platinum-resistant ovarian cancer (TRIAS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Oncol.* 19 (9), 1247–1258. doi:10.1016/s1470-2045(18)30372-3

Coleman, R. L., Brady, M. F., Herzog, T. J., Sabbatini, P., Armstrong, D. K., Walker, J. L., et al. (2017). Bevacizumab and paclitaxel-carboplatin chemotherapy and secondary cytoreduction in recurrent, platinum-sensitive ovarian cancer (NRG Oncology/Gynecologic Oncology Group study GOG-0213): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* 18 (6), 779–791. doi:10.1016/s1470-2045(17)30279-6

Davidson, B. A., and Secord, A. A. (2014). Profile of pazopanib and its potential in the treatment of epithelial ovarian cancer. *Int. J. Womens Health* 6, 289–300. doi:10.2147/ijwh.S49781

du Bois, A., Floquet, A., Kim, J. W., Rau, J., del Campo, J. M., Friedlander, M., et al. (2014). Incorporation of pazopanib in maintenance therapy of ovarian cancer. *J. Clin. Oncol.* 32 (30), 3374–3382. doi:10.1200/jco.2014.55.7348

du Bois, A., Kristensen, G., Ray-Coquard, I., Reuss, A., Pignata, S., Colombo, N., et al. (2016). Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol.* 17 (1), 78–89. doi:10.1016/s1470-2045(15)00366-6

du Bois, A., Vergote, I., Wimberger, P., Ray-Coquard, I., Harter, P., Curtis, L. B., et al. (2012). Open-label feasibility study of pazopanib, carboplatin, and paclitaxel in women with newly diagnosed, untreated, gynaecologic tumours: a phase I/II trial of the AGO study group. *Br. J. Cancer* 106 (4), 629–632. doi:10.1038/bjc.2011.608

Duska, L. R., Petroni, G. R., Varhegyi, N., Brown, J., Jelovac, D., Moore, K. N., et al. (2020). A randomized phase II evaluation of weekly gemcitabine plus pazopanib versus weekly gemcitabine alone in the treatment of persistent or recurrent epithelial ovarian, fallopian tube or primary peritoneal carcinoma. *Gynecol. Oncol.* 157 (3), 585–592. doi:10.1016/j.ygyno.2019.10.014

Duval, S., and Tweedie, R. (2000). Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56 (2), 455–463. doi:10.1111/j.0006-341x.2000.00455.x

Fernando, N. T., Koch, M., Rothrock, C., Gollogly, L. K., D'Amore, P. A., Ryeom, S., et al. (2008). Tumor escape from endogenous, extracellular matrix-associated angiogenesis inhibitors by up-regulation of multiple proangiogenic factors. *Clin. Cancer Res.* 14 (5), 1529–1539. doi:10.1158/1078-0432.Ccr-07-4126

Ferron, G., De Rauglaudre, G., Becourt, S., Delanoy, N., Joly, F., Lortholary, A., et al. (2023). Neoadjuvant chemotherapy with or without nintedanib for advanced epithelial ovarian cancer: lessons from the GINECO double-blind randomized phase II CHIVA trial. *Gynecol. Oncol.* 170, 186–194. doi:10.1016/j.ygyno.2023.01.008

Garzon, S., Laganà, A. S., Casarin, J., Raffaelli, R., Cromi, A., Franchi, M., et al. (2020). Secondary and tertiary ovarian cancer recurrence: what is the best management? *Gland. Surg.* 9 (4), 1118–1129. doi:10.21037/gs-20-325

Gore, M., Hackshaw, A., Brady, W. E., Penson, R. T., Zaino, R., McCluggage, W. G., et al. (2019). An international, phase III randomized trial in patients with mucinous epithelial ovarian cancer (mEOC/GOG 0241) with long-term follow-up: and experience of conducting a clinical trial in a rare gynecological tumor. *Gynecol. Oncol.* 153 (3), 541–548. doi:10.1016/j.ygyno.2019.03.256

Gotlieb, W. H., Amant, F., Advani, S., Goswami, C., Hirte, H., Provencher, D., et al. (2012). Intravenous aflibercept for treatment of recurrent symptomatic malignant ascites in patients with advanced ovarian cancer: a phase 2, randomised, double-blind, placebo-controlled study. *Lancet Oncol.* 13 (2), 154–162. doi:10.1016/s1470-2045(11)70338-2

Guo, C., Yan, C., Qu, L., Du, R., and Lin, J. (2021). The efficacy and toxicity of angiogenesis inhibitors for ovarian cancer: a meta-analysis of randomized controlled trials. *Arch. Gynecol. Obstet.* 303 (2), 285–311. doi:10.1007/s00404-020-05865-z

Hall, M. R., Dehbi, H. M., Banerjee, S., Lord, R., Clamp, A., Ledermann, J. A., et al. (2020). A phase II randomised, placebo-controlled trial of low dose (metronomic) cyclophosphamide and nintedanib (BIBF1120) in advanced ovarian, fallopian tube or primary peritoneal cancer. *Gynecol. Oncol.* 159 (3), 692–698. doi:10.1016/j.ygyno.2020.09.048

Helali, A. E., Wong, C. H. L., Choi, H. C. W., Chan, W. W. L., Dickson, N., Siu, S. W. K., et al. (2022). A comprehensive systematic review and network meta-analysis: the role of anti-angiogenic agents in advanced epithelial ovarian cancer. *Sci. Rep.* 12 (1), 3803. doi:10.1038/s41598-022-07731-1

Herzog, T. J., Scambia, G., Kim, B. G., Lhommé, C., Markowska, J., Ray-Coquard, I., et al. (2013). A randomized phase II trial of maintenance therapy with Sorafenib in front-line ovarian carcinoma. *Gynecol. Oncol.* 130 (1), 25–30. doi:10.1016/j.ygyno.2013.04.011

Higgins, J. P., and Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21 (11), 1539–1558. doi:10.1002/sim.1186

Hope, J. M., and Blank, S. V. (2010). Current status of maintenance therapy for advanced ovarian cancer. *Int. J. Womens Health* 1, 173–180. doi:10.2147/ijwh.s4661

IntHout, J., Ioannidis, J. P., Rovers, M. M., and Goeman, J. J. (2016). Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open* 6 (7), e010247. doi:10.1136/bmjopen-2015-010247

Ionescu, C., Berindan-Neagoe, I., Burz, C., Balacescu, O., Balacescu, L., Tudoran, O., et al. (2011). The clinical implications of platelet derived growth factor B, vascular endothelial growth factor and basic fibroblast growth factor in colorectal cancer. *J. buon* 16 (2), 274–276.

Jadad, A. R., Moore, R. A., Carroll, D., Jenkinson, C., Reynolds, D. J., Gavaghan, D. J., et al. (1996). Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin. Trials* 17 (1), 1–12. doi:10.1016/0197-2456(95)00134-4

Jászai, J., and Schmidt, M. H. H. (2019). Trends and challenges in tumor anti-angiogenic therapies. *Cells* 8 (9), 1102. doi:10.3390/cells8091102

Karlan, B. Y., Oza, A. M., Richardson, G. E., Provencher, D. M., Hansen, V. L., Buck, M., et al. (2012). Randomized, double-blind, placebo-controlled phase II study of AMG 386 combined with weekly paclitaxel in patients with recurrent ovarian cancer. *J. Clin. Oncol.* 30 (4), 362–371. doi:10.1200/jco.2010.34.3178

Khalique, S., and Banerjee, S. (2017). Nintedanib in ovarian cancer. *Expert Opin. Investig. Drugs* 26 (9), 1073–1081. doi:10.1080/13543784.2017.1353599

Kim, J. W., Mahner, S., Wu, L. Y., Shoji, T., Kim, B. G., Zhu, J. Q., et al. (2018). Pazopanib maintenance therapy in east asian women with advanced epithelial ovarian cancer: results from AGO-OVAR16 and an east asian study. *Int. J. Gynecol. Cancer* 28 (1), 2–10. doi:10.1097/igc.0000000000000602

Ledermann, J. A., Embleton, A. C., Raja, F., Perren, T. J., Jayson, G. C., Rustin, G. J. S., et al. (2016). Cediranib in patients with relapsed platinum-sensitive ovarian cancer (ICON6): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 387 (10023), 1066–1074. doi:10.1016/s0140-6736(15)01167-8

Ledermann, J. A., Embleton-Thirsk, A. C., Perren, T. J., Jayson, G. C., Rustin, G. J. S., Kaye, S. B., et al. (2021). Cediranib in addition to chemotherapy for women with relapsed platinum-sensitive ovarian cancer (ICON6): overall survival results of a phase III randomised trial. *ESMO Open* 6 (2), 100043. doi:10.1016/j.esmoop.2020.100043

Ledermann, J. A., Hackshaw, A., Kaye, S., Jayson, G., Gabra, H., McNeish, I., et al. (2011). Randomized phase II placebo-controlled trial of maintenance therapy using the oral triple angiokinase inhibitor BIBF 1120 after chemotherapy for relapsed ovarian cancer. *J. Clin. Oncol.* 29 (28), 3798–3804. doi:10.1200/jco.2010.33.5208

Ledermann, J. A., Raja, F. A., Fotopoulou, C., Gonzalez-Martin, A., Colombo, N., Sessa, C., et al. (2013). Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 24, vi24–32. Suppl 6. doi:10.1093/annonc/mdt333

Liu, J. F., Barry, W. T., Birrer, M., Lee, J. M., Buckanovich, R. J., Fleming, G. F., et al. (2014). Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. *Lancet Oncol.* 15 (11), 1207–1214. doi:10.1016/s1470-2045(14)70391-2

Liu, J. F., Barry, W. T., Birrer, M., Lee, J. M., Buckanovich, R. J., Fleming, G. F., et al. (2019). Overall survival and updated progression-free survival outcomes in a randomized phase II study of combination cediranib and olaparib versus olaparib in relapsed platinum-sensitive ovarian cancer. *Ann. Oncol.* 30 (4), 551–557. doi:10.1093/annonc/mdz018

Liu, J. F., Brady, M. F., Matulonis, U. A., Miller, A., Kohn, E. C., Swisher, E. M., et al. (2022). Olaparib with or without cediranib versus platinum-based chemotherapy in recurrent platinum-sensitive ovarian cancer (NRG-GY004): a randomized, open-label, phase III trial. *J. Clin. Oncol.* 40 (19), 2138–2147. doi:10.1200/jco.21.02011

Mahner, S., Woelber, L., Mueller, V., Witzel, I., Prieske, K., Grimm, D., et al. (2015). Beyond bevacizumab: an outlook to new anti-angiogenics for the treatment of ovarian cancer. *Front. Oncol.* 5, 211. doi:10.3389/fonc.2015.00211

Marth, C., Vergote, I., Scambia, G., Oberaigner, W., Clamp, A., Berger, R., et al. (2017). ENGOT-ov-6/TRINOVA-2: randomised, double-blind, phase 3 study of pegylated liposomal doxorubicin plus trebananib or placebo in women with recurrent partially platinum-sensitive or resistant ovarian cancer. *Eur. J. Cancer* 70, 111–121. doi:10.1016/j.ejca.2016.09.004

McGee, J., Bookman, M., Harter, P., Marth, C., McNeish, I., Moore, K. N., et al. (2017). Fifth ovarian cancer Consensus conference: individualized therapy and patient factors. *Ann. Oncol.* 28 (4), 702–710. doi:10.1093/annonc/mdx010

Mei, C., Gong, W., Wang, X., Lv, Y., Zhang, Y., Wu, S., et al. (2023). Anti-angiogenic therapy in ovarian cancer: current understandings and prospects of precision medicine. *Front. Pharmacol.* 14, 1147717. doi:10.3389/fphar.2023.1147717

Merritt, W. M., Nick, A. M., Carroll, A. R., Lu, C., Matsuo, K., Dumble, M., et al. (2010). Bridging the gap between cytotoxic and biologic therapy with metronomic topotecan and pazopanib in ovarian cancer. *Mol. Cancer Ther.* 9 (4), 985–995. doi:10.1158/1535-7163.Mct-09-0967

Monk, B. J., Minion, L. E., and Coleman, R. L. (2016a). Anti-angiogenic agents in ovarian cancer: past, present, and future. *Ann. Oncol.* 27, i33–i39. Suppl 1. doi:10.1093/annonc/mdw093

Monk, B. J., Poveda, A., Vergote, I., Raspagliesi, F., Fujiwara, K., Bae, D. S., et al. (2016b). Final results of a phase 3 study of trebananib plus weekly paclitaxel in recurrent ovarian cancer (TRINOVA-1): long-term survival, impact of ascites, and progression-free survival-2. *Gynecol. Oncol.* 143 (1), 27–34. doi:10.1016/j.ygyno.2016.07.112

Mullen, M. M., Kuroki, L. M., and Thaker, P. H. (2019). Novel treatment options in platinum-sensitive recurrent ovarian cancer: a review. *Gynecol. Oncol.* 152 (2), 416–425. doi:10.1016/j.ygyno.2018.10.023

Nicum, S., McGregor, N., Austin, R., Collins, L., Dutton, S., McNeish, I., et al. (2024). Results of a randomised Phase II trial of olaparib, chemotherapy or olaparib and cediranib in patients with platinum-resistant ovarian cancer. *Br. J. Cancer* 130 (6), 941–950. doi:10.1038/s41416-023-02567-6

Nusrat, O., Belotte, J., Fletcher, N. M., Memaj, I., Saed, M. G., Diamond, M. P., et al. (2016). The role of angiogenesis in the persistence of chemoresistance in epithelial ovarian cancer. *Reprod. Sci.* 23 (11), 1484–1492. doi:10.1177/1933719116645191

Oza, A. M., Cook, A. D., Pfisterer, J., Embleton, A., Ledermann, J. A., Pujade-Lauraine, E., et al. (2015). Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. *Lancet Oncol.* 16 (8), 928–936. doi:10.1016/s1470-2045(15)00086-8

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj* 372, n71. doi:10.1136/bmj.n71

Pignata, S., Lorusso, D., Joly, F., Gallo, C., Colombo, N., Sessa, C., et al. (2021). Carboplatin-based doublet plus bevacizumab beyond progression versus carboplatin-based doublet alone in patients with platinum-sensitive ovarian cancer: a randomised, phase 3 trial. *Lancet Oncol.* 22 (2), 267–276. doi:10.1016/s1470-2045(20)30637-9

Pignata, S., Lorusso, D., Scambia, G., Sambataro, D., Tamberi, S., Cinieri, S., et al. (2015). Pazopanib plus weekly paclitaxel versus weekly paclitaxel alone for platinum-resistant or platinum-refractory advanced ovarian cancer (MITO 11): a randomised, open-label, phase 2 trial. *Lancet Oncol.* 16 (5), 561–568. doi:10.1016/s1470-2045(15)70115-4

Plummer, R., Madi, A., Jeffels, M., Richly, H., Nokay, B., Rubin, S., et al. (2013). A Phase I study of pazopanib in combination with gemcitabine in patients with advanced solid tumors. *Cancer Chemother. Pharmacol.* 71 (1), 93–101. doi:10.1007/s00280-012-1982-z

Pujade-Lauraine, E., Hilpert, F., Weber, B., Reuss, A., Poveda, A., Kristensen, G., et al. (2014). Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial. *J. Clin. Oncol.* 32 (13), 1302–1308. doi:10.1200/jco.2013.51.4489

Ray-Coquard, I., Cibula, D., Mirza, M. R., Reuss, A., Ricci, C., Colombo, N., et al. (2020). Final results from GCIG/ENGOT/AGO-OVAR 12, a randomised placebo-controlled phase III trial of nintedanib combined with chemotherapy for newly diagnosed advanced ovarian cancer. *Int. J. Cancer* 146 (2), 439–448. doi:10.1002/ijc.32606

Reinthaller, A. (2016). Antiangiogenic therapies in ovarian cancer. *Memo* 9 (3), 139–143. doi:10.1007/s12254-016-0282-4

Richardson, D. L., Sill, M. W., Coleman, R. L., Sood, A. K., Pearl, M. L., Kehoe, S. M., et al. (2018). Paclitaxel with and without pazopanib for persistent or recurrent ovarian cancer: a randomized clinical trial. *JAMA Oncol.* 4 (2), 196–202. doi:10.1001/jamaoncol.2017.4218

Roque, D. M., Siegel, E. R., Buza, N., Bellone, S., Silasi, D. A., Huang, G. S., et al. (2022). Randomised phase II trial of weekly ixabepilone ± biweekly bevacizumab for platinum-resistant or refractory ovarian/fallopian tube/primary peritoneal cancer. *Br. J. Cancer* 126 (12), 1695–1703. doi:10.1038/s41416-022-01717-6

Ruscito, I., Gasparri, M. L., Marchetti, C., De Medici, C., Bracchi, C., Palaia, I., et al. (2016). Cediranib in ovarian cancer: state of the art and future perspectives. *Tumour Biol.* 37 (3), 2833–2839. doi:10.1007/s13277-015-4781-4

Saman, H., Raza, S. S., Uddin, S., and Rasul, K. (2020). Inducing angiogenesis, a key step in cancer vascularization, and treatment approaches. *Cancers (Basel)* 12 (5), 1172. doi:10.3390/cancers12051172

Shoji, T., Enomoto, T., Abe, M., Okamoto, A., Nagasawa, T., Oishi, T., et al. (2022). Efficacy and safety of standard of care with/without bevacizumab for platinum-resistant ovarian/fallopian tube/peritoneal cancer previously treated with bevacizumab: the Japanese Gynecologic Oncology Group study JGOG3023. *Cancer Sci.* 113 (1), 240–250. doi:10.1111/cas.15185

Siegel, R. L., Miller, K. D., Fuchs, H. E., and Jemal, A. (2021). Cancer statistics, 2021. *CA Cancer J. Clin.* 71 (1), 7–33. doi:10.3322/caac.21654

Singh, N., Badrun, D., and Ghatage, P. (2020). State of the art and up-and-coming angiogenesis inhibitors for ovarian cancer. *Expert Opin. Pharmacother.* 21 (13), 1579–1590. doi:10.1080/14656566.2020.1775813

Teleanu, R. I., Chircov, C., Grumezescu, A. M., and Teleanu, D. M. (2019). Tumor angiogenesis and anti-angiogenic strategies for cancer treatment. *J. Clin. Med.* 9 (1), 84. doi:10.3390/jcm9010084

Tewari, K. S., Burger, R. A., Enserro, D., Norquist, B. M., Swisher, E. M., Brady, M. F., et al. (2019). Final overall survival of a randomized trial of bevacizumab for primary treatment of ovarian cancer. *J. Clin. Oncol.* 37 (26), 2317–2328. doi:10.1200/jco.19.01009

Tierney, J. F., Stewart, L. A., Ghersi, D., Burdett, S., and Sydes, M. R. (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 8, 16. doi:10.1186/1745-6215-8-16

Timke, C., Zieher, H., Roth, A., Hauser, K., Lipson, K. E., Weber, K. J., et al. (2008). Combination of vascular endothelial growth factor receptor/platelet-derived growth factor receptor inhibition markedly improves radiation tumor therapy. *Clin. Cancer Res.* 14 (7), 2210–2219. doi:10.1158/1078-0432.Ccr-07-1893

Vergote, I., du Bois, A., Floquet, A., Rau, J., Kim, J. W., Del Campo, J. M., et al. (2019a). Overall survival results of AGO-OVAR16: a phase 3 study of maintenance pazopanib versus placebo in women who have not progressed after first-line chemotherapy for advanced ovarian cancer. *Gynecol. Oncol.* 155 (2), 186–191. doi:10.1016/j.ygyno.2019.08.024

Vergote, I., Scambia, G., O'Malley, D. M., Van Calster, B., Park, S. Y., Del Campo, J. M., et al. (2019b). Trebananib or placebo plus carboplatin and paclitaxel as first-line treatment for advanced ovarian cancer (TRINOVA-3/ENGOT-ov2/GOG-3001): a randomised, double-blind, phase 3 trial. *Lancet Oncol.* 20 (6), 862–876. doi:10.1016/s1470-2045(19)30178-0

Wang, H., Xu, T., Zheng, L., and Li, G. (2018). Angiogenesis inhibitors for the treatment of ovarian cancer: an updated systematic review and meta-analysis of randomized controlled trials. *Int. J. Gynecol. Cancer* 28 (5), 903–914. doi:10.1097/igc.0000000000001258

Wang, T., Tang, J., Yang, H., Yin, R., Zhang, J., Zhou, Q., et al. (2022). Effect of apatinib plus pegylated liposomal doxorubicin vs pegylated liposomal doxorubicin alone on platinum-resistant recurrent ovarian cancer: the APPROVE randomized clinical trial. *JAMA Oncol.* 8 (8), 1169–1176. doi:10.1001/jamaoncol.2022.2253

Wang, Z., Huang, Y., Long, L., Zhou, L., Huang, Y., Gan, L., et al. (2021). Apatinib treatment efficiently delays biochemical-only recurrent ovarian cancer progression. *J. Ovarian Res.* 14 (1), 91. doi:10.1186/s13048-021-00843-8

Wetterslev, J., Jakobsen, J. C., and Gluud, C. (2017). Trial Sequential Analysis in systematic reviews with meta-analysis. *BMC Med. Res. Methodol.* 17 (1), 39. doi:10.1186/s12874-017-0315-7

Xie, M., Zhong, Y., Yang, Y., Shen, F., and Nie, Y. (2022). Extended adjuvant endocrine therapy for women with hormone receptor-positive early breast cancer: a meta-analysis with trial sequential analysis of randomized controlled trials. *Front. Oncol.* 12, 1039320. doi:10.3389/fonc.2022.1039320

Yi, S., Zeng, L., Kuang, Y., Cao, Z., Zheng, C., Zhang, Y., et al. (2017). Antiangiogenic drugs used with chemotherapy for patients with recurrent ovarian cancer: a meta-analysis. *Onco Targets Ther.* 10, 973–984. doi:10.2147/ott.S119879

Zhang, C., and Zhao, W. (2022). The efficacy and safety of angiogenesis inhibitors for recurrent ovarian cancer: a meta-analysis. *J. Ovarian Res.* 15 (1), 99. doi:10.1186/s13048-022-01028-7

Zhang, Y., Fang, H., Wang, X., Wang, H., Pan, G., and Chen, J. (2023). The efficacy and safety of pazopanib plus chemotherapy in treating recurrent or persistent ovarian cancer: a systematic review and meta-analysis. *Am. J. Clin. Oncol.* 46 (6), 254–262. doi:10.1097/coc.0000000000000999

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Identification of potential novel N6-methyladenosine effector-related lncRNA biomarkers for serous ovarian carcinoma: a machine learning-based exploration in the framework of 3P medicine

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Background: Serous ovarian carcinoma (SOC) is considered the most lethal gynecological malignancy. The current lack of reliable prognostic biomarkers for SOC reduces the efficacy of predictive, preventive, and personalized medicine (PPPM/3PM) in patients with SOC, leading to unsatisfactory therapeutic outcomes. N6-methyladenosine (m^6A) modification-associated long noncoding RNAs (lncRNAs) are effective predictors of SOC. In this study, an effective risk prediction model for SOC was constructed based on m^6A modification-associated lncRNAs.

Methods: Transcriptomic data and clinical information of patients with SOC were downloaded from The Cancer Genome Atlas. Candidate lncRNAs were identified using univariate and multivariate and least absolute shrinkage and selection operator-penalized Cox regression analyses. The molecular mechanisms of m^6A effector-related lncRNAs were explored via Gene Ontology, pathway analysis, gene set enrichment analysis, and gene set variation analysis (GSVA). The extent of immune cell infiltration was assessed using various algorithms, including CIBERSORT, Microenvironment Cell Populations counter, xCell, European Prospective Investigation into Cancer and Nutrition, and GSVA. The calcPhenotype algorithm was used to predict responses to the drugs commonly

used in ovarian carcinoma therapy. *In vitro* experiments, such as migration and invasion Transwell assays, wound healing assays, and dot blot assays, were conducted to elucidate the functional roles of candidate lncRNAs.

Results: Six m^{6}A effector-related lncRNAs that were markedly associated with prognosis were used to establish an m^{6}A effector-related lncRNA risk model (m^{6}A-LRM) for SOC. Immune microenvironment analysis suggested that the high-risk group exhibited a proinflammatory state and displayed increased sensitivity to immunotherapy. A nomogram was constructed with the m^{6}A effector-related lncRNAs to assess the prognostic value of the model. Sixteen drugs potentially targeting m^{6}A effector-related lncRNAs were identified. Furthermore, we developed an online web application for clinicians and researchers (https://leley.shinyapps.io/OC_m6A_lnc/). Overexpression of the lncRNA RP11-508M8.1 promoted SOC cell migration and invasion. *METTL3* is an upstream regulator of RP11-508M8.1. The preliminary regulatory axis METTL3/m^{6}A/RP11-508M8.1/hsa-miR-1270/ARSD underlying SOC was identified via a combination of *in vitro* and bioinformatic analyses.

Conclusion: In this study, we propose an innovative prognostic risk model and provide novel insights into the mechanism underlying the role of m^{6}A-related lncRNAs in SOC. Incorporating the m^{6}A-LRM into PPPM may help identify high-risk patients and personalize treatment as early as possible.

KEYWORDS

m^{6}A modification, immunotherapy, biomarker, RP11-508M8.1, predictive, preventive, personalized medicine (PPPM/3PM)

1 Introduction

Ovarian carcinoma (OC) is the most lethal gynecological cancer, with serous ovarian carcinoma (SOC) accounting for most of the reported OC cases (Kotsopoulos et al., 2014; Bowtell et al., 2015). Most patients with SOC are diagnosed at an advanced stage due to the concealed anatomical location of the ovaries and the absence of obvious or specific early clinical symptoms. High recurrence rates and drug resistance lead to poor prognoses for patients with SOC (Siegel et al., 2019). Given the complexity, heterogeneity, and refractory nature of SOC, using predictive, preventive, personalized medicine (PPPM/3PM) may help predict patient prognosis, identify tumor characteristics, and optimize treatment plans. PPPM has become a research hotspot in precision cancer medicine, especially, multi-omics and network-based search for prognostic markers that may facilitate accurate diagnosis and treatment (Cheng and Zhan, 2017). However, the outcomes of PPPM for SOC remain unsatisfactory.

Recent advances in immunotherapy, as exemplified by the use of immune checkpoint inhibitors (ICIs), has resulted in its incorporation into the treatment regimens for a range of advanced cancers (Murciano-Goroff et al., 2020; Zhang and Zhang, 2020). The degree of immune cell infiltration into the tumor microenvironment (TME) is strongly associated with the efficacy of cancer immunotherapy (Binnewies et al., 2018; Duan et al., 2020). Currently, the spatial distribution of tumor-infiltrating immune cells is used to classify tumors as "hot tumors," which are sensitive to immunotherapy (such as those presenting an immune-inflamed phenotype), and "cold tumors," which are less sensitive to immunotherapy (such as those presenting immune-excluded and immune-desert phenotypes) (Duan et al., 2020; Liu and Sun, 2021). The landscape of SOC is complex and demonstrates potential immunogenicity (Yang et al., 2020; Morand et al., 2021). Nevertheless, the rate of response to immunotherapy in SOC remains suboptimal, necessitating the identification of ideal biomarkers that would facilitate precise selection of immunotherapy regimens for patients.

Long noncoding RNAs (lncRNAs) are a class of RNA molecules that are longer than 200 nucleotides and have limited or no protein-coding capacity (Liu et al., 2020; Statello et al., 2021). LncRNAs regulate the proliferation, apoptosis, metastasis, and drug resistance of tumor cells (Luo et al., 2017; Peng et al., 2017; Muller et al., 2019; Bhat et al., 2020; Wei et al., 2020), and their abnormal expression is closely associated with the severity of malignancy in various cancers, including SOC. Moreover, research has shown that ncRNAs could have a potential dynamic role in future cancer therapeutics, supporting personalized treatment decisions and modern precision medicine (Soureas et al., 2023). N6-methyladenosine (m^{6}A), a dynamic and reversible post-transcriptional modification commonly found on mRNAs and lncRNAs (Chen et al., 2020), is a promising clinically relevant biomarker and therapeutic target (Huang et al., 2016; Zhao and Cui, 2019). It is regulated by m^{6}A effectors, such as methyltransferases (i.e., writers), demethyltransferases (i.e., erasers), and m^{6}A-binding proteins (i.e., readers) (Fu et al., 2014). Numerous studies have demonstrated that m^{6}A and its effectors play an essential role in cellular metabolism (Liu et al., 2019), immunomodulation (Shulman and Stern-Ginossar, 2020), malignant progression of tumors (Hou et al., 2021), and drug resistance (Mehrdad et al., 2023). In addition, several studies have been devoted to the development of small-molecule inhibitors for m^{6}A modification to improve the efficacy of chemotherapy, radiotherapy, and immunotherapy (Gu et al., 2020; Deng et al., 2023).

Several studies have reported interactions between m^{6}A modifications and lncRNAs (Ma et al., 2019; Yi et al., 2020). m^{6}A modifications affect the functions of lncRNAs through an m^{6}A-switch, thereby inhibiting transcription, mediating competing endogenous RNA (ceRNA) effects, and regulating lncRNA stability or degradation (Jin and Fan, 2023; Mehrdad et al., 2023); for example, METTL14-mediated m^{6}A methylation and TINCR lncRNA regulation in pyroptosis and diabetic cardiomyopathy (Meng et al., 2022). The combination of the m^{6}A reader YTHDC1 and lncRNA *XIST* promotes lncRNA *XIST*-mediated gene repression (Patil et al., 2016). Yang et al. found that the m^{6}A-modified *linc1281* functions as a ceRNA to sequester let-7 miRNAs, thereby exerting regulatory effects on the differentiation of mouse embryonic stem cells (Yang et al., 2018). The m^{6}A eraser ALKBH5 promotes the invasion and metastasis of gastric cancer (GC) by removing the m^{6}A modification on the lncRNA NEAT1 (Zhang et al., 2019). In addition, lncRNAs may also regulate the functions of cancer-associated m^{6}A effectors (Yi et al., 2020). For example, the interplay between the lncRNA LINC00470 and METTL3 contributes to the advancement of GC by enhancing their interaction with the PTEN mRNA and diminishing its stability (Yan et al., 2020). Wang X. et al. reported that the lncRNA GAS5-AS1 enhances the stability of the tumor suppressor GAS5 by interacting with ALKBH5, which removes m^{6}A modification on GAS5, thereby inhibiting the proliferation, migration, and invasion of cervical cancer cells (Wang X. et al., 2019). Additionally, the lncRNA LIN28B-AS1 enhances the stability of *LIN28B* mRNA by interacting with the m^{6}A reader IGF2BP1, thereby promoting the proliferation and metastasis of lung adenocarcinoma (Wang C. et al., 2019).

Given the complexity of the mechanisms underlying the interaction between m^{6}A modifications and lncRNAs, an increasing number of studies have investigated their potential applications in the diagnosis, prognosis, and treatment of tumors and determining the sensitivity of cancer cells to chemotherapeutic agents (Jin and Fan, 2023). A previous study accurately predicted the 5-year survival of patients with GC by stratifying their overall survival (OS) using a risk prediction model based on 11 m^{6}A-associated lncRNAs (Wang H et al., 2021). Similarly, a risk prediction model constructed with m^{6}A-associated lncRNAs has been used to effectively assess the prognosis of patients with lung adenocarcinoma and predict their response to immunotherapy (Xu et al., 2021). Furthermore, m^{6}A effector-related lncRNAs have also been used to establish prediction models for colon adenocarcinoma (Zhang et al., 2021), clear cell renal cell carcinoma (Qiu et al., 2021), breast cancer (Zhang et al., 2020), and pancreatic ductal adenocarcinoma (Hu and Chen, 2021). Thus, m^{6}A effector-related lncRNAs may serve as prognostic biomarkers of various cancers and could potentially guide effective and precise individualized treatment. However, the association between m^{6}A effector-related lncRNAs and the diagnosis and prognosis of patients with SOC remains unclear. Further studies on the interactions between m^{6}A modification and lncRNAs as well as their biological roles in SOC may help reveal the potential of m^{6}A effector-related lncRNAs in PPPM.

In this study, we identified six m^{6}A effector-related lncRNAs via Pearson's correlation, univariate and multivariate Cox regression, and least absolute shrinkage and selection operator (LASSO)-penalized Cox regression analyses using transcriptomic and clinical data of patients with SOC obtained from TCGA database. These six lncRNAs were then used to establish an effective risk prediction model for SOC and develop a web link for clinicians and researchers. Subsequently, we used this newly developed risk model to explore immune-related factors, the TME, and the immunotherapeutic response in SOC. Several drugs capable of potentially targeting m^{6}A effector-related lncRNAs were identified. In addition, one risky lncRNA was selected, and its role and correlation with the m^{6}A effectors in SOC was explored. Our findings could potentially enhance PPPM implementation, enable target prevention, facilitate prognostic assessment, and provide potential biomarkers that may supplement clinical diagnosis as well as treatment in patients with SOC.

2 Materials and methods

2.1 Gene expression profiles and clinical information of patients with SOC

Transcriptomic and mutational data of patients with SOC were downloaded from TCGA using the "TCGAbiolinks" package in R, in September 2021. Information regarding the neoantigen load and mutation burden of patients with SOC was downloaded from The Cancer Immunome Atlas database (TCIA, https://tcia.at/). Genes were annotated using the GENCODE database (https://www.gencodegenes.org). Corresponding clinical information was downloaded from the cBioPortal database (https://www.cbioportal.org). Samples with missing OS values were excluded. As previously reported (Xu et al., 2021), data were randomly divided into training and testing sets in a ratio of 6:4 (Supplementary Table S1). The total data were used as the validation set. The expression of genes was normalized using fragments per kilobase of exon model per million mapped fragments.

The m^{6}A effectors included 12 m^{6}A writers (CBLL1, METTL14, METTL16, METTL3, METTL5, VIRMA, RBM15, RBM15B, TRMT112, WTAP, ZC3H13, and ZCCHC4), 19 m^{6}A readers (ELAVL1, EIF3A, FMR1, G3BP1, G3BP2, HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP2, IGF2BP3, LRPPRC, RBMX, PRRC2A, SND1, YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3), and 2 m^{6}A erasers (ALKBH5 and FTO), as described in previous studies (Zhang Z. et al., 2021; Wang X et al., 2021; Xu et al., 2021). The gene expression profiles (GEPs) of coding genes (including m^{6}A effectors) and lncRNAs needed for subsequent analyses were obtained from TCGA. Pearson's correlation analysis was performed to determine the association between m^{6}A effectors and lncRNAs via "Hmisc" (R package) and visualized using "ggsankey" (R package).

2.2 Establishment and validation of a risk score model

The training set was used to construct the m^{6}A effector-related lncRNA risk model (m^{6}A-LRM). LncRNAs were screened via univariate Cox regression, LASSO Cox regression (using the penalty parameter estimated by 10-fold cross-validation), and multivariate Cox regression analyses using the "survival" and

"glmnet" packages in R. Receiver operating characteristic (ROC) curves were analyzed and visualized using the "ROCR" package in R. The prognostic risk score was calculated as follows:

$$\begin{aligned} m^6A - \text{LRM risk score} = {} & \text{coefficient}(\text{lncRNA1}) \times \text{expression}(\text{lncRNA1}) \\ & + \text{coefficient}(\text{lncRNA2}) \times \text{expression}(\text{lncRNA2}) \\ & + \ldots + \text{coefficient}(\text{lncRNAn}) \\ & \times \text{expression}(\text{lncRNAn}) \end{aligned}$$

Patients in the training, testing, and validation sets were divided into low- and high-risk groups based on the cut-off risk score using the "surv_cutpoint" function of the "survminer" package in R. Both the testing and validation sets were used to validate m^{6}A-LRM, and the results were visualized using the "survminer" package in R. Univariate and multivariate Cox regression analyses were conducted to evaluate the independent effect of m^{6}A-LRM using the "survival" and "survminer" packages in R. Principal components analysis was performed for effective dimensionality reduction, model identification, and grouping using the "prcomp" function and visualized using the "scatterplot3d" package in R. Mutation information was summed, compared, and visualized using the "maftools" package in R. Nomograms were constructed using the "rms" package in R. The results of decision curve analysis and calibration plots were visualized using the "rms" package in R. The time-dependent area under the ROC curve (AUC) was analyzed and visualized using the "timeROC" and "pROC" packages in R.

2.3 Functional and pathway enrichment analyses

Differentially expressed genes (DEGs) between groups were analyzed using "limma" (R package). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted using the KEGG Orthology Based Annotation System (KOBAS, http://bioinfo.org/kobas) database and visualized via "Goplot" and "ggplot2" (R packages). Gene Set Enrichment Analysis (GSEA) was performed to determine potential pathways using "clusterProfiler" (R package) and visualized using "ggplot" and "enrichplot" (R packages). In addition, a single GSEA of miRNA target genes was analyzed using the "GSVA" package in R via "c2.cp.reactome.v2023.1.Hs.symbols.gmt" (https://www.gsea-msigdb.org/gsea/index.jsp).

2.4 Tumor immune microenvironment characteristics and drug response prediction

Differences between the TMEs of high- and low-risk patients were explored by comparing GEPs in "immune_response.gmt" using the "GSVA" (Hanzelmann et al., 2013) package in R (https://www.gsea-msigdb.org/gsea/index.jsp); these were visualized using "ComplexHeatmap" (R package). Immune cell infiltration was estimated using multiple algorithms based on the GEPs, including cell-type identification by estimating the relative subsets of RNA transcripts (CIBERSORT) (Newman et al., 2015), Microenvironment Cell Populations counter (MCPcounter) (Becht et al., 2016), European Prospective Investigation into Cancer and Nutrition (EPIC) (Racle and Gfeller, 2020), and ssGSEA (Charoentong et al., 2017), and "GSVA" (R package). Pro- and anti-inflammatory cytokine ratios of the subgroups were also compared based on the average expression levels of marker genes (Li et al., 2019).

Responses to various therapeutic drugs were predicted using "oncoPredict" (R package) based on the Genomics of Drug Sensitivity in Cancer (http://www.cancerrxgene.org) database. Correlations between lncRNAs and specific drugs were analyzed using information from the LncMAP database (http://bio-bigdata.hrbmu.edu.cn/LncMAP/) and visualized using Cytoscape (version 3.9.0, http://www.cytoscape.org/23).

2.5 Cell culture

The HEK293T cell line (293T), and OC cell lines (CAOV3, and HEY) were purchased from Meisen CTCC (Zhejiang Meisen Cell Technology Co., Ltd., Hangzhou, China). All cell lines were cultured in Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, United States), enriched with 10% fetal bovine serum (epizyme, Shanghai, China), at 37°C and 5% CO_2.

2.6 Generation of *RP11-508M8.1*-overexpressing cell line

We designed and synthesized the full sequence of *RP11-508M8.1 in vitro* and cloned it into the pCDH-EF1-MCS-IRES-puro vector. LncRNA-overexpressing lentivirus vectors and corresponding negative control lentiviruses were generated by packaging in 293T cells, and the viral particles were harvested after 60 h. OC cell lines were infected with the lentivirus. HEY and CAOV3 cells in good condition were selected, counted, and cultured in 10 cm cell culture dishes at 37°C and 5% CO_2 overnight. The medium was discarded the following day, and 2 mL lentivirus and 2 mL complete culture medium were added to each dish. Infection was terminated after 18 h, and the medium was replaced with complete culture medium. After 48 h of virus addition, 0.5 mg/mL puromycin was used for screening.

2.7 Detection of candidate lncRNAs via reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

For RNA purification, cells were lysed in TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY, United States). RNA was extracted from each sample using the RNeasy Mini kit (Qiagen, Hilden, Germany). The extracted RNA was further digested with DNase I (Invitrogen, Waltham, MA, United States) to remove residual DNA. Total extracted RNA was stored at −80°C until use.

RT-qPCR was performed using a QuantStudio 6 Real-Time PCR instrument (Thermo Fisher Scientific Inc.); the reaction mixture comprised 1 μL diluted cDNA, 18.2 μL of 1 × SYBR Green PCR Master Mix, and 0.4 μL each of the forward and reverse primers (10 μmol). The PCR amplification conditions were as follows: 95°C for 5 min, followed by 40 cycles each at 95°C for 10 s and 60°C for

30 s. All samples were tested in triplicate. The relative levels of lncRNAs in cells was calculated using the following equation:

$$\text{Amount of target} = 2^{-\Delta Ct}, \text{where } \Delta Ct = Ct_{lncRNA} - Ct_{GAPDH}$$

Gene-specific primers for lncRNA and the housekeeping gene *GAPDH* are listed in Supplementary Table S2. Three primers were designed for the RP11-508M8.1 sequence to identify the overexpression of lncRNA.

2.8 EdU staining assay

The proliferation ability of *RP11-508M8.1* in OC cells was determined using an EdU assay kit (Cell Light EdU DNA imaging Kit, RiboBio). A total of 1×10^4 cells were seeded in 96-well plates, incubated overnight, and treated with EdU (50 μMol) for 2 h. Subsequently, the plate was removed, and the remainder of the experiment was conducted according to the instructions provided with the kit. Finally, five visual fields were randomly selected under a fluorescence microscope to acquire images as well as to calculate the proportion of EdU-positive cells.

2.9 Migration and invasion Transwell assays

Cell migration and invasion experiments were performed using a Transwell chamber (3422, Corning, United States). The invasion experiment required the addition of Matrigel (BD Pharmingen, San Jose, CA, United States) to the bottom of the chamber in advance, followed by a subsequent experiment post-solidification. A total of 2×10^5 cells were suspended in serum-free medium in the upper chamber. For the migration assay, 1×10^5 cells were suspended in serum-free medium in the upper chamber, followed by the addition of 600 μL complete medium to each culture hole. The Transwell chamber was placed in the plate and returned to the incubator for further culture for 6 (HEY) or 8 h (CAOV3). After removing the chamber, the cells were fixed with 4% paraformaldehyde for 10 min and stained with 0.1% crystal purple for 10 min. The operational procedure for the invasion experiment was consistent with that for the migration assay. Each chamber was photographed under a microscope (Leica, London, United Kingdom).

2.10 Wound healing assay

When the cells seeded in six-well plates reached 100% confluence, the plates were removed. Scratches were made on each plate, and the cells were rinsed gently with phosphate-buffered saline to remove floating cells. Cell culture was continued with a medium containing 2% fetal bovine serum. Representative images of cells at 0 and 24 h were obtained using a microscope, and the confluence of cells was calculated using ImageJ 1.53a (Schneider et al., 2012) to observe the invasion and migration abilities of *RP11-508M8.1* in OC cell lines.

2.11 Cell transfection and Western blotting

Small interfering RNA (siRNA) against METTL3 was synthesized by RiboBio (Guangzhou, China; Supplementary Table S3) and then transfected using Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, United States) according to the manufacturer's instructions. After 48 h of transfection, cells were lysed in RIPA lysis buffer supplemented with a proteasome inhibitor. Following whole cell lysis, proteins separated via SDS-PAGE (12%) were transferred onto a PVDF membrane. The membrane was then blocked with skimmed milk and incubated with specific primary and secondary antibodies (anti-METT3: huabio; anti-GAPDH: Proteintech). Finally, protein expression was visualized using a Bio-Rad ChemiDoc Touch Imaging System.

2.12 RNA sequencing and analysis

Cells were collected, and total cellular RNA was extracted as described above. One microgram of total RNA was used for library preparation; poly (A) mRNA was isolated using Oligo (dT) beads, and mRNA fragmentation was performed using divalent cations under high temperature. Priming was performed using Random Primers. First- and second-strands of cDNA were synthesized; then, double-stranded cDNA was purified, treated to repair both ends, and subjected to dA-tailing in a single reaction. Subsequently, T-A ligation was performed to add adaptors to both the ends. Size selection of adaptor-ligated DNA was performed using DNA Clean Beads. Each sample was amplified via PCR using P5 and P7 primers, and the PCR products were validated. Libraries with different indices were then multiplexed and loaded on an Illumina HiSeq/Illumina Novaseq/MGI2000 instrument for sequencing using the 2 × 150 paired-end (PE) configuration according to the manufacturer's instructions.

Pass filter data in the fastq format were processed using Cutadapt (V1.9.1, phred cutoff: 20, error rate: 0.1, adapter overlap: 1 bp, min. length: 75, proportion of N: 0.1) to remove technical sequences, including adapters, PCR primers or fragments thereof, and bases of quality lower than 20, to obtain high-quality clean data. First, human GRCh38 genome sequences and annotation files of relative species were downloaded from ENSEMBL. Then, Hisat2 (v2.0.1) was used to index the reference genome sequences. Finally, clean data were aligned to the reference genome via the Hisat2 software (v2.0.1). The initial transcripts in the fasta format were converted from a known gff annotation file and indexed properly. Next, using the file as the reference gene file, gene and isoform expression levels were estimated from cleaned pair-end data via HTSeq (v0.6.1). DEGs between groups were determined using "DESeq2" (R package) based on $p < 0.05$ and foldchange ≥1.5.

2.13 Dot blot assay

First, total RNA was denatured at 65°C for 5 min and transferred on to a nitrocellulose membrane (Millipore, United States) according to experimental requirements. Next, the membrane was cross-linked using UV for 30 min and washed in Phosphate Buffered Saline with Tween at room temperature for 10 min to remove the unbound RNA and

subsequently sealed with milk at room temperature for 1 h. Finally, the membrane was incubated overnight with an m^{6}A antibody (1:1,000, Synaptic Systems, Germany) at 4°C and a horseradish peroxidase-conjugated secondary antibody (1:1,000, Cell Signaling Technology, United States) at room temperature for 1 h. After washing, the signal of the membrane was detected using a chemiluminescence system (Bio-Rad). The membrane was stained with 0.02% methylene blue (MB) dissolved in 0.3 M sodium acetate solution (pH 5.2), and images were acquired.

2.14 m^{6}A RNA immunoprecipitation-qRT-PCR (m^{6}A MeRIP-qRT-PCR)

METTL3 expression in ovarian cancer cells was knocked down using siRNA (Supplementary Table S3), and an m^{6}A-modified RNA enrichment analysis was performed on the control and *METTL3*-knockdown cell samples according to the instructions of the riboMeRIP m^{6}A Transcriptome Profiling Kit (C11051-1, RiboBio China). Briefly, 50 μg total RNA was extracted and segmented into 100–150 nt fragments, and magnetic beads with anti-m^{6}A were prepared using 1/10 segmented RNA as input. The remaining segmented RNA required for MeRIP reaction solution was prepared, rotated and mixed at 4°C, and incubated for 2 h. Finally, the methylated RNA bound to the m^{6}A antibody was eluted and recovered. RT-qPCR was used to detect *RP11-508M8.1* expression as well as to analyze the data following normalization to the input.

2.15 Identification of the lncRNA-miRNA-mRNA regulatory axis

Potential target miRNAs for candidate lncRNAs were predicted using RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid), which was also used to predict the secondary structures of lncRNAs. Key miRNAs associated with the candidate lncRNA *RP11-508M8.1* were further screened based on the following criteria: (i) the miRNAs were significantly associated with survival in OC according to the ONCOMIR (https://www.oncomir.org) database and (ii) miRNA seed region (5′-> 3′) with the 2–7 bp was strictly matched with that of lncRNA. Moreover, potential target genes of these key miRNAs were further screened according to the following criteria: (i) the miRTarBase database (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/ index.php) was used to predict the target genes for the key miRNAs; (ii) the target genes were further identified by combining with the DEGs (|foldchange| > 1.5 and $p < 0.05$) in cell lines overexpressing *RP11-508M8.1*; (iii) Kaplan–Meier (KM) survival analysis was applied to filter the prognosis-related mRNAs; (iv) mRNA expression levels in patients with OC were aberrant.

2.16 Statistical analysis

Continuous variables were analyzed using Student's *t*-test or the nonparametric Wilcoxon test. Prognostic analyses were performed using KM survival and univariate Cox analyses. Data were analyzed using R 4.0.1 (http://www.r-project.org/). *p*-values < 0.05 were considered statistically significant.

3 Results

3.1 Construction of the m^{6}A-LRM for patients with SOC

The detailed procedure for identifying m^{6}A effector-related lncRNAs is illustrated in Figure 1. GEP data for 33 m^{6}A effectors and 15,900 lncRNAs of patients with SOC were obtained from TCGA. A total of 2,244 m^{6}A effector-related lncRNAs were identified based on Pearson's correlation analysis (|R| > 0.3 and $p < 0.001$). The m^{6}A effectors and their related lncRNAs were visualized in a correlation network (Figure 2A). Among the 2,244 m^{6}A effector-related lncRNAs in the training set, 895 lncRNAs that were significantly correlated with OS were identified using univariate Cox regression analysis ($p < 0.05$; Supplementary Table S4). Subsequently, we performed LASSO Cox regression analysis to identify candidate lncRNAs associated with the prognosis of patients with SOC. As a result, 13 m^{6}A effector-related lncRNAs were selected based on the λ minimization method (Figures 2B, C). Model self-rating indicated that these 13 lncRNAs had significant diagnostic value (AUC = 0.802) as well as discriminatory power in the training set (Figures 2D, E). Multivariate Cox regression analysis, which was performed to control confounding factors, detected six m^{6}A effector-related lncRNAs that were independently correlated with OS. Among them, RP11-508M8.1 and AC138761.4 were identified as risk factors [hazard ratio (HR) > 1, $p < 0.05$], whereas AL513211.1, LINC02384, MYCNOS, and AC072062.3 were identified as protective factors (HR < 1, $p < 0.05$; Figure 2F; Supplementary Figure S1).

Subsequently, the m^{6}A-LRM was constructed based on the above-mentioned six lncRNAs, the GEPs and regression coefficients of which were used to calculate prognostic risk scores in the training set. The concordance index of the m^{6}A-LRM was 0.672 ± 0.025 (Figure 2F), indicating a favorable prognostic value. Surprisingly, the correlations between m^{6}A effectors and candidate lncRNAs were complex, suggesting interactions and a crosstalk (Figure 2G). Patients with SOC were stratified into low- and high-risk groups based on the risk scores. The distribution of risk scores from the m^{6}A-LRM and survival status of patients in the training set are shown in Figure 3A. High-risk patients had significantly shorter OS than low-risk patients ($p < 0.001$, Figure 3B).

3.2 External validation of the prognostic model m^{6}A-LRM

To validate the prognostic ability of the m^{6}A-LRM, risk scores in the testing and validation sets were determined. The distributions of risk scores, survival status, and survival time of patients with SOC are depicted (Figures 3C, E). As expected, the high-risk patients with SOC had shorter OS than the low-risk patients ($P_{\text{testing set}} < 0.001$, $P_{\text{validation set}} < 0.001$; Figures 3D, F). Furthermore, the AUC values for 1-, 3-, 5-, 10-year OS estimated using the m^{6}A-LRM were stable over time (Supplementary Figure S2). Furthermore, principal components analysis was performed to analyze the discriminatory power of the m^{6}A-LRM for low- and high-risk patients with SOC using

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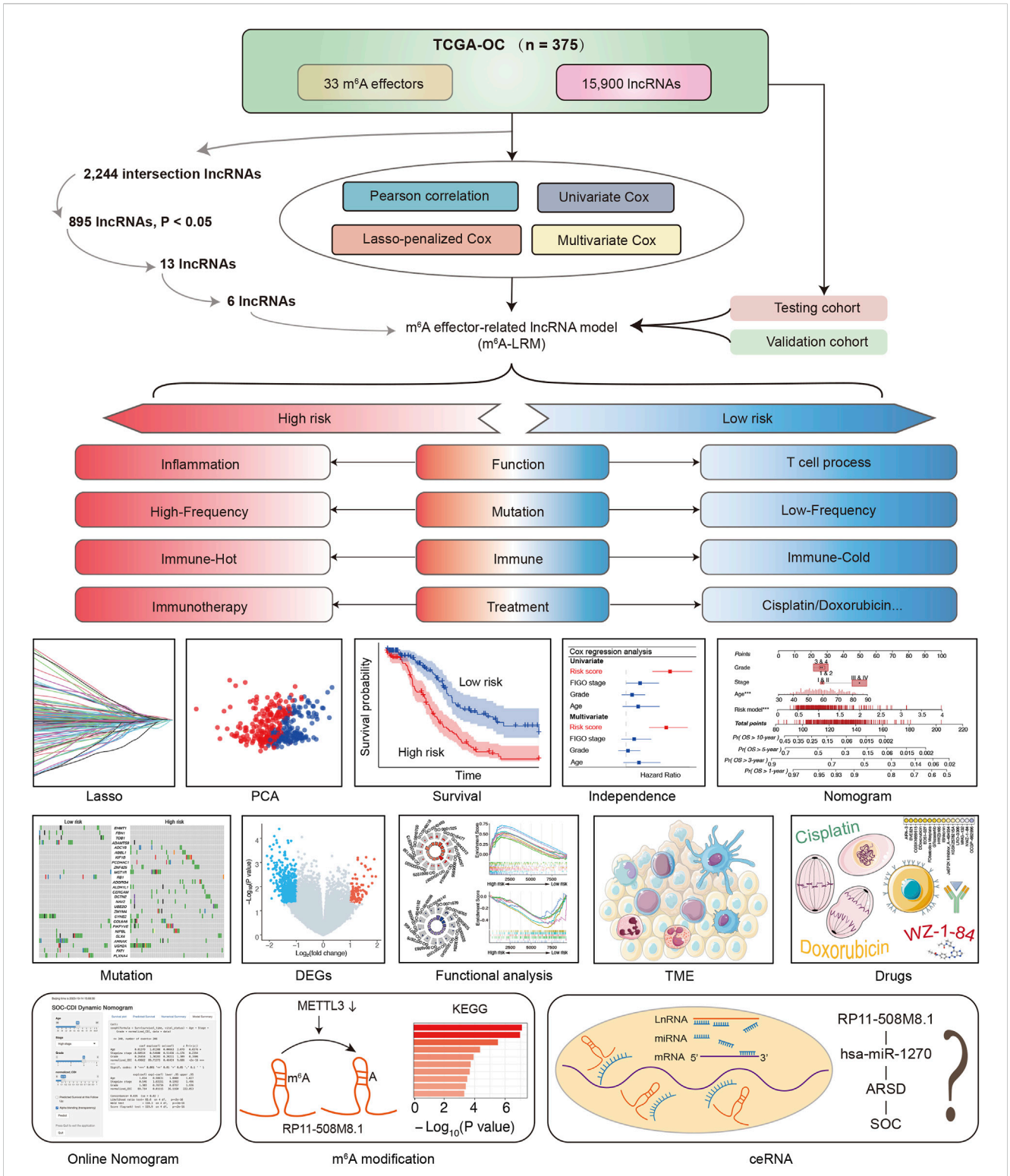

FIGURE 1
Schematic of the study.

GEPs obtained from the following: all RNA-seq data, coding genes, 33 m⁶A effectors, 6 m⁶A effector-related lncRNAs, and m⁶A-LRM. These GEPs did not effectively discriminate between patients with SOC in the low- and high-risk groups, except for the m⁶A-LRM (Figure 3G). Interestingly, the m⁶A-LRM showed remarkable discriminatory power and provided an efficient prognostic signature in patients with SOC.

To evaluate whether the m⁶A-LRM shows potential as an independent prognosis estimator for patients with SOC, univariate and multivariate Cox regression analyses were conducted on the

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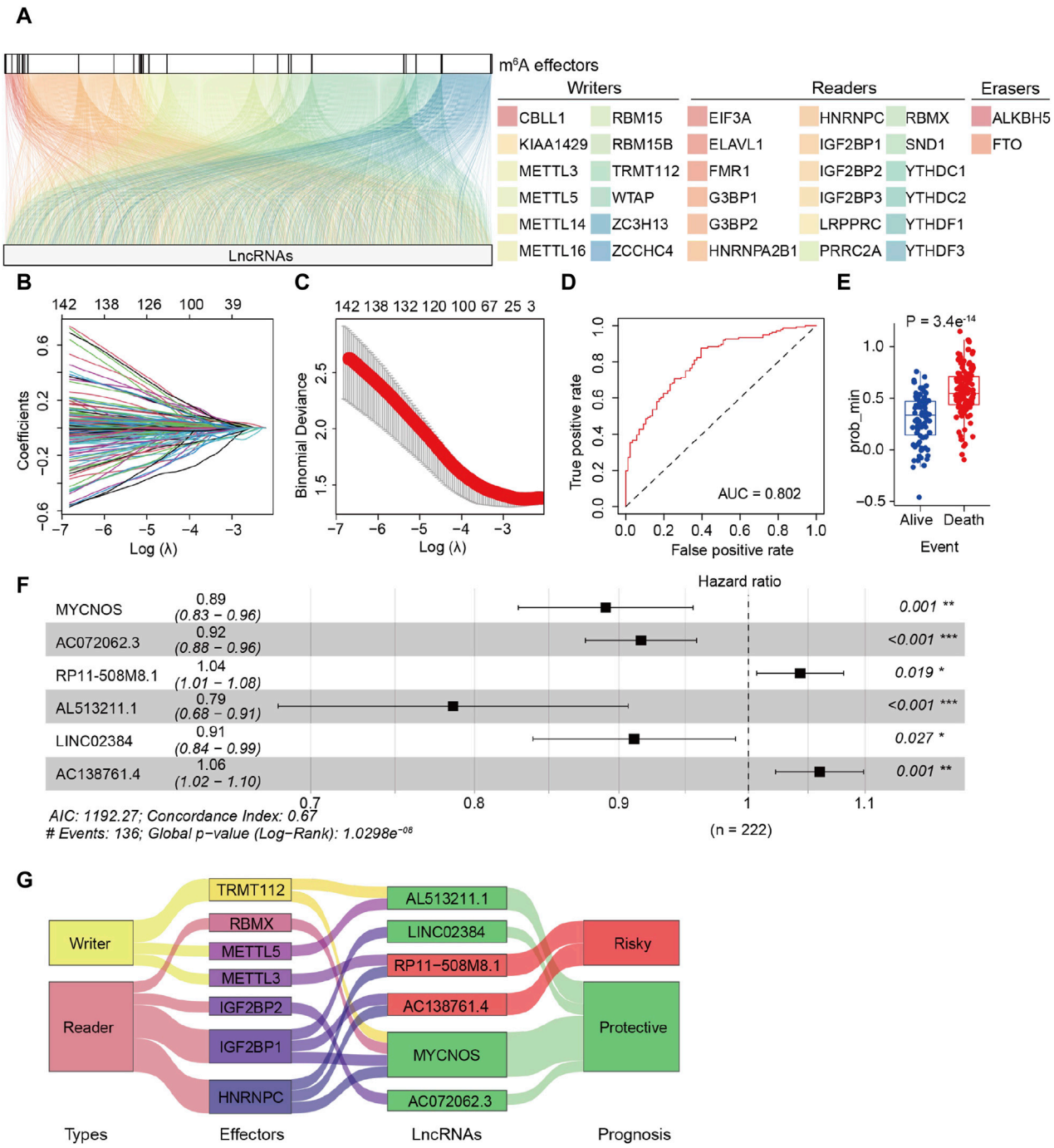

FIGURE 2
Identification of m^{6}A effector-related lncRNAs in patients with serous ovarian carcinoma (SOC). **(A)** Relationship between 33 m^{6}A effectors and related lncRNAs (YTHDF2 is not shown because there was no significant relationship between YTHDF2 and lncRNAs). **(B)** LASSO coefficient plots of overall survival (OS)-related lncRNAs. Perpendicular imaginary lines were drawn at the value chosen via 10-fold cross-validation. **(C)** Tuning parameters (log λ) of OS-related lncRNAs were selected to cross-verify the error curve. According to the minimal criterion (left vertical line) and 1-se criterion (right vertical line), perpendicular imaginary lines were drawn at the optimal value. **(D)** ROC curves of the model in internal validation. **(E)** Predictive discrimination of the model based on the results from minimal criterion (left panel) and 1-se criterion (right panel). **(F)** Multivariate cox regression analysis of six independent lncRNAs associated with prognosis; two were risk factors and the other four were protective factors. **(G)** Sankey diagram for correlations between 33 m^{6}A effectors and 6 prognostic m^{6}A effector-related lncRNAs; the diagram shows that IGF2BP1 and HNRNPC are correlated to risk-related and protective lncRNAs. In addition, different effectors were related to the same lncRNAs (METTL3/HNRNPC to RP11-508M8.1, TRMT112/RBMX/IGF2BP1/HNRNPC to MYCNOS).

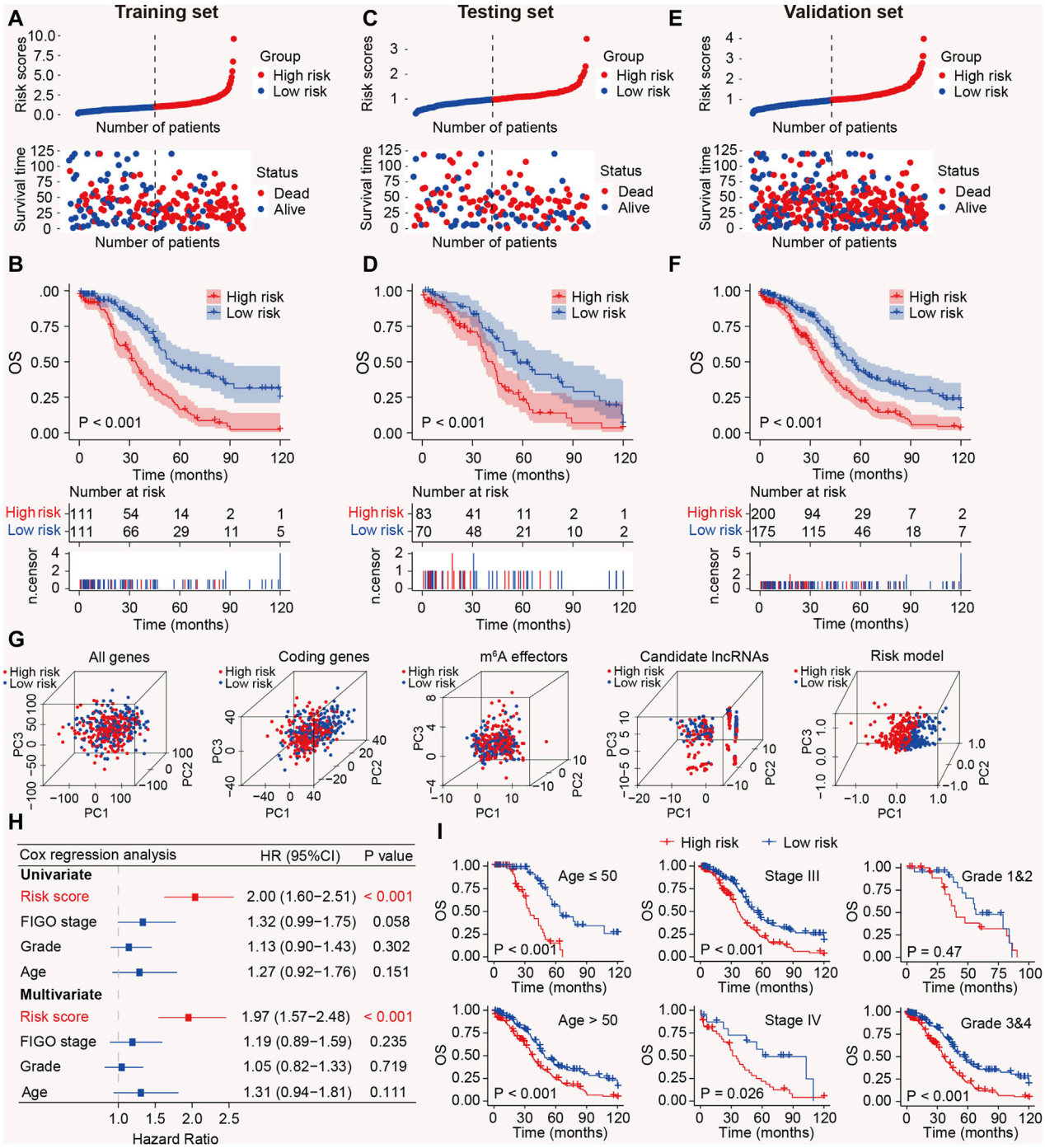

FIGURE 3
Prognostic value of the m^{6}A-LRM in training, testing, and validation sets. **(A)** Distribution of m^{6}A-LRM-based risk scores (upper panel), survival status, and survival time between high- and low-risk patients (bottom panel). Blue represents the low-risk group, whereas red represents the high-risk group. **(B)** KM analysis of survival of patients in the training set in the high- and low-risk groups. **(C,E)** Distribution of risk scores, survival status, and survival time of patients divided by m^{6}A-LRM in the **(C)** testing set and **(E)** validation set. **(D,F)** KM survival analysis in the **(D)** testing and **(F)** validation sets. **(G)** Principal components analysis between the high- and low-risk groups based on following data: (1) All gene expression profiles, (2) Expression profiles of coding genes, (3) Expression profiles of 33 m^{6}A effectors, (4), Expression profiles of six m^{6}A effector-related lncRNAs, (5) m^{6}A-LRM. **(H)** Univariate and multivariate analyses of clinical features and risk scores with OS; CI, confidence interval. **(I)** Prognostic ability of the risk score in distinguishing between the OS of patients ≤50 years of age and those aged >50 years (left panel). Prognostic ability of the risk score to distinguish between the OS of patients with SOC with stage III and stage IV (Middle panel). Prognostic ability of the risk score to distinguish between the OS of SOC patients with grades 1 and 2 or grades 3 and 4 (Right panel).

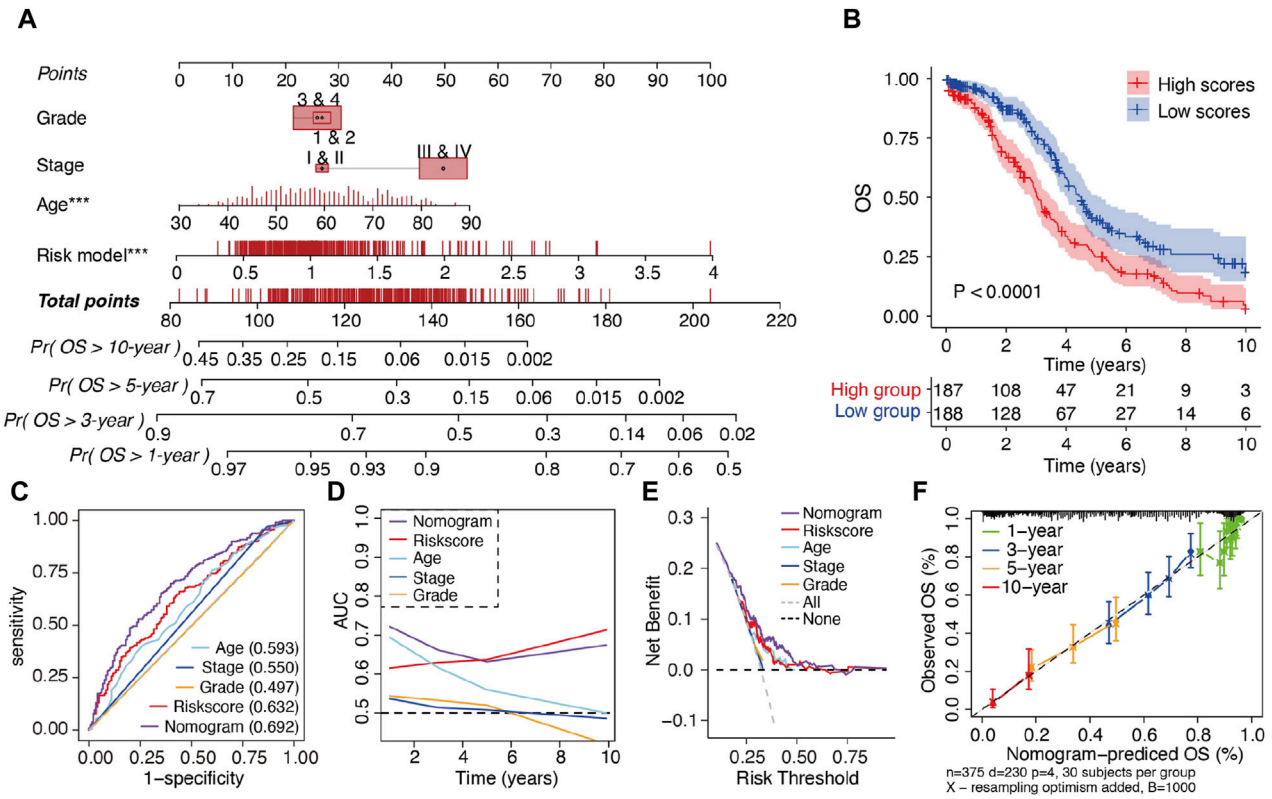

FIGURE 4
Construction and assessment of a prognostic nomogram. **(A)** Nomogram constructed using the m^{6}A-LRM risk score and clinical features for 1-, 3-, 5-, and 10-year overall survival (OS). **(B)** KM survival analysis of patients in the high- and low-nomogram scores groups. **(C)** ROC curves of clinical features, m^{6}A-LRM, and nomogram for OS. **(D)** Time-dependent AUC of the nomogram, m^{6}A-LRM, and clinical features. **(E)** Decision curve analyses (DCAs) of the nomogram, m^{6}A-LRM, and clinical features. **(F)** Calibration plot of the nomogram, showing the correlation between the actual and predicted 1-, 3-, 5-, and 10-year OS in SOC.

m^{6}A-LRM risk score, the patients' International Federation of Gynecology and Obstetrics (FIGO) stage, tumor grade, and age. Only the m^{6}A-LRM risk score was found to be an independent prognostic risk factor for patients with SOC ($p < 0.001$; Figure 3H). Univariate Cox regression analysis revealed that the m^{6}A-LRM risk score had HR and 95% confidence interval (CI) values of 2.00 and 1.60–2.51, respectively, similar to those obtained using the multivariate Cox regression analysis (1.97 and 1.57–2.48, respectively). These results highlighted the m^{6}A-LRM risk score as the key independent prognostic factor for patients with SOC. Moreover, based on their clinicopathological characteristics, patients were stratified into low- and high-risk groups in the validation set. According to classification by patients' age, FIGO stage, and grade, the OS of low-risk patients was longer than that of high-risk patients (Figure 3I).

3.3 Nomogram construction and evaluation

To enhance the clinical applicability of the m^{6}A-LRM, a nomogram consisting of the m^{6}A-LRM risk score, FIGO stage, tumor grade, and age of patients was constructed for predicting the 1-, 3-, 5- and 10-year OS in SOC (Figure 4A). Stratification of patients into low- and high-risk groups, based on their nomogram scores, indicated that the OS of patients with low nomogram scores was longer than that of patients with high nomogram scores (Figure 4B). Additionally, the nomogram (0.692) as well as the m^{6}A-LRM (0.632) had higher ROC values than those of the other clinicopathological characteristics (Figure 4C). Moreover, the AUC value of the nomogram was greater than that of other clinical features and similar to that corresponding to m^{6}A-LRM over time (Figure 4D). Compared with clinical characteristics alone, the nomogram showed a predominant predictive ability for SOC (Figure 4E). The calibration charts further displayed that the 1-, 3-, 5- and 10-year survival curves were ideally consistent between the actual and predicted OS (Figure 4F), confirming its prognostic value. Moreover, we established a user-friendly web link for clinicians (https://leley.shinyapps.io/OC_m6A_lnc/). These results suggest that the nomogram can be effectively used to assess the prognosis of patients with SOC.

3.4 Functional enrichment analysis of the m^{6}A-related lncRNAs between low- and high-risk patients with SOC

To explore the underlying molecular mechanisms of m^{6}A-related lncRNAs, GO, pathway, GSEA, and GSVA analyses were performed. DEGs were identified based on fold change >1.5 and $p < 0.001$. GO analysis revealed that the most significantly altered pathways in the high-risk subgroup were those

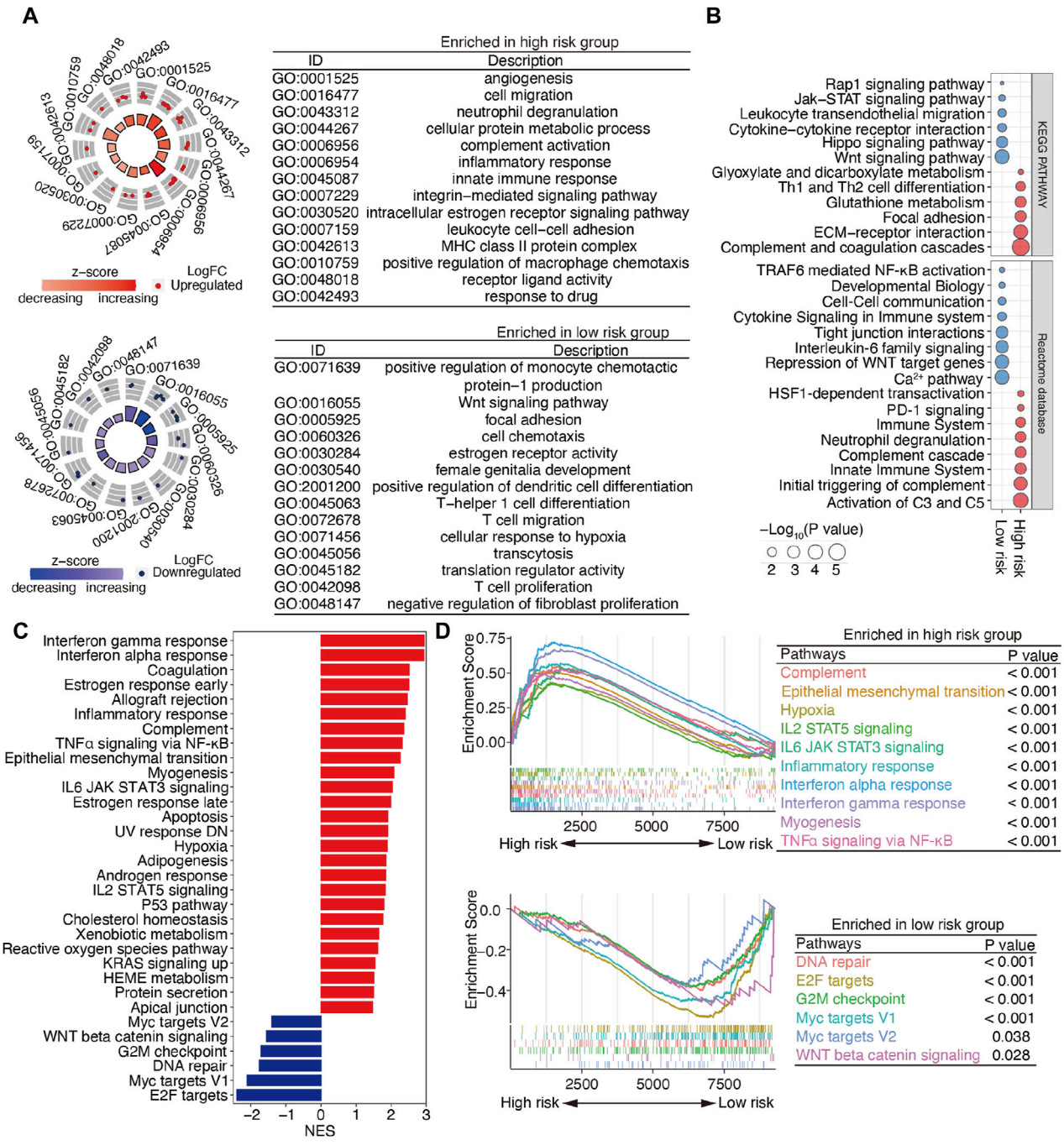

FIGURE 5
Functional enrichment analysis of m^{6}A-related lncRNAs between the low- and high-risk patients with SOC. **(A)** GO terms are displayed by GOCircle plots. Red and blue dots represent the genes upregulated in the high-risk and low-risk groups separately. **(B)** Pathway analyses based on the KEGG and Reactome databases. **(C)** GSEA enrichment analysis. **(D)** GSEA plots for the two subgroups of patients with SOC. Top panel, pathways enriched the high-risk group; bottom panel, pathways enriched in the low-risk group.

mainly associated with angiogenesis, cell migration, neutrophil degranulation, innate immune response, the integrin-mediated signaling pathway, and the MHC class II protein complex. T-cell-related pathways, including T-helper 1 cell differentiation, T-cell migration, and T-cell proliferation, positive regulation of monocyte chemotactic protein-1 production, the Wnt signaling pathway, and negative regulation of fibroblast proliferation, were mainly converged in the low-risk group (Figure 5A). Pathway analyses based on two databases confirmed these findings and showed some extent of overlap with the GO analysis results (Figure 5B). A GSEA, conducted to clarify the specific roles of these pathways according to the risk categories, revealed that DEGs were enriched in inflammation-related pathways, including the interferon-gamma response, interferon-alpha response, inflammatory response, TNFα signaling via NF-κB, IL6 JAK STAT3 signaling and IL2 STAT5 signaling, the epithelial mesenchymal transition

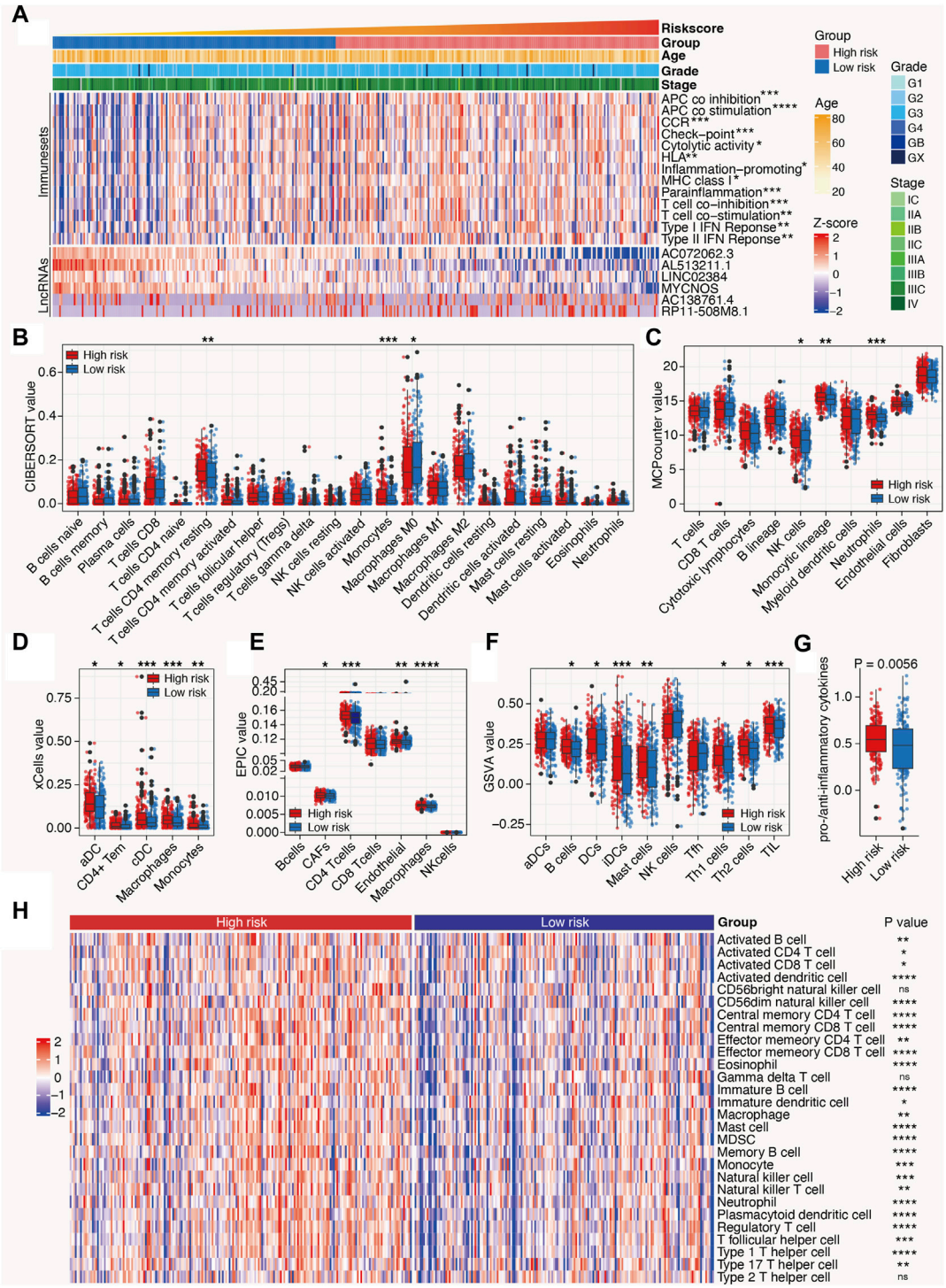

FIGURE 6
Tumor immune microenvironment characteristics of the m^{6}A-related lncRNAs in SOC. **(A)** Heat map depicts the distribution of clinicopathological features and risk scores calculated by the m^{6}A-LRM; value of immune sets and expression levels of lncRNAs included in the m^{6}A-LRM. **(B–F,H)** Deconvolution algorithms of the CIBERSORT **(B)** and EPIC **(E)** algorithms based on the expression levels of marker genes, including MCPcounter **(C)**, xCell **(D)**, GSVA **(F)**, and ssGSEA **(H)**, were applied to estimate the immune cell infiltration status between the high- and low-risk groups. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. **(G)** Ratios of pro-to anti-inflammatory cytokines.

(EMT), and the hypoxia and reactive oxygen species pathway (Figure 5C).

Importantly, the upregulated genes were enriched in the EMT and inflammation-related pathways, whereas the downregulated genes were enriched in DNA repair and WNT beta-catenin signaling (Figure 5D). These findings suggest that DEGs between high- and low-risk groups are implicated in the cancer–immunity pathway.

3.5 Characteristics of m^{6}A-related lncRNAs in the tumor immune microenvironment in SOC

Owing to the close relationship between m^{6}A-related lncRNAs and the immune process, the differences between the immunological data and tumor-infiltrating immune cells associated with high- and low-risk SOC were compared. The high-risk patients with SOC had higher scores for immune sets than the low-risk patients (Figure 6A). Multiple algorithms, including CIBERSORT, MCPcounter, xCell, EPIC, and GSVA, were used to evaluate the extent of infiltration of immune cells. The expression levels of CD4$^+$ T cells, monocytes, dendritic cells, B cells, Th1 cells, Th2 cells, and Tumor-infiltrating lymphocytes (TILs) in the high-risk subgroup were higher than those in the low-risk subgroup. In addition, the ratio of pro-to anti-inflammatory cytokines in the high-risk subgroup was elevated compared with that in the low-risk group ($p < 0.05$; Figures 6B–G). ssGSEA algorithms for approximately 28 immune cells were also used to substantiate the above-mentioned findings. Consistent with these results, the heat maps showed that most immune cells were enriched in the high-risk group, indicating a proinflammatory status in the high-risk group and an immune-inhibiting environment in the low-risk group (Figure 6H). These results indicate that the high-risk group is characterized by an activated immune phenotype, whereas the low-risk group exhibits a suppressed immune phenotype.

3.6 Mutational landscape of m^{6}A-related lncRNAs in SOC

Considering that hot tumors are more susceptible to immune therapy, we anticipated that patients with SOC in the high-risk group (as defined by the m^{6}A-LRM) may respond to immune therapies more readily than those in the low-risk group. Previous studies have indicated that high levels of somatic mutations and neoantigens may signify a greater probability of a favorable chemotherapeutic response. We investigated the variability observed between the mutation statuses of these two groups. First, the top 20 genes with high mutation frequencies in low- and high-risk patients with SOC were identified and compared. A higher mutational rate of *USH2A* was observed in the high-risk group, while a higher mutational rate of *SYNE2* was observed in the low-risk group, with the other genes not showing any statistically significant differences (Supplementary Figure S3A). Next, we identified differentially mutated genes and found generally greater mutational rates in the high-risk group, indicating that the m^{6}A-LRM did not affect frequently mutated genes but instead exerted an additive effect on those with low-frequency mutations (Figure 7A; Supplementary Figure S3A). Moreover, *TP53* had the highest mutation frequency in patients with SOC (89% and 92% in the low- and high-risk groups with gene mutation, respectively). However, no significant differences were observed in the tumor mutational burden (TMB), *TP53* mutations, and neoantigens between the low- and high-risk groups (Supplementary Figures S3A–C).

The prognostic ability of m^{6}A-LRM for the TMB, *TP53* mutations, and neoantigens in patients with SOC were further explored. *TP53* effectively distinguished the survival statuses of patients with SOC (Riskscore-H + *TP53* mutation vs. Riskscore-H + wild *TP53*, $p = 0.460$; Riskscore-L + *TP53* mutation vs. Riskscore-L + wild *TP53*, $p = 0.955$; Figure 7B). Interestingly, the TMB also effectively distinguished between the survival statuses of patients with SOC (Riskscore-H + TMB-H vs. Riskscore-H + TMB-L, $p = 0.011$; Riskscore-L + TMB-H vs. Riskscore-L + TMB-L, $p < 0.001$; Figure 7C), as did neoantigens in patients with SOC with low-risk scores (Riskscore-L + NEO-H vs. Riskscore-L + NEO-L, $p = 0.019$, Figure 7D).

Furthermore, patients with SOC with high neoantigen levels in the high-risk group showed a propensity for higher OS compared with those with low neoantigen levels without significant differences (Riskscore-H + NEO-H vs. Riskscore-H + NEO-L, $p = 0.071$, Figure 7D). The m^{6}A-LRM showed significant effectiveness for classifying patients who had the same *TP53*, TMB, and neoantigen status (Riskscore-H + *TP53* mutation vs. Riskscore-L + *TP53* mutation, $p < 0.001$; Riskscore-L + wild *TP53* vs. Riskscore-L + wild *TP53*, $p < 0.001$; Riskscore-H + TMB-H vs. Riskscore-L + TMB-H, $p < 0.001$; Riskscore-H + TMB-L vs. Riskscore-L + TMB-L, $p = 0.022$; Riskscore-H + NEO-H vs. Riskscore-L + NEO-H, $p < 0.001$; Riskscore-H + NEO-L vs. Riskscore-L + NEO-L, $p = 0.008$, Figures 7B–D), confirming the superiority of m^{6}A-LRM over the currently available biomarkers. Additionally, we found that combining the risk scores with TMB and neoantigens increased the accuracy of prognosis estimation of patients with SOC (Riskscore-H + TMB-L vs. Riskscore-L + TMB-H, $p < 0.001$; Riskscore-H + NEO-L vs. Riskscore-L + NEO-H, $p < 0.001$, Figures 7C, D). These results indicated that the prognostic value of the m^{6}A-LRM was superior to that of the TMB and neoantigens in patients with SOC.

3.7 Estimation of drug sensitivity and identification of novel compounds that target m^{6}A-related lncRNAs in SOC

Considering the above-mentioned findings, we explored the association between m^{6}A-related lncRNAs and immunotherapy. First, we compared the expression of immune checkpoints between the two subgroups. As expected, the high-risk patients were more likely to respond positively to immunotherapy than the low-risk patients and showed high expression of immune checkpoint targets, except for CD200 (Figure 8A), which suggested that risk classification based on the m^{6}A-LRM may serve as an indicator for response to immunotherapy.

Next, we investigated the association between the lncRNAs utilized in the m^{6}A-LRM and drug compounds to identify potential drugs targeting m^{6}A effector-related lncRNAs. Interactions between lncRNAs and these drugs were predicted, resulting in the identification of 26 lncRNA-drug pairs (Supplementary Table S5); then, the complex interactions between them were observed (Figure 8B; Supplementary Figure S4). Considering its potential role in modifying immunotherapy, we used the calcPhenotype algorithm to predict the response of a common drug used for OC treatment based on the half-maximal inhibitory concentration (IC_{50}) to explore the clinical use of the m^{6}A-LRM. The results indicated that of the 16 commonly used drugs, six (cisplatin, gemcitabine, vinorelbine, doxorubicin, camptothecin, and irinotecan) had lower IC_{50} values ($p < 0.05$, Figure 8C) in the low-risk group. Furthermore, there was no

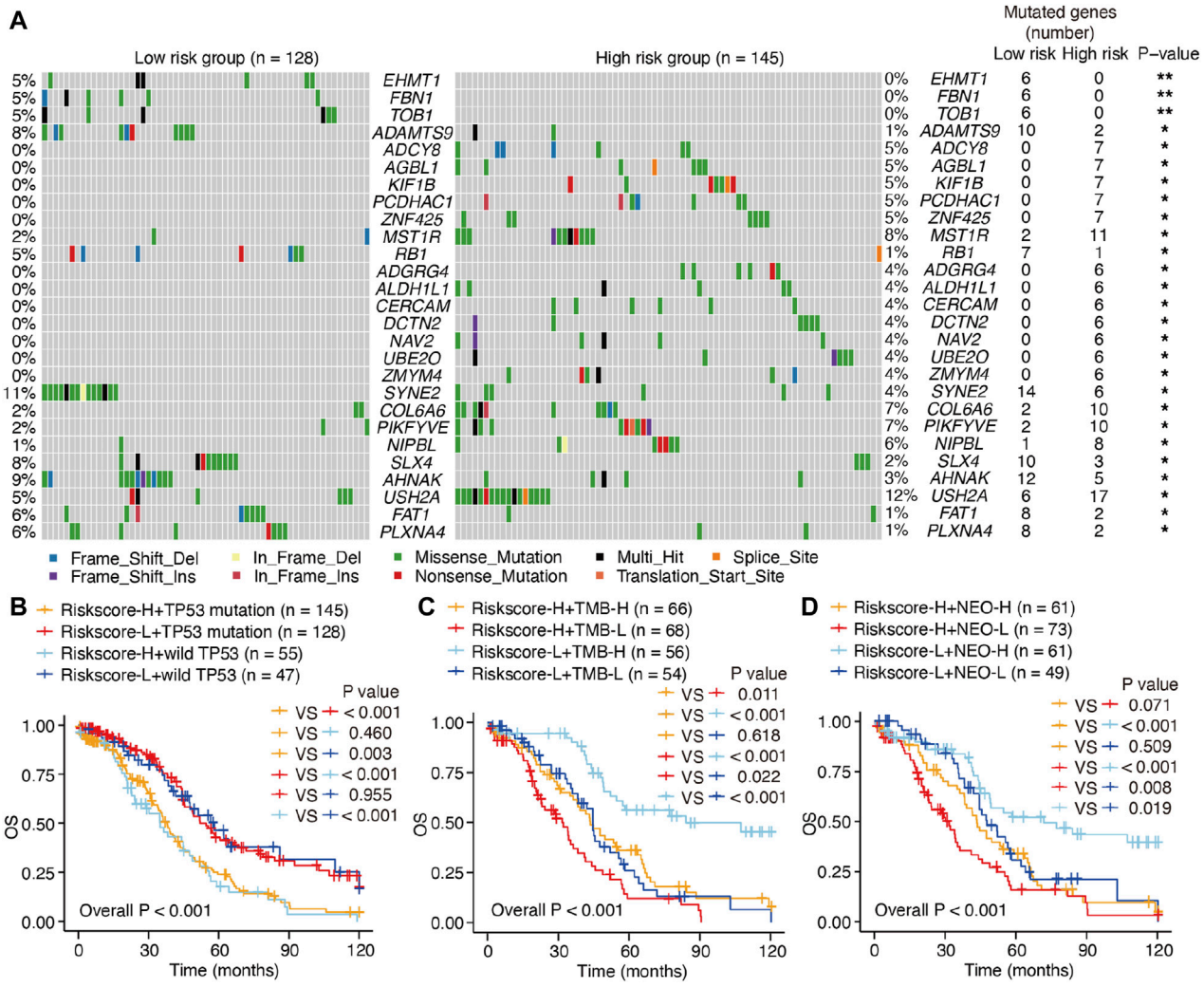

FIGURE 7
Mutations and survival in low- and high-risk patients with SOC. **(A)** Waterfall plot of differentiated somatic mutation features between the high- and low-risk groups. **(B–D)** KM survival analysis of patients classified via m^{6}A-LRM risk scores, combining *TP53* mutation status, TMB loads, and neoantigens levels. TMB, tumor mutational burden; NEO, neoantigen; H, high; L, low.

significant difference between the IC_{50} values of other 10 drugs (Supplementary Figure S4). To further explore drugs that may potentially target SOC, we screened the Genomics of Drug Sensitivity in Cancer database. The top 10 potential compounds that exhibited significant differences in efficacy between the high- and low-risk groups are shown (Figures 8C, D). Seven compounds, including doxorubicin, displayed lower IC_{50} values in the low-risk group (Figure 8C), whereas three drugs exhibited greater sensitivity in the high-risk patients. These results indicate that the m^{6}A-LRM has potential for predicting the sensitivities of certain drugs beneficial to different groups of patients with SOC.

3.8 Cytological function of RP11-508M8.1 in OC cells

RP11-508M8.1 is closely related to METTL3 and HNRNPC, while AC138761.4 is closely related to IGF2BP1 and HNRNPC. Previous studies have shown that METTL3 is the only catalytic subunit of the m^{6}A methyltransferase complex that plays critical roles in various cancers (Deng et al., 2022; Fang et al., 2022). This information indicates that RP11-508M8.1 may play an important role in SOC. Thus, we initially selected RP11-508M8.1 and investigated its mechanism in ovarian cancer. First, in a previous study, we detected the expression of RP11-508M8.1 in normal ovaries and ovarian cancer cell lines (Ye et al., 2022). Then, to explore the functions of a candidate lncRNA, two stable SOC cell lines (HEY and CAOV3) overexpressing RP11-508M8.1 were successfully constructed (Figure 9A). Overexpression of *RP11-508M8.1* resulted in only minor effects on the proliferation of OC cell lines (Figure 9B) but significantly promoted the migration and invasion of HEY and CAOV3 (Figure 9C). Furthermore, the wound healing assay revealed that overexpression of *RP11-508M8.1* may enhance the wound-healing ability of OC cells (Figure 9D). To further explore the association between the m^{6}A modification effector and lncRNA, we detected the expression of *METTL3*, which is associated with RP11-508M8.1 (Figure 2G). Overexpression of *RP11-508M8.1* did

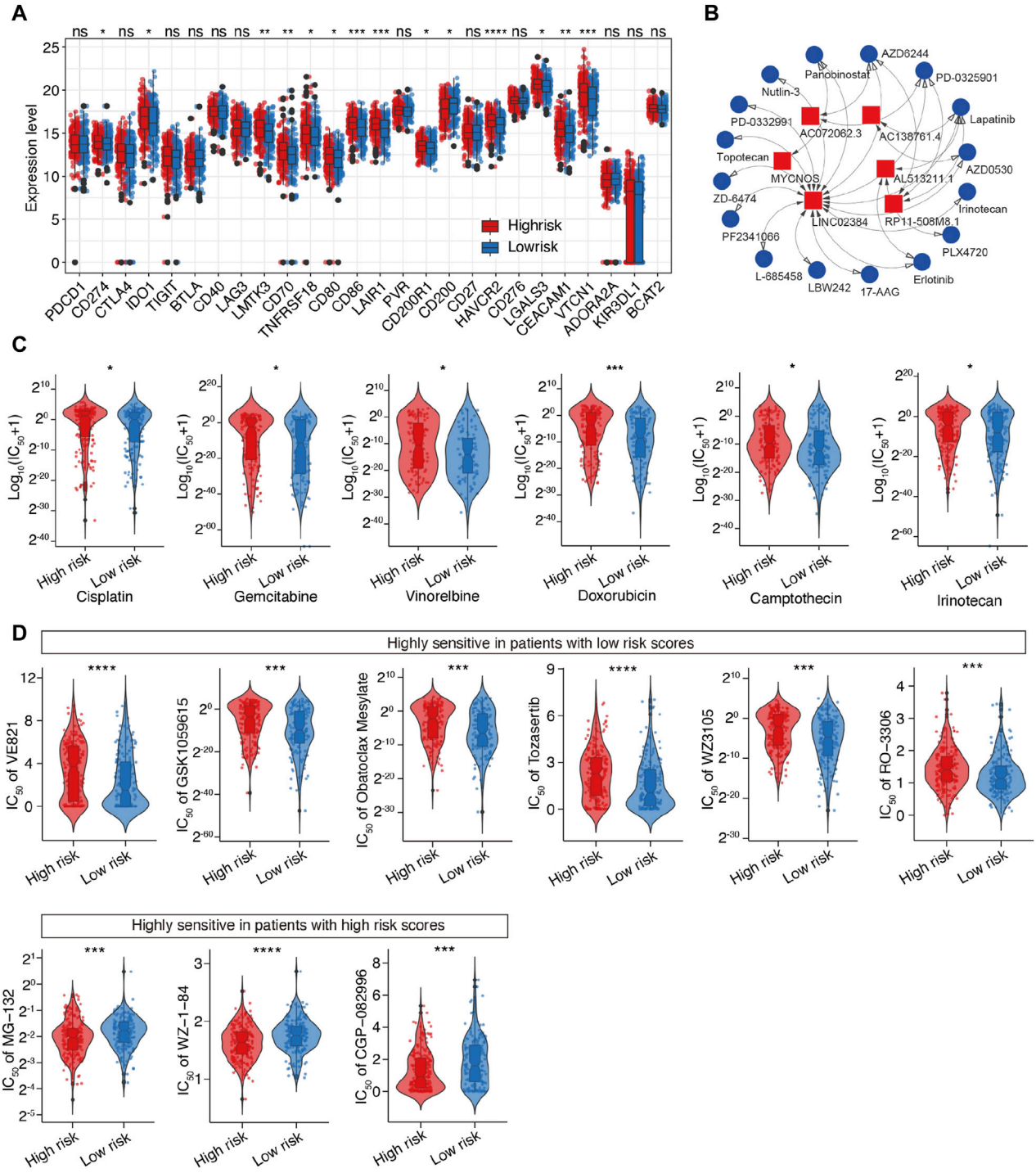

FIGURE 8
Prediction of the therapeutic response for distinct subgroups and screening of potential drugs targeting m^{6}A-related lncRNAs in SOC. **(A)** Differential analysis of immune checkpoints between the two groups. **(B)** Interaction between 6 lncRNAs and 16 SOC-targeting compounds; LINC02384 exhibited the strongest correlation with compounds. **(C)** Evaluation of the sensitivities of various drug, including six common drugs for SOC. Y-axis represents the half maximal inhibitory concentration (IC_{50}). **(D)** Top 10 novel candidate compounds targeting SOC according to the m^{6}A-LRM (The result of Doxorubicin is shown in this figure **(C)**].

not alter the expression of *METTL3* (Figure 9E). However, the RNA expression of RP11-508M8.1 in HEY and CAOV3 was increased following *METTL3* knockdown (Figures 9F, G). These results indicated that *METTL3* is an upstream regulator of RP11-508M8.1. Furthermore, to determine the manner in which *METTL3* regulates RP11-508M8.1 expression, we conducted a dot blot assay, which revealed that *METTL3* knockdown reduced the m^{6}A level of RNA in CAOV3 (Figure 10A). Next, m^{6}A MeRIP-qRT-PCR was used to analyze *METTL3* expression via m^{6}A-dependent regulation of *RP11-508M8.1* expression; we found that *RP11-508M8.1* was immunoprecipitated by

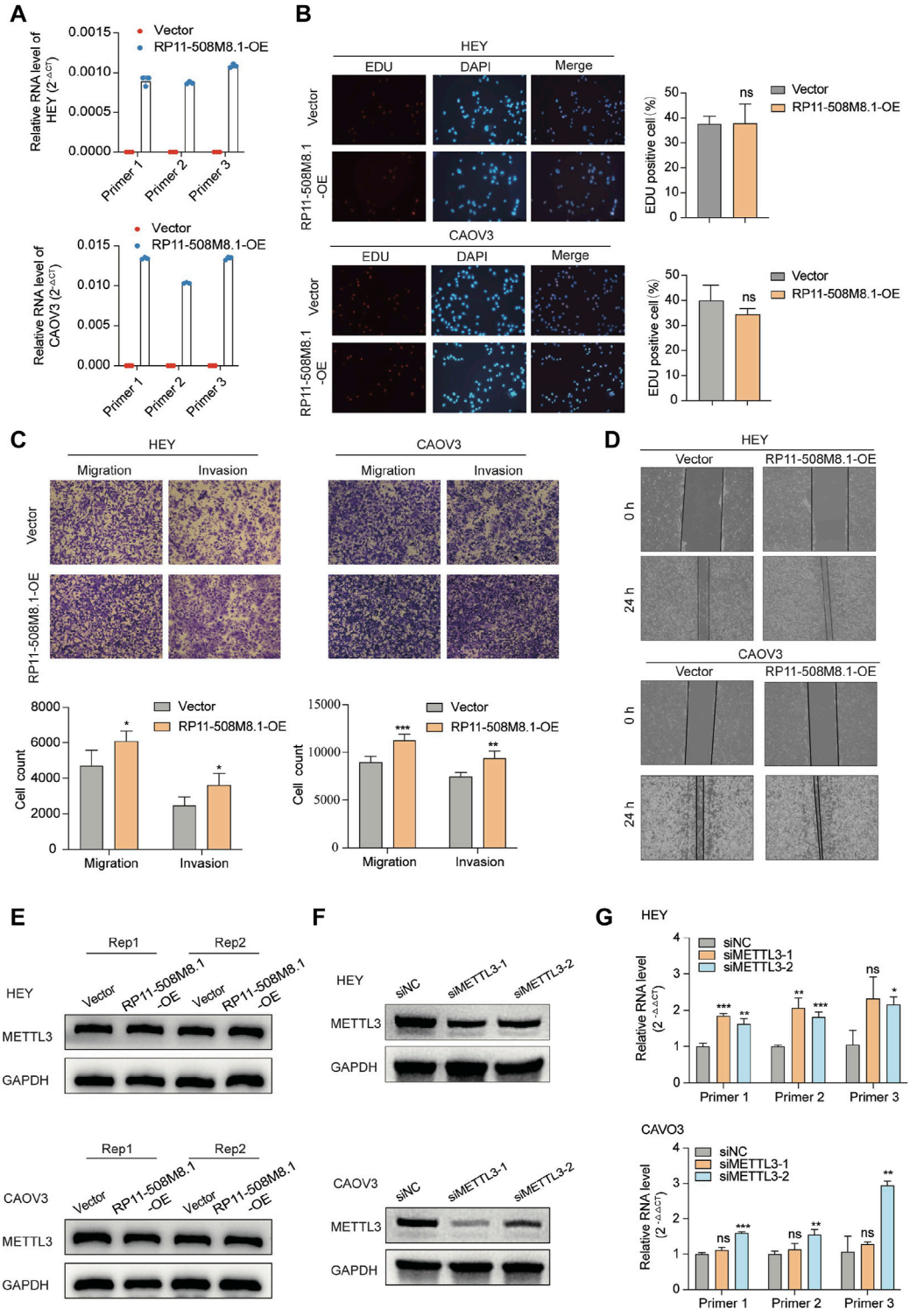

FIGURE 9
RP11-508M8.1 promotes migration and invasion of ovarian cancer cells *in vitro*. **(A)** RT-qPCR assay was used to detect the overexpression efficiency of *RP11-508M8.1* in CAOV3 and HEY OC cell lines with stable overexpression of *RP11-508M8.1* and a negative control. **(B)** Proliferation ability of HEY and CAOV3 cells overexpressing *RP11-508M8.1* was detected via an EdU assay. **(C)** Transwell representative images (upper) and quantitative results (lower) showed that overexpression of *RP11-508M8.1* enhanced the migration and invasion abilities of ovarian cancer cells. **(D)** Wound healing assay demonstrated that increased expression of *RP11-508M8.1* promoted the wound-healing ability of OC cells. Data are presented as mean ± SD. **(E)** Protein levels of METTL3 in OC cells overexpressing *RP11-508M8.1* (Rep: repeat). **(F)** Knockdown of METTL3 expression in CAVO3 and HEY cells via siRNA. **(G)** Relative RNA levels of *RP11-508M8.1* in METTL3-knockdown OC cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

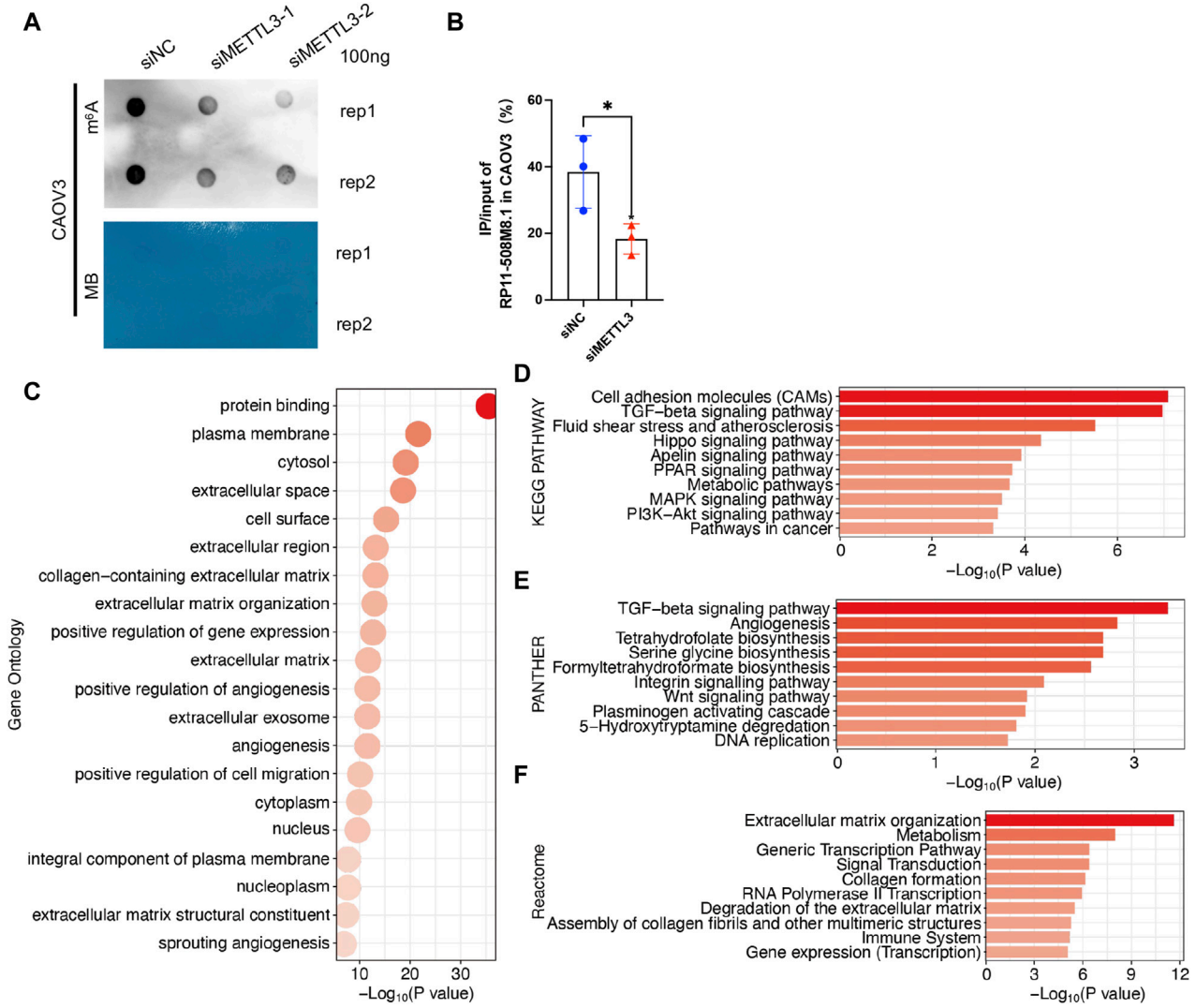

FIGURE 10
Potential function of RP11-508M8.1 in patients with SOC. **(A)** m^{6}A dot blot assay in CAOV3 with knockdown of METTL3. Methylene blue (MB) stain as the loading control. **(B)** Detection of the *RP11-508M8.1* m^{6}A modified levels in METTL3-knockdown CAOV3 and the normal control using MeRIP qRT-PCR. *$p < 0.05$. **(C)** GO analysis of DEGs in CAOV3 cells overexpressing RP11-508M8.1. **(D–F)** Pathway analyses of DEGs in CAOV3 cells overexpressing RP11-508M8.1. Based on the KEGG **(D)**, PANTHER **(E)**, and Reactome **(F)** databases.

m^{6}A-MeRIP, suggesting the existence of m^{6}A modification in *RP11-508M8.1*. *METTL3* knockdown significantly reduced the m^{6}A enrichment level in *RP11-508M8.1* (Figure 10B). These results indicate that METTL3 may exert regulatory control over m^{6}A modification, thereby modulating *RP11-508M8.1* expression.

To further explore the potential effects exerted by RP11-508M8.1 on SOC, we performed RNAseq analysis of OC cells overexpressing *RP11-508M8.1*. DEGs were identified based on fold change >1.5 and $p < 0.001$, and then GO and pathway analyses were performed. GO analysis identified the processes that were most significantly altered by RP11-508M8.1; they were mainly related to cell migration, including the plasma membrane, extracellular space, extracellular region, extracellular matrix organization, and positive regulation of cell migration (Figure 10C). Pathway analyses based on three databases confirmed these findings. DEGs were enriched in the extracellular matrix and cell movement pathways, such as cell adhesion molecules, the TGF-beta signaling pathway, angiogenesis, the Wnt signaling pathway, extracellular matrix organization, and extracellular matrix degradation (Figures 10D–F). These results indicated that RP11-508M8.1 may play an oncogenic role by affecting extracellular matrix organization and cell migration.

3.9 Identification of a ceRNA regulatory axis

RP11-508M8.1 may be involved in the progression of OC. The potential molecular mechanism underlying the role of RP11-508M8.1 in SOC was subsequently investigated using a regulation axis of ceRNA interactions (Figure 11A). The RNAhybrid and ONCOMIR databases predicted the presence of a total of seven miRNAs (hsa-miR-1270, has-miR-1301-3p, hsa-miR-3605-5p, hsa-miR-363-3p, hsa-miR-892b, hsa-miR-205-3p, and hsa-let-7f-2-3p)

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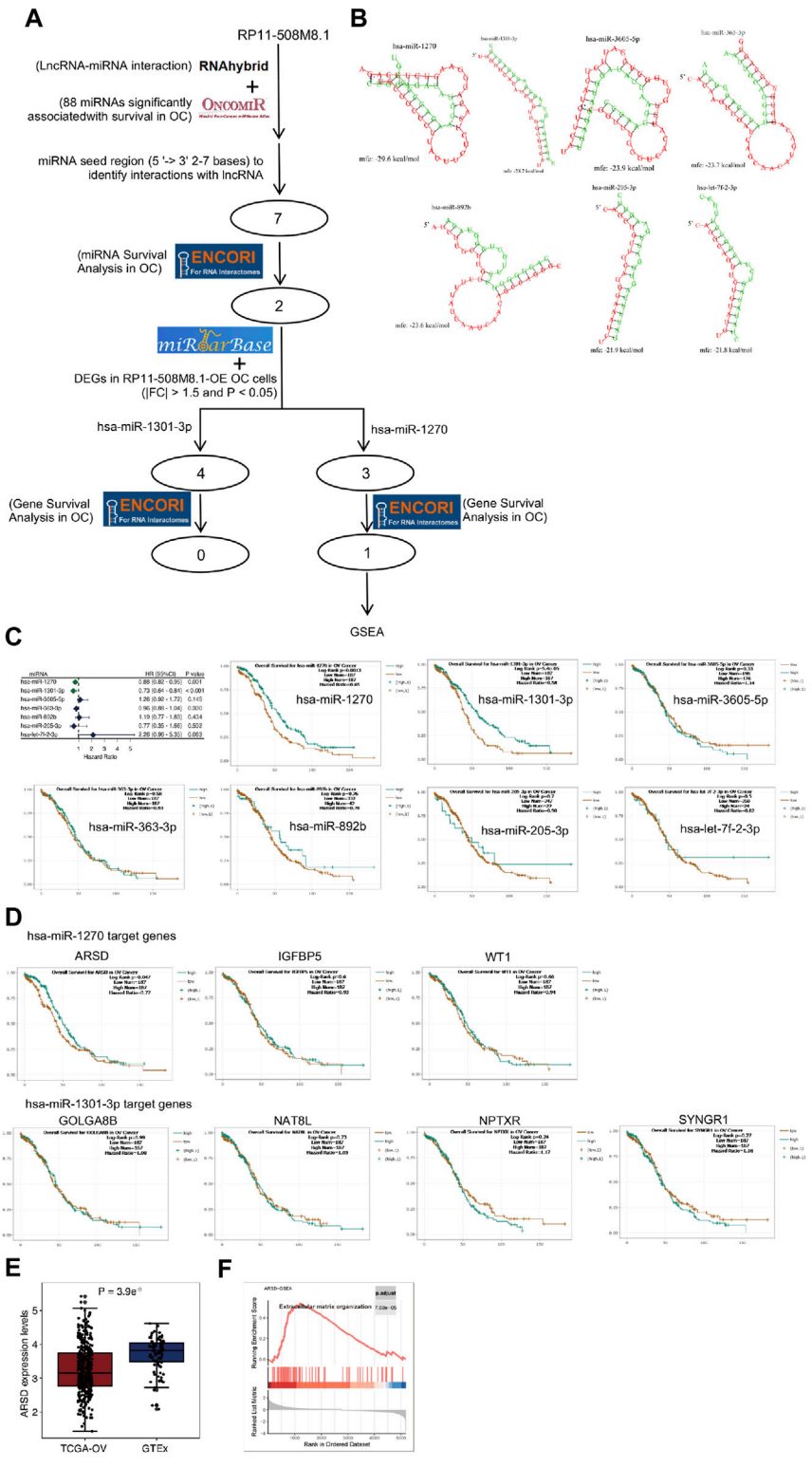

FIGURE 11
Construction of an lncRNA-miRNA-mRNA regulatory network and bioinformatic analysis. **(A)** Data analysis-based overview of the lncRNA-miRNA-mRNA regulatory axis. **(B)** Seven miRNA targets of the lncRNA PR11-508M8.1. **(C)** Univariate Cox regression and KM survival analysis of the highly connected miRNAs based on TCGA and ENCORI databases. **(D)** The correlation between survival possibility and the expression of hsa-miR-1270 targets (ARSD, IGFBP5, and WT1) and has-miR-1301-3p targets (GOLGA8B, NAT8L, NPTXR, and SYNGR1) is shown via KM analysis using data from the ENCORI database. **(E)** Expression of ARSD in SOC and normal ovary tissues. **(F)** Single gene enrichment analysis of ARSD.

that bind to *RP11-508M8.1* under the following conditions: miRNA seed region (5′- > 3′) and 2–7 bp should be closely matched with the lncRNA to execute screening (Figure 11B). However, univariate Cox regression and KM analyses showed that only hsa-miR-1270 and has-miR-1301-3p expression was correlated with the survival of patients with OC (Figure 11C). Furthermore, analysis of data from the miRTarBase database indicated that the gene targets binding to the miRNAs hsa-miR-1270 and has-miR-1301-3p may constitute the miRNA-mRNA axis. We combined the DEGs in *RP11-508M8.1*-OE OC cells to filter the genes. A total of three (*ARSD, IGFBP5,* and *WT1*) and four (*GOLGA8B, NAT8L, NPTXR,* and *SYNGR1*) gene targets were found to bind hsa-miR-1270 and has-miR-1301-3p, respectively (Figure 11D). The ENCORI database, which was used to perform gene survival analysis in OC, showed that ARSD expression (hsa-miR-1270 targeted gene) alone was significantly associated with survival and that patients with higher ARSD expression had a higher survival possibility, with an HR < 1 (Figure 11D). In addition, ARSD expression was also detected; the results suggested that ARSD was downregulated in OC tissues compared to that in normal tissues (Figure 11E). Moreover, ssGSEA showed that ARSD expression was correlated with the extracellular matrix organization pathway (Top one enrichment), indicating that ARSD may serve as a regulatory factor in tumorigenesis and tumor progression (Figure 11F). These findings indicated that the RP11-508M8.1/hsa-miR-1270/ARSD regulatory axis may be of importance in the progression of OC.

4 Discussion

4.1 Potential of m^{6}A-related lncRNAs as biomarkers in SOC to improve PPPM

High-grade SOC is the most prevalent and aggressive form of SOC, which is an intractable disease (Drumond-Bock and Bieniasz, 2021). Most patients with SOC are diagnosed at stage III or IV, which results in a significant reduction in their responsiveness to treatment as well as survival (Drumond-Bock and Bieniasz, 2021). Many studies have focused on identifying reliable early diagnostic biomarkers, novel therapeutic targets, and prognostic biomarkers to improve the prognosis of patients in advanced stages (van Zyl et al., 2018). However, the currently used imaging, histological evaluation, serum markers (i.e., CA125), and predictive models for managing SOC lack sensitivity and specificity, making it difficult to meet the needs of PPPM (Punzón-Jiménez et al., 2022). The m^{6}A modification, which is considered the most common modification among lncRNAs (Patil et al., 2018), has currently become the focus of attention of cancer researchers. The m^{6}A modification and its effectors influence the fate of RNA molecules via lncRNA regulation, often resulting in the onset and development of cancers (Dai et al., 2020; Huang et al., 2020). LncRNAs regulated by the m^{6}A modification have shown potential applicability in the diagnosis, treatment, and prognosis of various cancers (Dai et al., 2020), especially as diagnostic and prognostic tools in clinical settings, thereby facilitating the prediction, targeted prevention, and personalized treatment of SOC.

Considering that the functions of lncRNAs are dynamically regulated by m^{6}A writers, readers, and erasers (He et al., 2020) and that the role of lncRNAs in cancer has been attributed to integrated m^{6}A effector regulation (Lan et al., 2021), the present study analyzed a comprehensive set of m^{6}A effectors. In this study, we identified six m^{6}A effector-related lncRNAs (RP11-508M8.1, AC138761.4, AL513211.1, LINC02384, MYCNOS, and AC072062.3) and constructed a risk model, m^{6}A-LRM, to accurately predict the OS of patients with SOC as well as their response to treatment. Of these six lncRNAs, only MYCNOS and LINC02384 were extensively investigated. MYCNOS expression is associated with various cancers. For example, MYCNOS, which is upregulated in hepatocellular carcinoma cells and tissues, affects disease progression, shortens patient survival (Yu et al., 2020) and acts as an endogenous sponge of miR-216b, thereby regulating the expression of FOXM1 and promoting the proliferation of glioblastoma cells (Zhao et al., 2021). MYCNOS upregulation is associated with poor prognosis in neuroblastoma patients (Vadie et al., 2015). Interestingly, in this study, MYCNOS was identified as a protective factor in patients with SOC. However, lncRNAs reportedly play opposing roles in different cancers via crosstalks among multiple mechanisms (Fang and Fullwood, 2016; Goodall and Wickramasinghe, 2021); thus, the role of MYCNOS in SOC may require further investigation. Furthermore, studies have suggested that LINC02384, which stimulates melanoma progression by reducing the expression of the tumor-protecting miRNAs miR.891a.5p and miR.203b.3p (Zhang C. et al., 2021), may also act as a protective factor in renal cell carcinoma (Li et al., 2021) and breast cancer (Xu Z. J. et al., 2021). Although the results of the current study indicated that LINC02384 may act as a protective factor in SOC, data on the remaining four lncRNAs are lacking. The findings of subsequent univariate and multivariate analyses indicated that the m^{6}A-LRM may also be useful as an independent prognostic factor for SOC. Moreover, the m^{6}A-LRM may predict risks across different age groups. However, the risk model demonstrated predictive trends ($p > 0.05$) only when stratifying the OS of stage IV and grade 1 and 2 patients with SOC. This may be attributed to the limited sample size. The nomogram further indicated that risk models based on m^{6}A effector-related lncRNAs exhibited a strong association with SOC and may therefore serve as a valuable tool for effective risk stratification of patients with SOC.

4.2 Application of the m^{6}A-LRM in immunotherapy and chemotherapy

To explore the potential of the m^{6}A-LRM in predicting the immunotherapeutic response of SOC, we performed comparative analyses of tumor-infiltrating immune cell levels, ICIs expression, tumor mutations, and neoantigen loads. The TME, including immune cells, cytokines, and chemokines, exhibits high heterogeneity and plasticity, which evolve with tumor progression, thus forming a complex immune landscape (Hiam-Galvez et al., 2021; Liu and Sun, 2021). Dendritic cells initiate anti-tumor immunity by capturing and presenting tumor antigens, which activate CD8$^+$ and CD4$^+$ T cells (Jhunjhunwala et al., 2021). However, tumor cells remodel the TME to augment immune-suppressive cells, thereby evading immune surveillance (Cao et al., 2023; de Visser and Joyce, 2023). Growing evidence

suggests that m^{6}A modification regulates the metabolism and activation of immune cells as well as the processes associated with immune response, thereby playing a pivotal role in reshaping the TME and orchestrating immune evasion in tumors, which in turn undermines the efficacy of immunotherapy (Li X. et al., 2022; Cao et al., 2023). LncRNAs not only play a key regulatory role in the process of proliferation, migration, and invasion of cancer cells but also act as active participants in the immune system by regulating the development, differentiation, and function of various immune cells (Chen et al., 2017; Denaro et al., 2019; Zhang Y. et al., 2021). In this study, we constructed the m^{6}A-LRM using m^{6}A effector-related lncRNAs and applied the model to stratify the risk of patients with SOC. Moreover, we comprehensively analyzed the disparities between the TMEs of the high- and low-risk groups to determine immune cell infiltration patterns within the TME. We found that the counts of CD4$^+$ T cells, monocytes, dendritic cells, B cells, Th1 cells, Th2 cells, and TILs in the high-risk group were increased and the proportion of pro- and anti-inflammatory cytokines in the high-risk group was higher than that in the low-risk group, indicating an adaptive immune activation status. Considering the proinflammatory immune milieu observed in the high-risk group and the immunosuppression environment observed in the low-risk group, it is plausible that the high- and low-risk groups based on the m^{6}A-LRM signature may encompass "hot" and "cold" tumors, respectively. Thus, the high-risk group may display elevated responsiveness toward immunotherapeutic interventions.

Cancer cells may suppress the immune system by activating immune checkpoints, a class of immunosuppressive molecules that are expressed on immune cells and regulate the extent of immune activation (Darvin et al., 2018). ICIs, which exert an oncostatic effect by enhancing T-cell activation and proliferation, are emerging as potential therapeutic modalities for cancer (Darvin et al., 2018; Ribas and Wolchok, 2018). Many ICIs, such as cytotoxic T lymphocyte associate protein-4 (CTLA-4), programmed cell death-1 (PD-1), and programmed cell death-ligand 1 (PD-L1) antibodies, have been applied in clinical settings (Postow et al., 2015). The TMB is an independent biomarker used to determine the suitability of patients for immunotherapy. A higher TMB leads to tumorigenesis and more neoantigens, which in turn drive T cell-mediated antitumor immune responses; thus, patients with high TMB may benefit more from immunotherapy than patients with low TMB (Jardim et al., 2021). In the present study, we found that low-frequency mutations that were closer to the upper end of the low frequency range were prevalent in the high-risk group, indicating that the high-risk group was more suitable for immunotherapy. We also confirmed that the established m^{6}A-LRM had superior predictive power with respect to the prognosis of patients with SOC compared with that of the TMB and neoantigens. Thus, the m^{6}A-LRM shows potential as a novel prognostic marker for patients with SOC. Furthermore, the expression levels of immune checkpoints may be compared to assess their effectiveness in patients receiving immunotherapy. We found that the expression levels of IC-related genes, such as *HAVCR2, CD86, LAIR1,* and *VTCN1,* in the high-risk group were significantly higher than those in the low-risk group, thereby explaining the higher sensitivity shown by the high-risk group to immunotherapy and confirming the high-risk group as a "hot tumor" group.

Models based on the m^{6}A-LRM signature may also be used to predict the chemotherapy response of patients with SOC. Drug sensitivity experiments revealed that the susceptibility of the low-risk patients to conventional chemotherapeutic agents, such as cisplatin, gemcitabine, vinorelbine, doxorubicin, camptothecin, and irinotecan, was enhanced. Based on the close association between the m^{6}A-LRM and immunotherapy response, potential lncRNA-targeting chemicals were identified for future exploration. AZD6244, PD-0325901, and lapatinib were the top three drugs predicted as being capable of targeting multiple candidate lncRNAs. The MEK1/2 inhibitor AZD6244 reportedly inhibited the growth of clear cell ovarian carcinoma (Bartholomeusz et al., 2012). Sheppard et al. demonstrated that PF-04691502 and PD-0325901 synergistically inhibited the growth of OC cells (Sheppard et al., 2013). Meanwhile, treatment with nanocolloids of paclitaxel and lapatinib effectively overcame the multi-drug resistance of OC cells (Vergara et al., 2012). These findings imply that the m^{6}A-LRM may potentially be used to evaluate treatment response, assess prognostic risk, and develop personalized treatment strategies for individuals with SOC, thereby demonstrating a superior ability to improve PPPM in SOC.

4.3 Molecular mechanisms underlying the functions of m^{6}A-related lncRNAs

Functional enrichment analyses and variation landscapes of the high- and low-risk groups may provide insights into the effects and underlying molecular mechanisms of m^{6}A-related lncRNAs. Such experiments may help optimize the prediction model, further reveal the association between the immune microenvironment and m^{6}A-related lncRNAs, provide more treatment choices, and reveal the presence of additional SOC-related pathways.

Our study showed that the high-risk group was in a state of immunophenotype activation. Such immune signatures may be explained via molecular signatures. GSEA indicated that the upregulated genes in the high-risk group were significantly enriched in the EMT and inflammation-related pathways. EMT refers to the transformation of epithelioid cells into mesenchymal phenotypic cells, which is recognized as malignant cellular behavior that facilitates tumor metastasis (Huang et al., 2022). EMT interacts with the tumor immune microenvironment in a significant manner (Dongre and Weinberg, 2019). T lymphocytes and macrophages may induce cancer cell EMT, thereby facilitating the recruitment of various immune cells, including immunosuppressive regulatory T cells, to inhibit tumor immunity and promote PD-L1 expression in cancer cells (Dongre and Weinberg, 2019). EMT may well account for the poor prognoses and proinflammatory statuses observed in the high-risk group. The genes that were downregulated in the low-risk group were enriched in the DNA repair and WNT beta-catenin signaling pathways. DNA damage repair (DDR) maintains the genome integrity of cancer cells, which plays a role in cancer progression, while downregulation of the DNA repair pathway corresponded to better prognoses in the low-risk group in our study (Xie et al., 2021). However, upregulation of DNA repair genes is linked to a lack of immune cell infiltration, which is inconsistent with the immunophenotypic suppression observed in the low-risk group (Higgs et al., 2022). The association between

DDR and the immune microenvironment requires in-depth investigations, with particular reference to the treatment efficacy of DDR inhibitors combined with ICIs, which has attracted the attention of researchers (Sheng et al., 2020). The WNT beta-catenin signaling pathway is known to be associated with carcinogenicity. More importantly, the activation of WNT beta-catenin signaling is positively correlated with DDR and EMT, which jointly participate in cancer progression as well as in the shaping of the immune microenvironment (Hashemi et al., 2023). These pathways as well as a potential crosstalk between them are essential aspects of the molecular mechanism underlying the accurate prediction of tumor characteristics by the m^{6}A-LRM.

In terms of the differences between the mutation landscapes of the high- and low-risk groups, *USH2A* had a higher mutation frequency in the high-risk group. A study found that ICIs exhibit better efficacy in patients carrying *USH2A* missense mutations, thereby providing an important reference for treatment selection in high-risk patients (Yang et al., 2023). These findings are consistent with those of Sun et al., who suggested that the mutation of *USH2A* was associated with an increase in the TMB and antitumor immunity (Sun et al., 2021). *SYNE2* showed a higher mutation frequency in the low-risk group. A previous study suggested that ovarian cancer cell clusters with higher mutation burden tend to display high mutation rates of SYNE2 (Li L. et al., 2022), which is inconsistent with our results. We hypothesize that this discrepancy may be attributed to variances within the analyzed cohort and grouping. Specific reasons for these conflicting results warrant further research.

4.4 RP11-508M8.1 regulates the expression of ARSD via hsa-miR-1270

The results of this study indicated that RP11-508M8.1 was strongly associated with the m^{6}A-writer METTL3. METTL3 is a risk factor for SOC and a core m^{6}A methyltransferase that plays critical roles in various cancers (Zeng et al., 2020). The function of RP11-508M8.1 in OC was explored *in vitro*. Preliminary results indicated that PR11-508M8.1 promoted OC cell invasion and migration. Although PR11-508M8.1 overexpression did not alter METTL3 levels, downregulating METTL3 increased RP11-508M8.1 expression. These findings indicate that METTL3 may be an upstream regulator of RP11-508M8.1 and that the METTL3-m^{6}A-RP11-508M8.1 axis plays a role in the carcinogenicity mechanism underlying SOC.

ceRNA refers to RNA molecules such as mRNA, lncRNA, and circRNA that can competitively bind miRNAs to alter the transcriptional levels of miRNA-regulated mRNAs, thus exerting biological functions in cancer (Tay et al., 2014; Braga et al., 2020). In recent years, the ceRNA regulatory network has garnered significant attention as a novel mechanism underlying RNA interactions (Thomson and Dinger, 2016). Therefore, we investigated the ceRNA network of RP11-508M8.1 and established a novel lncRNA-miRNA-mRNA regulatory network, which has not been previously reported in relation to SOC. Our results indicated that RP11-508M8.1 may regulate ARSD expression by altering hsa-miR-1270 expression. This regulatory axis may activate protumor pathways (e.g., EMT, reactive oxygen species pathway, and extracellular matrix organization pathway). Previous research has shown that miR-1270 plays a novel tumor suppressor role in lung adenocarcinoma (Saproo et al., 2023). Hsa-miR-1270 suppresses the malignant progression of breast cancer by regulating gene expression (Hu et al., 2022). A previous study reported that ARSD exerts inhibitory effects on the proliferation and migration of breast cells by activating the Hippo/YAP pathway (Lin et al., 2021). Here, we identified ARSD as a potential protective factor in the context of SOC, exhibiting an anti-tumorigenic role. Furthermore, ARSD serves as a prognostic biomarker that facilitates the progression of glioma cells via the activation of the JAK2/STAT3 signaling pathway and infiltration of M2 macrophages (Song et al., 2023). Thus, ARSD may act as a potential novel biomarker that may improve the prognosis of patients with SOC.

4.5 Limitations

The current study was affected by some limitations. First, our data analysis was derived from TCGA data; thus, further large-scale investigations are required to corroborate our findings. Second, an increasing body of evidence indicates that various modification types may interact during tumorigenesis and progression, thereby establishing a complex regulatory network. Consequently, additional modifications should be incorporated into future studies to elucidate the specific molecular mechanisms underlying SOC. Third, the data used to analyze and construct the model were obtained from ovarian cancer samples, and the role played by N6-methyladenosine effector-related lncRNAs signature in other cancers remains to be explored. Fourth, the expression and biological function of RP11-508M8.1 *in vivo* must be verified in the future. Fifth, further investigation should be performed to elucidate the intricate regulatory network between m^{6}A effector HNRNPC and lncRNA RP11-508M8.1. Moreover, it is necessary to screen specific mutant cell lines to further explore the potential in the pathogenesis and progression of SOC. Finally, the ability of the developed risk model to predict the response to immunotherapy was only indirectly evaluated. These findings remain to be confirmed by future studies possibly involving *in vitro* drug sensitivity tests.

5 Conclusion

We constructed a novel risk prediction model for patients with SOC based on six m^{6}A effector-related lncRNAs, namely, RP11-508M8.1, AC138761.4, AL513211.1, LINC02384, MYCNOS, and AC072062.3. This novel risk prediction model effectively evaluated the survival rate and treatment response in relation to SOC. A free web application of the m^{6}A-LRM for researchers and clinicians was developed and may provide reference information for precision treatment, thereby facilitating the PPPM of SOC. The influence of m^{6}A-LRM on SOC was explored from multiple perspectives, and the association between m^{6}A effectors and key lncRNAs as well as the preliminary mechanisms underlying their effect on OC were explored via *in vitro* experimentation. In conclusion, we propose that the regulatory axis involving METTL3/m^{6}A/RP11-508M8.1/hsa-miR-1270/ARSD may represent one of the molecular mechanisms underlying SOC.

Data availability statement

The RNA-Seq datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA1119078.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

LY: Data curation, Formal Analysis, Methodology, Writing–original draft. XT: Methodology, Writing–original draft, Investigation. KP: Writing–original draft. XS: Methodology, Writing–original draft. BX: Writing–original draft. XY: Writing–original draft. LZ: Data curation, Writing–original draft. SF: Data curation, Writing–original draft. ST: Data curation, Writing–original draft. ZJ: Writing–original draft. XX: Supervision, Writing–review and editing. WL: Supervision, Writing–review and editing. GG: Conceptualization, Supervision, Project administration, Writing–review and editing, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1351929/full#supplementary-material

References

Bartholomeusz, C., Oishi, T., Saso, H., Akar, U., Liu, P., Kondo, K., et al. (2012). MEK1/2 inhibitor selumetinib (AZD6244) inhibits growth of ovarian clear cell carcinoma in a PEA-15-dependent manner in a mouse xenograft model. *Mol. Cancer Ther.* 11 (2), 360–369. doi:10.1158/1535-7163.MCT-11-0400

Becht, E., Giraldo, N. A., Lacroix, L., Buttard, B., Elarouci, N., Petitprez, F., et al. (2016). Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol.* 17 (1), 218. doi:10.1186/s13059-016-1070-5

Bhat, A. A., Younes, S. N., Raza, S. S., Zarif, L., Nisar, S., Ahmed, I., et al. (2020). Role of non-coding RNA networks in leukemia progression, metastasis and drug resistance. *Mol. Cancer* 19 (1), 57. doi:10.1186/s12943-020-01175-9

Binnewies, M., Roberts, E. W., Kersten, K., Chan, V., Fearon, D. F., Merad, M., et al. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med.* 24 (5), 541–550. doi:10.1038/s41591-018-0014-x

Bowtell, D. D., Böhm, S., Ahmed, A. A., Aspuria, P. J., Bast, R. C., Jr., Beral, V., et al. (2015). Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat. Rev. Cancer* 15 (11), 668–679. doi:10.1038/nrc4019

Braga, E. A., Fridman, M. V., Moscovtsev, A. A., Filippova, E. A., Dmitriev, A. A., and Kushlinskii, N. E. (2020). LncRNAs in ovarian cancer progression, metastasis, and main pathways: ceRNA and alternative mechanisms. *Int. J. Mol. Sci.* 21 (22), 8855. doi:10.3390/ijms21228855

Cao, X., Geng, Q., Fan, D., Wang, Q., Wang, X., Zhang, M., et al. (2023). m(6)A methylation: a process reshaping the tumour immune microenvironment and regulating immune evasion. *Mol. Cancer* 22 (1), 42. doi:10.1186/s12943-022-01704-8

Charoentong, P., Finotello, F., Angelova, M., Mayer, C., Efremova, M., Rieder, D., et al. (2017). Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep.* 18 (1), 248–262. doi:10.1016/j.celrep.2016.12.019

Chen, Y., Lin, Y., Shu, Y., He, J., and Gao, W. (2020). Interaction between N(6)-methyladenosine (m(6)A) modification and noncoding RNAs in cancer. *Mol. Cancer* 19 (1), 94. doi:10.1186/s12943-020-01207-4

Chen, Y. G., Satpathy, A. T., and Chang, H. Y. (2017). Gene regulation in the immune system by long noncoding RNAs. *Nat. Immunol.* 18 (9), 962–972. doi:10.1038/ni.3771

Cheng, T., and Zhan, X. (2017). Pattern recognition for predictive, preventive, and personalized medicine in cancer. *Epma J.* 8 (1), 51–60. doi:10.1007/s13167-017-0083-9

Dai, F., Wu, Y., Lu, Y., An, C., Zheng, X., Dai, L., et al. (2020). Crosstalk between RNA m(6)A modification and non-coding RNA contributes to cancer growth and progression. *Mol. Ther. Nucleic Acids* 22, 62–71. doi:10.1016/j.omtn.2020.08.004

Darvin, P., Toor, S. M., Sasidharan Nair, V., and Elkord, E. (2018). Immune checkpoint inhibitors: recent progress and potential biomarkers. *Exp. Mol. Med.* 50 (12), 1–11. doi:10.1038/s12276-018-0191-1

Denaro, N., Merlano, M. C., and Lo Nigro, C. (2019). Long noncoding RNAs as regulators of cancer immunity. *Mol. Oncol.* 13 (1), 61–73. doi:10.1002/1878-0261.12413

Deng, L. J., Deng, W. Q., Fan, S. R., Chen, M. F., Qi, M., Lyu, W. Y., et al. (2022). m6A modification: recent advances, anticancer targeted drug discovery and beyond. *Mol. Cancer* 21 (1), 52. doi:10.1186/s12943-022-01510-2

Deng, X. L., Qing, Y., David, H., Huang, H. l., and Chen, J. J. (2023). The roles and implications of RNA m6A modification in cancer. *Nat. Rev. Clin. Oncol.* 20 (8), 507–526. doi:10.1038/s41571-023-00774-x

De Visser, K. E., and Joyce, J. A. (2023). The evolving tumor microenvironment: from cancer initiation to metastatic outgrowth. *Cancer Cell* 41 (3), 374–403. doi:10.1016/j.ccell.2023.02.016

Dongre, A., and Weinberg, R. A. (2019). New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat. Rev. Mol. Cell Biol.* 20 (2), 69–84. doi:10.1038/s41580-018-0080-4

Drumond-Bock, A. L., and Bieniasz, M. (2021). The role of distinct BRD4 isoforms and their contribution to high-grade serous ovarian carcinoma pathogenesis. *Mol. Cancer* 20 (1), 145. doi:10.1186/s12943-021-01424-5

Duan, Q., Zhang, H., Zheng, J., and Zhang, L. (2020). Turning cold into hot: firing up the tumor microenvironment. *Trends Cancer* 6 (7), 605–618. doi:10.1016/j.trecan.2020.02.022

Fang, Y., and Fullwood, M. J. (2016). Roles, functions, and mechanisms of long non-coding RNAs in cancer. *Genomics Proteomics Bioinforma.* 14 (1), 42–54. doi:10.1016/j.gpb.2015.09.006

Fang, Z., Mei, W. T., Qu, C., Lu, J. D., Shang, L., Cao, F., et al. (2022). Role of m6A writers, erasers and readers in cancer. *Exp. Hematol. Oncol.* 11 (1), 45. doi:10.1186/s40164-022-00298-7

Fu, Y., Dominissini, D., Rechavi, G., and He, C. (2014). Gene expression regulation mediated through reversible m^{6}A RNA methylation. *Nat. Rev. Genet.* 15 (5), 293–306. doi:10.1038/nrg3724

Goodall, G. J., and Wickramasinghe, V. O. (2021). RNA in cancer. *Nat. Rev. Cancer* 21 (1), 22–36. doi:10.1038/s41568-020-00306-0

Gu, C., Shi, X., Dai, C. Y., Shen, F., Geatano, R., Chen, J. F., et al. (2020). RNA m6A modification in cancers: molecular mechanisms and potential clinical applications. *Innov. (Camb)* 1 (3), 100066. doi:10.1016/j.xinn.2020.100066

Hanzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinforma.* 14, 7. doi:10.1186/1471-2105-14-7

Hashemi, M., Hasani, S., Hajimazdarany, S., Ghadyani, F., Olyaee, Y., Khodadadi, M., et al. (2023). Biological functions and molecular interactions of Wnt/β-catenin in breast cancer: revisiting signaling networks. *Int. J. Biol. Macromol.* 232, 123377. doi:10.1016/j.ijbiomac.2023.123377

He, R. Z., Jiang, J., and Luo, D. X. (2020). The functions of N6-methyladenosine modification in lncRNAs. *Genes Dis.* 7 (4), 598–605. doi:10.1016/j.gendis.2020.03.005

Hiam-Galvez, K. J., Allen, B. M., and Spitzer, M. H. (2021). Systemic immunity in cancer. *Nat. Rev. Cancer* 21 (6), 345–359. doi:10.1038/s41568-021-00347-z

Higgs, E. F., Bao, R., Hatogai, K., and Gajewski, T. F. (2022). Wilms tumor reveals DNA repair gene hyperexpression is linked to lack of tumor immune infiltration. *J. Immunother. Cancer* 10 (6), e004797. doi:10.1136/jitc-2022-004797

Hou, P., Meng, S., Li, M., Lin, T., Chu, S., Li, Z., et al. (2021). LINC00460/DHX9/IGF2BP2 complex promotes colorectal cancer proliferation and metastasis by mediating HMGA1 mRNA stability depending on m6A modification. *J. Exp. Clin. Cancer Res.* 40 (1), 52. doi:10.1186/s13046-021-01857-2

Hu, S., Song, Y., Zhou, Y., Jiao, Y., and Wang, S. (2022). MiR-1270 suppresses the malignant progression of breast cancer via targeting MMD2. *J. Healthc. Eng.* 2022, 3677720. doi:10.1155/2022/3677720

Hu, Y. H., and Chen, Y. P. (2021). N6-methylandenosine-related lncRNAs play an important role in the prognosis and immune microenvironment of pancreatic ductal adenocarcinoma. *Sci. Rep.* 11 (1), 17844. doi:10.1038/s41598-021-97362-9

Huang, H., Weng, H., and Chen, J. (2020). m(6)A Modification in coding and non-coding RNAs: roles and therapeutic implications in cancer. *Cancer Cell* 37 (3), 270–288. doi:10.1016/j.ccell.2020.02.004

Huang, W., Qi, C. B., Lv, S. W., Xie, M., Feng, Y. Q., Huang, W. H., et al. (2016). Determination of DNA and RNA methylation in circulating tumor cells by mass spectrometry. *Anal. Chem.* 88 (2), 1378–1384. doi:10.1021/acs.analchem.5b03962

Huang, Y., Hong, W., and Wei, X. (2022). The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis. *J. Hematol. Oncol.* 15 (1), 129. doi:10.1186/s13045-022-01347-8

Jardim, D. L., Goodman, A., de Melo Gagliato, D., and Kurzrock, R. (2021). The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell* 39 (2), 154–173. doi:10.1016/j.ccell.2020.10.001

Jhunjhunwala, S., Hammer, C., and Delamarre, L. (2021). Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat. Rev. Cancer* 21 (5), 298–312. doi:10.1038/s41568-021-00339-z

Jin, Y. Z., and Fan, Z. P. (2023). New insights into the interaction between m6A modification and lncRNA in cancer drug resistance. *Cell Prolif.*, e13578. doi:10.1111/cpr.13578

Kotsopoulos, I. C., Papanikolaou, A., Lambropoulos, A. F., Papazisis, K. T., Tsolakidis, D., Touplikioti, P., et al. (2014). Serous ovarian cancer signaling pathways. *Int. J. Gynecol. Cancer* 24 (3), 410–417. doi:10.1097/IGC.0000000000000079

Lan, Y., Liu, B., and Guo, H. (2021). The role of M(6)A modification in the regulation of tumor-related lncRNAs. *Mol. Ther. Nucleic Acids* 24, 768–779. doi:10.1016/j.omtn.2021.04.002

Li, L., Chen, D., Luo, X., Wang, Z., Yu, H., Gao, W., et al. (2022a). Identification of CD8(+) T cell related biomarkers in ovarian cancer. *Front. Genet.* 13, 860161. doi:10.3389/fgene.2022.860161

Li, L., Li, M., Jiang, Z., and Wang, X. (2019). ARID1A mutations are associated with increased immune activity in gastrointestinal cancer. *Cells* 8 (7), 678. doi:10.3390/cells8070678

Li, N., Zhang, H. Y., Hu, K. Y., and Chu, J. F. (2021). A novel long non-coding RNA-based prognostic signature for renal cell carcinoma patients with stage IV and histological grade G4. *Bioengineered* 12 (1), 6275–6285. doi:10.1080/21655979.2021.1971022

Li, X., Ma, S., Deng, Y., Yi, P., and Yu, J. (2022b). Targeting the RNA m(6)A modification for cancer immunotherapy. *Mol. Cancer* 21 (1), 76. doi:10.1186/s12943-022-01558-0

Lin, Y., Li, C., Xiong, W., Fan, L., Pan, H., and Li, Y. (2021). ARSD, a novel ERα downstream target gene, inhibits proliferation and migration of breast cancer cells via activating Hippo/YAP pathway. *Cell Death Dis.* 12 (11), 1042. doi:10.1038/s41419-021-04338-8

Liu, H., Xu, Y., Yao, B., Sui, T., Lai, L., and Li, Z. (2020). A novel N6-methyladenosine (m6A)-dependent fate decision for the lncRNA THOR. *Cell Death Dis.* 11 (8), 613. doi:10.1038/s41419-020-02833-y

Liu, Y., You, Y., Lu, Z., Yang, J., Li, P., Liu, L., et al. (2019). N6-methyladenosine RNA modification-mediated cellular metabolism rewiring inhibits viral replication. *Science* 365, 1171–1176. doi:10.1126/science.aax4468

Liu, Y. T., and Sun, Z. J. (2021). Turning cold tumors into hot tumors by improving T-cell infiltration. *Theranostics* 11 (11), 5365–5386. doi:10.7150/thno.58390

Luo, P., Liu, X. F., Wang, Y. C., Li, N. D., Liao, S. J., Yu, M. X., et al. (2017). Prognostic value of abnormally expressed lncRNAs in ovarian carcinoma: a systematic review and meta-analysis. *Oncotarget* 8, 23927–23936. doi:10.18632/oncotarget.14760

Ma, S., Chen, C., Ji, X., Liu, J., Zhou, Q., Wang, G., et al. (2019). The interplay between m6A RNA methylation and noncoding RNA in cancer. *J. Hematol. Oncol.* 12 (1), 121. doi:10.1186/s13045-019-0805-7

Mehrdad, H., Pouria, D., Mohammad, A. Z., Rasoul, R., Neda, Z., Mehrsa, B., et al. (2023). Non-coding RNA-Mediated N6-Methyladenosine (m6A) deposition: a pivotal regulator of cancer, impacting key signaling pathways in carcinogenesis and therapy response. *Noncoding RNA Res.* 9 (1), 84–104. doi:10.1016/j.ncrna.2023.11.005

Meng, L. P., Lin, H., Huang, X. X., Weng, J. F., Peng, F., and Wu, S. J. (2022). METTL14 suppresses pyroptosis and diabetic cardiomyopathy by downregulating TINCR lncRNA. *Cell Death Dis.* 13 (1), 38. doi:10.1038/s41419-021-04484-z

Morand, S., Devanaboyina, M., Staats, H., Stanbery, L., and Nemunaitis, J. (2021). Ovarian cancer immunotherapy and personalized medicine. *Int. J. Mol. Sci.* 22 (12), 6532. doi:10.3390/ijms22126532

Muller, V., Oliveira-Ferrer, L., Steinbach, B., Pantel, K., and Schwarzenbach, H. (2019). Interplay of lncRNA H19/miR-675 and lncRNA NEAT1/miR-204 in breast cancer. *Mol. Oncol.* 13 (5), 1137–1149. doi:10.1002/1878-0261.12472

Murciano-Goroff, Y. R., Warner, A. B., and Wolchok, J. D. (2020). The future of cancer immunotherapy: microenvironment-targeting combinations. *Cell Res.* 30 (6), 507–519. doi:10.1038/s41422-020-0337-2

Newman, A. M., Liu, C. L., Green, M. R., Gentles, A. J., Feng, W., Xu, Y., et al. (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nat. Methods* 12 (5), 453–457. doi:10.1038/nmeth.3337

Patil, D. P., Chen, C. K., Pickering, B. F., Chow, A., Jackson, C., Guttman, M., et al. (2016). m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature* 537 (7620), 369–373. doi:10.1038/nature19342

Patil, D. P., Pickering, B. F., and Jaffrey, S. R. (2018). Reading m(6)A in the transcriptome: m(6)a-binding proteins. *Trends Cell Biol.* 28 (2), 113–127. doi:10.1016/j.tcb.2017.10.001

Peng, W. X., Koirala, P., and Mo, Y. Y. (2017). LncRNA-mediated regulation of cell signaling in cancer. *Oncogene* 36 (41), 5661–5667. doi:10.1038/onc.2017.184

Postow, M. A., Callahan, M. K., and Wolchok, J. D. (2015). Immune checkpoint blockade in cancer therapy. *J. Clin. Oncol.* 33 (17), 1974–1982. doi:10.1200/JCO.2014.59.4358

Punzón-Jiménez, P., Lago, V., Domingo, S., Simón, C., and Mas, A. (2022). Molecular management of high-grade serous ovarian carcinoma. *Int. J. Mol. Sci.* 23 (22), 13777. doi:10.3390/ijms232213777

Qiu, Y., Wang, X., Fan, Z., Zhan, S., Jiang, X., and Huang, J. (2021). Integrated analysis on the N6-methyladenosine-related long noncoding RNAs prognostic signature, immune checkpoints, and immune cell infiltration in clear cell renal cell carcinoma. *Immun. Inflamm. Dis.* 9, 1596–1612. doi:10.1002/iid3.513

Racle, J., and Gfeller, D. (2020). EPIC: a tool to estimate the proportions of different cell types from bulk gene expression data. *Methods Mol. Biol.* 2120, 233–248. doi:10.1007/978-1-0716-0327-7_17

Ribas, A., and Wolchok, J. D. (2018). Cancer immunotherapy using checkpoint blockade. *Science* 359 (6382), 1350–1355. doi:10.1126/science.aar4060

Saproo, S., Sarkar, S. S., Gupta, E., Chattopadhyay, S., Charaya, A., Kalra, S., et al. (2023). MiR-330-5p and miR-1270 target essential components of RNA polymerase I

transcription and exhibit a novel tumor suppressor role in lung adenocarcinoma. *Cancer Gene Ther.* 30 (2), 288–301. doi:10.1038/s41417-022-00544-4

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9 (7), 671–675. doi:10.1038/nmeth.2089

Sheng, H., Huang, Y., Xiao, Y., Zhu, Z., Shen, M., Zhou, P., et al. (2020). ATR inhibitor AZD6738 enhances the antitumor activity of radiotherapy and immune checkpoint inhibitors by potentiating the tumor immune microenvironment in hepatocellular carcinoma. *J. Immunother. Cancer* 8 (1), e000340. doi:10.1136/jitc-2019-000340

Sheppard, K. E., Cullinane, C., Hannan, K. M., Wall, M., Chan, J., Barber, F., et al. (2013). Synergistic inhibition of ovarian cancer cell growth by combining selective PI3K/mTOR and RAS/ERK pathway inhibitors. *Eur. J. Cancer* 49 (18), 3936–3944. doi:10.1016/j.ejca.2013.08.007

Shulman, Z., and Stern-Ginossar, N. (2020). The RNA modification N6-methyladenosine as a novel regulator of the immune system. *Nat. Immunol.* 21, 501–512. doi:10.1038/s41590-020-0650-4

Siegel, R. L., Miller, K. D., and Jemal, A. (2019). Cancer statistics, 2019. *CA Cancer J. Clin.* 69 (1), 7–34. doi:10.3322/caac.21551

Song, Z., Zhao, Z., Zhu, S., Jin, Q., Zhang, S., Wang, Z., et al. (2023). Arylsulfatase D is a prognostic biomarker that promotes glioma cells progression through JAK2/STAT3 pathway and M2 macrophage infiltration. *Front. Oncol.* 13, 1228426. doi:10.3389/fonc.2023.1228426

Soureas, K., Papadimitriou, M. A., Panoutsopoulou, K., Pilala, K. M., Scorilas, A., and Avgeris, M. (2023). Cancer quiescence: non-coding RNAs in the spotlight. *Trends Mol. Med.* 29, 843–858. doi:10.1016/j.molmed.2023.07.003

Statello, L., Guo, C. J., Chen, L. L., and Huarte, M. (2021). Gene regulation by long non-coding RNAs and its biological functions. *Nat. Rev. Mol. Cell Biol.* 22 (2), 96–118. doi:10.1038/s41580-020-00315-9

Sun, Y., Li, L., Yao, W., Liu, X., Yang, Y., Ma, B., et al. (2021). USH2A mutation is associated with tumor mutation burden and antitumor immunity in patients with colon adenocarcinoma. *Front. Genet.* 12, 762160. doi:10.3389/fgene.2021.762160

Tay, Y., Rinn, J., and Pandolfi, P. P. (2014). The multilayered complexity of ceRNA crosstalk and competition. *Nature* 505 (7483), 344–352. doi:10.1038/nature12986

Thomson, D. W., and Dinger, M. E. (2016). Endogenous microRNA sponges: evidence and controversy. *Nat. Rev. Genet.* 17 (5), 272–283. doi:10.1038/nrg.2016.20

Vadie, N., Saayman, S., Lenox, A., Ackley, A., Clemson, M., Burdach, J., et al. (2015). MYCNOS functions as an antisense RNA regulating MYCN. *RNA Biol.* 12 (8), 893–899. doi:10.1080/15476286.2015.1063773

van Zyl, B., Tang, D., and Bowden, N. A. (2018). Biomarkers of platinum resistance in ovarian cancer: what can we use to improve treatment. *Endocr. Relat. Cancer* 25 (5), R303–R318. doi:10.1530/ERC-17-0336

Vergara, D., Bellomo, C., Zhang, X., Vergaro, V., Tinelli, A., Lorusso, V., et al. (2012). Lapatinib/Paclitaxel polyelectrolyte nanocapsules for overcoming multidrug resistance in ovarian cancer. *Nanomedicine* 8 (6), 891–899. doi:10.1016/j.nano.2011.10.014

Wang, C., Gu, Y., Zhang, E., Zhang, K., Qin, N., Dai, J., et al. (2019a). A cancer-testis non-coding RNA LIN28B-AS1 activates driver gene LIN28B by interacting with IGF2BP1 in lung adenocarcinoma. *Oncogene* 38 (10), 1611–1624. doi:10.1038/s41388-018-0548-x

Wang, H., Meng, Q., and Ma, B. (2021). Characterization of the prognostic m6A-related lncRNA signature in gastric cancer. *Front. Oncol.* 11, 630260. doi:10.3389/fonc.2021.630260

Wang, X., Ma, R., Zhang, X., Cui, L., Ding, Y., Shi, W., et al. (2021). Crosstalk between N6-methyladenosine modification and circular RNAs: current understanding and future directions. *Mol. Cancer* 20 (1), 121. doi:10.1186/s12943-021-01415-6

Wang, X., Zhang, J., and Wang, Y. (2019b). Long noncoding RNA GAS5-AS1 suppresses growth and metastasis of cervical cancer by increasing GAS5 stability. *Am. J. Transl. Res.* 11 (8), 4909–4921.

Wei, L., Sun, J., Zhang, N., Zheng, Y., Wang, X., Lv, L., et al. (2020). Noncoding RNAs in gastric cancer: implications for drug resistance. *Mol. Cancer* 19 (1), 62. doi:10.1186/s12943-020-01185-7

Xie, H., Wang, W., Qi, W., Jin, W., and Xia, B. (2021). Targeting DNA repair response promotes immunotherapy in ovarian cancer: rationale and clinical application. *Front. Immunol.* 12, 661115. doi:10.3389/fimmu.2021.661115

Xu, F., Huang, X., Li, Y., Chen, Y., and Lin, L. (2021). m(6)A-related lncRNAs are potential biomarkers for predicting prognoses and immune responses in patients with LUAD. *Mol. Ther. Nucleic Acids* 24, 780–791. doi:10.1016/j.omtn.2021.04.003

Xu, Z. J., Jiang, S. X., Ma, J., Tang, D. S., Yan, C. S., and Fang, K. (2021c). Comprehensive analysis of ferroptosis-related lncRNAs in breast cancer patients reveals prognostic value and relationship with tumor immune microenvironment. *Front. Surg.* 8, 742360. doi:10.3389/fsurg.2021.742360

Yan, J., Huang, X., Zhang, X., Chen, Z., Ye, C., Xiang, W., et al. (2020). LncRNA LINC00470 promotes the degradation of PTEN mRNA to facilitate malignant behavior in gastric cancer cells. *Biochem. Biophys. Res. Commun.* 521 (4), 887–893. doi:10.1016/j.bbrc.2019.11.016

Yang, C., Xia, B. R., Zhang, Z. C., Zhang, Y. J., Lou, G., and Jin, W. L. (2020). Immunotherapy for ovarian cancer: adjuvant, combination, and neoadjuvant. *Front. Immunol.* 11, 577869. doi:10.3389/fimmu.2020.577869

Yang, D., Feng, Y., Lu, H., Chen, K., Xu, J., Li, P., et al. (2023). USH2A mutation and specific driver mutation subtypes are associated with clinical efficacy of immune checkpoint inhibitors in lung cancer. *J. Zhejiang Univ. Sci. B* 24 (2), 143–156. doi:10.1631/jzus.B2200292

Yang, D. D., Qiao, J., Wang, G. Y., Lan, Y. Y., Li, G. P., Guo, X. D., et al. (2018). N6-Methyladenosine modification of lincRNA 1281 is critically required for mESC differentiation potential. *Nucleic Acids Res.* 46 (8), 3906–3920. doi:10.1093/nar/gky130

Ye, L. L., Pan, K., Fang, S., Wu, S. N., Chen, S., Tang, S. S., et al. (2022). Four types of RNA modification writer-related lncRNAs are effective predictors of prognosis and immunotherapy response in serous ovarian carcinoma. *Front. Immunol.* 13, 863484. doi:10.3389/fimmu.2022.863484

Yi, Y. C., Chen, X. Y., Zhang, J., and Zhu, J. S. (2020). Novel insights into the interplay between m(6)A modification and noncoding RNAs in cancer. *Mol. Cancer* 19 (1), 121. doi:10.1186/s12943-020-01233-2

Yu, J., Ou, Z., Lei, Y., Chen, L., Su, Q., and Zhang, K. (2020). LncRNA MYCNOS facilitates proliferation and invasion in hepatocellular carcinoma by regulating miR-340. *Hum. Cell* 33 (1), 148–158. doi:10.1007/s13577-019-00303-y

Zeng, C. W., Huang, W. X., Li, Y. Q., and Weng, H. Y. (2020). Roles of METTL3 in cancer: mechanisms and therapeutic targeting. *J. Hematol. Oncol.* 13 (1), 117. doi:10.1186/s13045-020-00951-w

Zhang, B., Gu, Y., and Jiang, G. (2020). Expression and prognostic characteristics of m(6) A RNA methylation regulators in breast cancer. *Front. Genet.* 11, 604597. doi:10.3389/fgene.2020.604597

Zhang, C., Dang, D., Cong, L. L., Sun, H. Y., and Cong, X. L. (2021a). Pivotal factors associated with the immunosuppressive tumor microenvironment and melanoma metastasis. *Cancer Med.* 10 (14), 4710–4720. doi:10.1002/cam4.3963

Zhang, J., Guo, S., Piao, H. Y., Wang, Y., Wu, Y., Meng, X. Y., et al. (2019). ALKBH5 promotes invasion and metastasis of gastric cancer by decreasing methylation of the lncRNA NEAT1. *J. Physiol. Biochem.* 75 (3), 379–389. doi:10.1007/s13105-019-00690-8

Zhang, P., Liu, G., and Lu, L. (2021). N6-Methylandenosine-related lncRNA signature is a novel biomarkers of prognosis and immune response in colon adenocarcinoma patients. *Front. Cell Dev. Biol.* 9, 703629. doi:10.3389/fcell.2021.703629

Zhang, Y., Mao, Q., Xia, Q., Cheng, J., Huang, Z., Li, Y., et al. (2021b). Noncoding RNAs link metabolic reprogramming to immune microenvironment in cancers. *J. Hematol. Oncol.* 14 (1), 169. doi:10.1186/s13045-021-01179-y

Zhang, Y., and Zhang, Z. (2020). The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol. Immunol.* 17 (8), 807–821. doi:10.1038/s41423-020-0488-6

Zhang, Z., Zhang, C., Yang, Z., Zhang, G., Wu, P., Luo, Y., et al. (2021c). m(6)A regulators as predictive biomarkers for chemotherapy benefit and potential therapeutic targets for overcoming chemotherapy resistance in small-cell lung cancer. *J. Hematol. Oncol.* 14 (1), 190. doi:10.1186/s13045-021-01173-4

Zhao, P., Li, T., Wang, Y., Wang, Y., Gu, Q., and Li, Z. (2021). LncRNA MYCNOS promotes glioblastoma cell proliferation by regulating miR-216b/FOXM1 axis. *Metab. Brain Dis.* 36 (6), 1185–1189. doi:10.1007/s11011-021-00729-0

Zhao, X., and Cui, L. (2019). Development and validation of a m(6)A RNA methylation regulators-based signature for predicting the prognosis of head and neck squamous cell carcinoma. *Am. J. Cancer Res.* 9 (10), 2156–2169.

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New hopes and promises in the treatment of ovarian cancer focusing on targeted treatment—a narrative review

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Unfortunately, ovarian cancer is still diagnosed most often only in an advanced stage and is also the most lethal gynecological cancer. Another problem is the fact that treated patients have a high risk of disease recurrence. Moreover, ovarian cancer is very diverse in terms of molecular, histological features and mutations. Many patients may also develop platinum resistance, resulting in poor response to subsequent lines of treatment. To improve the prognosis of patients with ovarian cancer, it is expected to make better existing and implement new, promising treatment methods. Targeted therapies seem very promising. Currently, bevacizumab - a VEGF inhibitor and therapy with olaparib - a polyADP-ribose polymerase inhibitor are approved. Other methods worth considering in the future include: folate receptor α, immune checkpoints or other immunotherapy methods. To improve the treatment of ovarian cancer, it is also important to ameliorate the determination of molecular features to describe and understand which group of patients will benefit most from a given treatment method. This is important because a larger group of patients treated for ovarian cancer can have a greater chance of surviving longer without recurrence.

KEYWORDS

ovarian cancer, targeted treatment, angiogenesis inhibitors, folate receptor inhibitors, PARP inhibitors, bevacizumab, immune checkpoint inhibitors immunotherapy

1 Introduction

Despite continuous progress in gynecological oncology, statistics are still unfavorable for ovarian cancer. It is the third most common gynecological cancer in the world with the highest mortality rate among cancers of the female reproductive system (Höhn et al., 2020). Moreover, according to Global Cancer Statistics 2020, up to 24,000 women will be diagnosed with ovarian cancer every year (Sung et al., 2021). Finally, most of these patients learn about the disease only in its advanced stage, where the 5-year survival rate is less than 30% (Zachou et al., 2023).

The goal of primary ovarian cancer treatment is surgical removal of the tumor and assessment of the cancer's advancement along with possible adjuvant chemotherapy. The emphasis is placed not only on prolonging survival and delaying relapse, but also on improving the woman's quality of life, which also has a significant impact on the

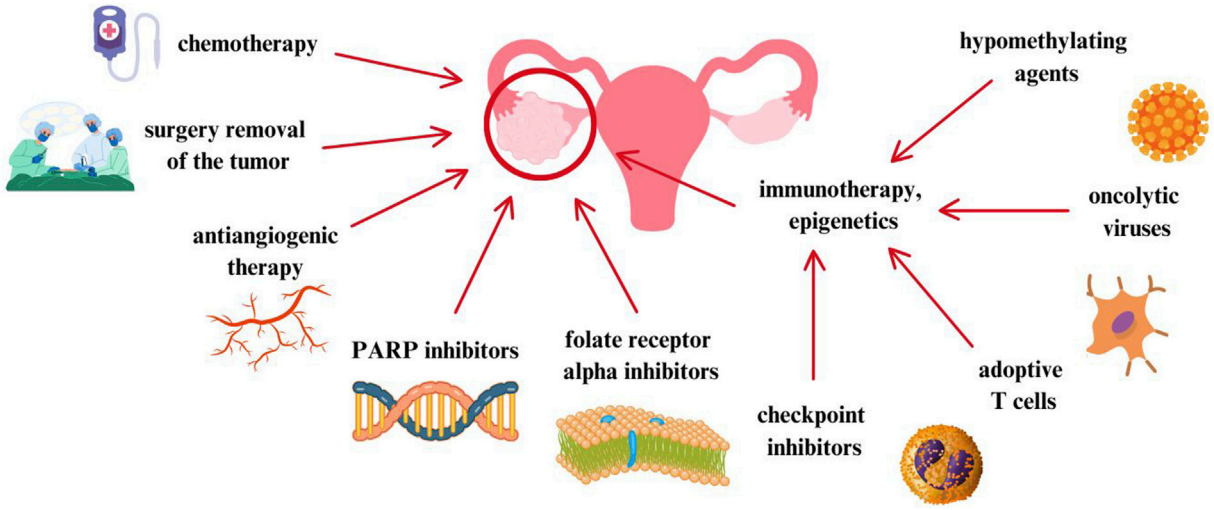

FIGURE 1
Ovarian cancer treatment methods described in the article (Burger et al., 2011; Coleman et al., 2017a; Slade, 2020; Arend et al., 2021; Heo, 2023).

effectiveness of treatment. Unfortunately, it turns out that up to 70% of patients treated with standard platinum chemotherapy will have a recurrence of the disease within 18–28 months (Armstrong et al., 2021; Fan et al., 2023).

Considering these alarming data, it is extremely important to develop new treatment methods and conduct further randomized clinical trials. New therapies and treatment strategies are based on molecular features, tumor cell proliferation, escape from immune surveillance or death signals. For this purpose, increasingly well-known standards have become the subject of discussion and interest, such as: antiangiogenic therapy with bevacizumab. The key here is to inhibit VEGF and thus the proliferation of endothelial cells (Coleman et al., 2017a). 15% of women with ovarian cancer have a BRCA1 and/or BRCA2 mutation (Slade, 2020). In these patients, poly(ADP-ribose) polymerase (PARP) inhibitors are used, which are a promising treatment method for women with this mutation. Although both angiogenesis inhibitors and PARP inhibitors have benefits, unfortunately they only delay the recurrence of ovarian cancer. Moreover, it turns out that immune checkpoint inhibitors are also not associated with benefits for patients with ovarian cancer (Arend et al., 2021). In turn, folate receptor alpha (FRα) is expressed in tissues on the plasma membrane of epithelial cells of the ovary and fallopian tube. Mirvetuximab soravtansine, a folate receptor inhibitor, is approved by the FDA for the treatment of women with platinum-resistant ovarian cancer (Heo, 2023). In Figure 1, ovarian cancer treatment methods described in the article are performed.

Although none of the methods described above has yet cured ovarian cancer, it is important to develop more and more clinical trials to improve the therapy and quality of life of patients with ovarian cancer. In this narrative review, we discuss and evaluate the latest treatments for ovarian cancer. We made a detailed review of angiogenesis inhibitors, folate receptor inhibitors, PARP inhibitors and, finally, immunotherapy. We believe that the following work will provide valuable tips for gynecologists and oncologists in selecting the best treatment strategy for patients.

2 Methods

A search was performed in January 2024 with no time restrictions for searching articles. The studies cited in this review were selected from the PubMed, Scopus and Google Scholar databases. Terms used by us to find articles were created by combining all words connected with ovarian cancer and available treatment methods by using Boolean operator "OR". We used keywords: ovarian cancer, bevacizumab, cediranib, nintedanib, pazopanib, olaparib, niraparib, rucaparib, mirvetuximab soravtansine, farletuzumab, vintafolide, checkpoint inhibitors, adoptive T cell transfer, therapeutic vaccines, oncolytic viruses. Moreover, we also used more specific terms relating to epidemiology and etiology of ovarian cancer, using "epidemiology" and "etiology".

Our aim was to create a narrative review, however, we used a paper selection to find appropriate articles. The inclusion criteria were studies evaluating the treatment of ovarian cancer, manuscripts written in English, retrospective studies, clinical trials and metanalyses. The exclusion criteria were manuscripts that did not investigate the treatment of ovarian cancer, articles not written in English, conference abstracts, document types including review and systematic review, technical report, editorial, letter and duplicated papers. Manuscripts with non-available full-text were also not taken into account.

3 Antiangiogenic therapy

Malignant tumors are characterized by uninhibited cell proliferation, which leads to the formation and spread of metastases. In this tumor development, cancer cells require the supply of oxygen and nutrients, which leads to the induction of angiogenesis. This process is the creation of new blood vessels from existing ones, thanks to which the metabolic needs of the tumor are met (Carmeliet and Jain, 2000; Ferrara et al., 2004; Burger et al.,

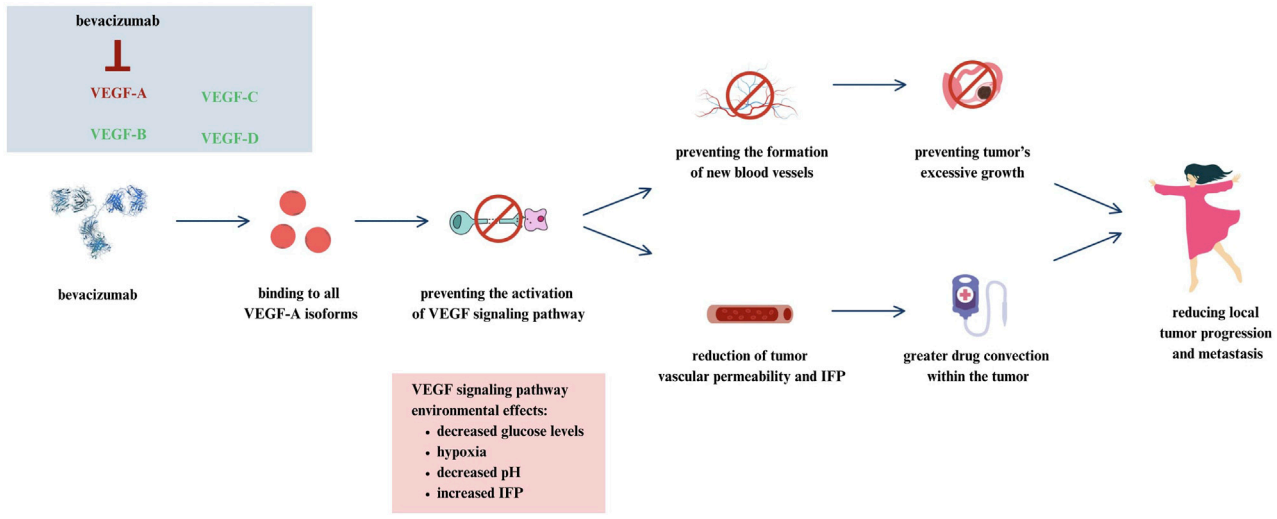

FIGURE 2
Mechanism of action for bevacizumab in ovarian cancer (Żak et al., 2024).

2011; Xu et al., 2012; Li et al., 2016; Hegde et al., 2018). Angiogenesis promotes tumor progression and worse prognosis, including ovarian cancer. Therefore, antiangiogenic therapy has been the subject of interest in numerous clinical trials for over 20 years (Burger et al., 2011). In this chapter, we analyzed the latest and most important research on the use and effectiveness of antiangiogenic therapy in the treatment of ovarian cancer.

3.1 Bevacizumab

Bevacizumab is an anti-VEGF antibody whose mechanism of action is based on the inhibition of angiogenesis, thus depriving the tumor of the ability to grow and develop. Bevacizumab for the treatment of stage III or IV epithelial ovarian cancer was approved by the EMA in 2011 and by the FDA in 2018. This medicine is used for 15 months. It is one of the first drugs whose therapy is based on targeting the tumor microenvironment (Nakai and Matsumura, 2022; Żak et al., 2024). The exact mechanism of action for bevacizumab in the treatment of ovarian cancer is shown in Figure 2.

Why is bevacizumab so special? This is the first targeted therapy in almost 40 years to treat advanced ovarian cancer (Garcia et al., 2020). In this context, the results of the GOG-218 study are important. This is a double-blind, placebo-controlled phase III study. In this study, 1,873 women were included in the group receiving chemotherapy (paclitaxel and carboplatin) with placebo in cycles 2 to 22, in the group receiving bevacizumab at a dose of 15 mg/kg body weight. in cycles 2 to 6 and placebo from 7 to 22 together with chemotherapy, and to the group receiving chemotherapy with bevacizumab in cycles 2 to 22. The median PFS in these groups was 10.3 months, 11.2 months and 14.1 months (Burger et al., 2011).

Moreover, the results of the ICON-7 study seem interesting. It was an international, open-label, randomized phase III trial. 1,528 women were assigned to the group receiving chemotherapy alone or chemotherapy plus bevacizumab at a dose of 7.5 mg/kg body weight. every 3 weeks intravenously. The median OS in these groups was 44.6 and 45.4 months, and the median PFS was 34.5 and 36.3 months. This study confirmed the effectiveness of bevacizumab in the primary treatment of patients with ovarian cancer (Oza et al., 2015).

Moreover, it turns out that bevacizumab in combination with carboplatin also prolongs PFS in patients. Results of PAOLA-1 - a randomized, double-blind phase III study, showed the benefits of using olaparib together with bevacizumab (15 mg/kg every 3 weeks for 15 months). The median OS was 56.5 months in patients treated with the combination of olaparib and bevacizumab and 51.5 months in the group receiving placebo plus bevacizumab. Interestingly, the 5-year OS was higher in patients with HRD-added ovarian cancer, as it amounted to 65.5%. In patients with HRD-negative ovarian cancer, it was 48.4% (Ray-Coquard et al., 2023). The results of the PAOLA-1 study indicate the need for biomarker testing in patients with ovarian cancer.

AGO-OVAR 17 BOOD/GINECO OV118/ENGOT Ov15 is an open-label, randomized phase III study. In 927 patients qualified for the study, a comparison of treatment with bevacizumab and chemotherapy was assessed. First, the patients were treated with cytoreductive surgery with 6 cycles of chemotherapy with paclitaxel and bevacizumab at a dose of 15 mg/kg body weight. 1 time every 3 weeks. The median PFS was 26.0 months in patients treated with extended bevacizumab and 24.2 months in patients treated standardly. No differences were observed in median OS. Therefore, the study did not show that long-term bevacizumab treatment had a significant impact on PFS or OS (Pfisterer et al., 2023).

The results of the study, which aimed to evaluate the use of the combination of bevacizumab and mirvetuximab soravtensine in patients with platinum-resistant ovarian cancer, are interesting. 94 patients were enrolled and received bevacizumab at a dose of 15 mg/kg body weight. intravenously once every 3 weeks and mirvetuximab soravtenise. 59% of patients were previously treated with bevacizumab, 52%

TABLE 1 Clinical trials with bevacizumab in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of bevacizumab	Results
GOG-218 (Burger et al., 2011)	2011	III	1873, (control therapy n = 625, bevacizumab-initiation therapy n = 625, bevacizumab-throughout therapy n = 623)	Bevacizumab-initiation: chemotherapy + bevacizumab (15 mg/kg), cycles 2–6, placebo, cycles 7–22; bevacizumab-throughout: chemotherapy + bevacizumab, cycles: 2–22	Control group, bevacizumab-initiation group, bevacizumab-throughout group: PFS 10.3, 11.2, 14.1 months; OS 39.3, 38.7, 39.7 months, respectively
ICON-7 (Oza et al., 2015)	2015	III	1,528, (standard chemotherapy n = 764, chemotherapy plus bevacizumab n = 764)	7.5 mg/kg every 3 weeks	Standard chemotherapy group, chemotherapy plus bevacizumab group: PFS 34.5, 36.3 months; OS 44.6, 45.4 months, respectively
PAOLA-1 (Ray-Coquard et al., 2023)	2023	III	806, (olaparib plus bevacizumab n = 537, placebo plus bevacizumab n = 269)	15 mg/kg every 3 weeks for 15 months	Olaparib plus bevacizumab, placebo plus bevacizumab: OS 56.5, 51.6 months, respectively
AGO-OVAR 17 BOOD/ GINECO OV118/ENGOT Ov15 (Pfisterer et al., 2023)	2023	III	927, (standard chemotherapy plus bevacizumab for 15 months n = 464, standard chemotherapy plus bevacizumab for 30 months n = 463)	15 mg/kg once every 3 weeks for 15 or 30 months	Standard chemotherapy plus bevacizumab for 15 months, standard chemotherapy plus bevacizumab for 30 months: PFS 24.2, 26.0 months; OS 60.4, 60.8 months, respectively
Gilbert et al. (Gilbert et al., 2023)	2023	Ib/II	94, (combination treatment with mirvetuximab soravtansine and bevacizumab n = 94)	15 mg/kg	PFS: 8.2 months, DOR: 9.7 months

PFS, progression-free survival; OS, overall survival; DOR, duration of response.

with ≥3 therapies, and 27% with PARP inhibitor therapy. The median PFS was 8.2 months in this study. Results were promising regardless of folate receptor alpha (FRAα) expression or prior treatment (Gilbert et al., 2023).

The clinical trials describing the efficacy of bevacizumab in patients with ovarian cancer are presented in Table 1.

3.2 Cediranib

Cediranib is another anti-aniogenic drug that is a multikinase inhibitor acting against VEGF receptor 3 (VEGFR1-VEGFR3). So far, the beneficial use of this inhibitor has been described in ovarian cancer, lung cancer, glioblastoma multiforme and kidney cancer (Matulonis et al., 2009; Goss et al., 2010; Batchelor et al., 2010; Mulders et al., 2012). So far, the results of studies have shown an increase in progression-free survival and overall survival in patients with ovarian cancer as a result of the use of cediranib in combination with chemotherapy and PARP inhibitors (Liu et al., 2019).

In 2021, the results of the ICON6 study were published - a three-arm, double-blind, placebo-controlled randomized trial, the aim of which was to examine the effectiveness of cediranib in 456 patients with recurrent platinum-sensitive ovarian cancer. Patients were randomly assigned to three arms in a 2:3:3 ratio. Arm A consisted of patients receiving chemotherapy with oral placebo and continuing supportive care. Patients in arm B received daily oral cediranib during chemotherapy and then received placebo during chemotherapy. In turn, patients in arm C received cediranib during chemotherapy and continued to take it as maintenance therapy. The daily dose of cediranib was 20 mg. The median follow-up period was 7 years for arm A and 83.7 months for arm C. The median survival in arm A was 19.9 months and in arm C 27.3 months. Moreover, in arm C, the time to death over 6 years was increased by an average of 4.8 months compared to arm A. The median survival time in arm B was similar to the results in arm C and amounted to 26.6 months. The reasons for discontinuing the drug in patients were symptoms such as diarrhea, neutropenia, voice changes or hypertension (Ledermann et al., 2021). Despite an increase in progression-free survival, cediranib caused toxic effects.

In platinum-sensitive ovarian cancer, there is evidence of beneficial effects when antiangiogenic agents are used synergistically with PARP inhibitors. Preclinical study by Kaplan et al. from 2019 showed that due to the ability of cediranib to increase sensitivity to PARP inhibition, it may be beneficial to combine it with olaparib in patients with ovarian cancer (Kaplan et al., 2019). Study NRG-GY004 is an open-label, randomized, phase 3 study designed to evaluate the activity of olaparib or olaparib plus cediranib compared with platinum chemotherapy in 565 patients with ovarian cancer. The median PFS was 10.3 months for platinum-based chemotherapy, 8.2 months for olaparib, and 10.4 months for olaparib plus cediranib (Liu et al., 2019). Although the median PFS in the group of patients using olaparib with cediranib was not significantly higher than in the group of patients using chemotherapy, the results of this study should be a reason to conduct further studies related to the use of non-chemotherapy-based therapy in patients, which may prevent potential toxicity. chemotherapy. This seems extremely important considering that in this study 20 patients withdrew from the study after being assigned to chemotherapy. Perhaps this was due to fear of the side effects of this therapy, which further emphasizes the need to continue looking for alternative methods of treating ovarian cancer.

TABLE 2 Clinical trials with cediranib in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of cediranib	Results
ICON6 (Ledermann et al., 2021)	2021	III	456, (chemotherapy plus placebo n = 118, chemotherapy plus cediranib with placebo maintenance n = 174, chemotherapy plus cediranib with cediranib maintenance n = 164)	Daily dose 20 mg	Chemotherapy plus placebo, chemotherapy plus cediranib with placebo maintenance, chemotherapy plus cediranib with cediranib maintenance: OS 19.9, 26.6, 27.3 months, respectively
NRG-GY004 (Liu et al., 2019)	2022	III	565, (platinum-based chemotherapy n = 187, olaparib alone n = 189, olaparib plus cediranib)	30 mg once daily	Platinum-based chemotherapy, olaparib alone, olaparib plus cediranib: PFS 10.3, 8.2, 10.4 months; OS 31.2, 29.2, 31.3, respectively

PFS, progression-free survival; OS, overall survival.

In summary, cediranib may increase the median PFS in female patients, but due to severe toxicity and the small number of studies, it seems important to further investigate its effectiveness in patients.

The clinical trials describing the efficacy of cediranib in patients with ovarian cancer are presented in Table 2.

3.3 Nintedanib

Nintedanib is another angiogenesis inhibitor, acting on VEGF 1-3, FGFR 1-3 and PDGFR α and β receptors, which has a shorter half-life of 10–15 h than bevacizumab (14–21 days). By targeting so many receptors, studies have demonstrated antitumor activity of nintedanib, as well as efficacy with docetaxel in patients with locally advanced and metastatic non-small cell lung cancer (Khalique and Banerjee, 2017). For this reason, over the last decade, further studies have been carried out to assess the effectiveness of nintedanib in the treatment of patients with ovarian cancer (Khalique and Banerjee, 2017; Wind et al., 2019).

AGO-OVAR 12 is a randomized phase III trial, the final results of which were presented in 2020. The study was designed to compare the effectiveness of administering nintedanib with carboplatin and paclitaxel in a group of 911 patients with a placebo group (455 patients) who received carboplatin and paclitaxel. Median follow-up was 60.9 months. The median OS was 62.0 months in the nintedanib group and 62.8 months in the placebo group. The median PFS for these patients was 17.6 and 16.6 months, respectively. The most common side effects were diarrhea (78% of patients taking nintedanib vs 26% of patients taking placebo), nausea (65% vs 53%), and alopecia (58% vs 62% (Ray Coquard et al., 2020). The results of this study did not demonstrate that adding nintedanib to chemotherapy contributed to improved OS. Improved OS was observed in patients with peritoneal disease/ascites, which may be due to M1-polarized macrophages, which have been reported to be associated with ascites (Madeddu et al., 2018).

In 2020, the results of METRO-BIBF were published - a randomized, placebo-controlled study aimed at examining the effectiveness and safety of the combination of nintedanib with oral cyclophosphamide in patients with recurrent ovarian cancer. To our knowledge, this is the first study to analyze the effectiveness of this therapy in patients treated early with other intensive methods. Patients received oral cyclophosphamide 100 mg once daily and were randomized 1:1 to also receive placebo (n = 55) or nintedanib (n = 59). 35 patients were previously treated with bevacizumab and 55 patients were previously treated with ≥5 cycles of chemotherapy. The median OS was 6.8 months for patients in the nintedanib group and 6.4 months for patients in the placebo group. In turn, the median PFS was 2.9 months for patients taking nintedanib and 2.6 months for patients taking placebo. Moreover, in the study, patients took 100 mg of cyclophosphamide, whereas in other studies the dose of cyclophosphamide was 50 mg daily. The most common side effects in patients are lymphopenia, neutropenia, diarrhea, vomiting and fatigue. Toxicity was 10% lower in patients taking cyclophosphamide alone than in patients taking cyclophosphamide plus nintedanib (Hall et al., 2020). The study did not show that nintedanib improved treatment outcomes in patients taking cyclophosphamide.

The CHIVA study is a double-blind randomized phase II study, the results of which were presented in January 2023. The aim of the study was to determine the effectiveness of nintedanib with neoadjuvant chemotherapy (NACT) in patients after interval debulking surgery (IDS) with advanced ovarian cancer. A total of 188 patients with newly diagnosed ovarian cancer, FIGO stage IIIC/IV, who were not eligible for surgical treatment, were included in the study. Patients received chemotherapy with carboplatin AUC plus paclitaxel at a dose of 175 mg/m2. every 21 days for three to four cycles before and two to three cycles after IDS (up to 8 cycles). 124 patients also received nintedanib 200 mg and 64 patients received placebo twice daily on days 2–21 every 3 weeks during NACT and thereafter as maintenance treatment for approximately 2 years. The median PFS in patients taking nintedanib was 14.14, while the median in patients taking placebo was 16.8 months. The median OS was 37.3 and 44.1 months, respectively. Moreover, 92% of patients in the nintedanib group experienced side effects such as widespread hematological or gastrointestinal events, compared to the placebo group, where these symptoms occurred in 69% of patients (Ferron et al., 2023). The study results showed a clear lack of improvement in efficacy when nintedanib was added to NACT. This is actually consistent with the results of other studies that also evaluated the effect of adding antiangiogenic therapy to NACT (Garcia Garcia et al., 2019). A limitation of this study is that the study included inoperable patients with multiple comorbidities and deteriorated condition, which may have influenced the final treatment outcome. It also seems important to focus on the toxicity of chemotherapy in subsequent studies.

The results of randomized trials did not show that the use of nintedanib led to a significant increase in median PFS and OS in

TABLE 3 Clinical trials with nintedanib in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of nintedanib	Results
AGO-OVAR 12 (Ray Coquard et al., 2020)	2020	III	1,366, (nintedanib group n = 911, placebo group n = 455)	200 mg twice daily on days 2–21 every 3 weeks for up to 120 weeks	Nintedanib group, placebo group: PFS 17.6, 16.6 months; OS 62.0, 62.8 months, respectively
METRO-BIBF (Hall et al., 2020)	2020	II	117, (oral cyclophosphamide plus nintedanib group n = 59, oral cyclophosphamide plus placebo group n = 58)	Starting dose was 200 mg twice daily	Oral cyclophosphamide plus nintedanib group, oral cyclophosphamide plus placebo group: PFS 2.9, 2.6 months; OS 6.8, 6.4 months, respectively
CHIVA (Ferron et al., 2023)	2023	II	188, (nintedanib group n = 124, placebo group n = 64)	200 mg on days 2–21 every 3 weeks during NACT and thereafter as maintenance treatment for approximately 2 years	Nintedanib group, placebo group: PFS 14.4, 16.8 months; OS 37.3, 44.1 months, respectively

NACT, neoadjuvant chemotherapy; PFS, progression-free survival; OS, overall survival.

patients with ovarian cancer. Moreover, in each of the described studies, patients experienced significant side effects related to its toxicity. Therefore, caution is required when adding antiangiogenic therapy to chemotherapy in the neoadjuvant treatment.

The clinical trials describing the efficacy of nintedanib in patients with ovarian cancer are presented in Table 3.

3.4 Pazopanib

Another option in the treatment of ovarian cancer seems to be pazopanib. It is a small molecule inhibitor of VEGFR 1-3, c-Kit and platelet-derived growth factor receptor α and β (PDGFRA and PDGFRB) (Du Bois et al., 2014). It is true that there are a limited number of studies assessing the effect of pazopanib on the treatment of patients with ovarian cancer, and the current ones indicate quite high toxicity of the therapy in the form of side effects such as diarrhea (Friedlander et al., 2018). Therefore, the latest studies are based on the use of pazopanib at a reduced dose, with the aim of reducing the likelihood of toxicity in patients.

In 2020, the results of NCT01610206 were published - an open-label, randomized, multi-site, phase 2 study that assessed the effectiveness of adding pazopanib to gemcitabine in 148 patients with platinum-resistant or sensitive ovarian cancer after ≤3 previous lines of chemotherapy. Patients were randomized 1:1 to receive gemcitabine 1,000 mg/m2 weekly on days 1, 2, and 8 intravenously for up to 21 days with or without pazopanib 800 mg orally daily. The median PFS was 2.9 in patients receiving gemcitabine alone and 5.3 months in patients receiving combination therapy with pazopanib. A significantly greater number of side effects such as anemia, neutropenia, thrombocytopenia, fatigue, elevated AST and hypertension occurred in the group of patients treated with combination therapy. Moreover, 14% of those treated with gemcitabine alone and 40% of those treated with gemcitabine plus pazopanib discontinued participation in the study due to side effects such as neutropenia, fatigue, or hepatotoxicity (Duska et al., 2020). Although the study showed an improvement in median PFS in patients using the combination therapy, a large number of side effects were also reported. Moreover, the limitation of this study is definitely the unselected patient population. Moreover, in this study, the high dose of pazopanib of 800 mg orally daily may have caused such significant toxicity, supporting the need for further studies using lower doses of pazopanib.

Sharma et al. in a 2021 study aimed to evaluate the use of oral metronomic therapy in 75 patients with platinum- or treatment-resistant epithelial ovarian cancer. 38 patients in group A received etoposide 50 mg from days 1–14 and cyclophosphamide 50 mg from days 1–28 every 4 weeks. In turn, 37 patients from group B received the same treatment in combination with pazopanib at a dose of 400 mg once daily. The median PFS was 3.4 months in patients in group A and 5.1 months in patients in group B. The median OS in group A was 11.2 months, and in group B it was "not achieved". Side effects occurred in 19 patients from group A and 22 patients from group B. Only in patients from group B, side effects such as hypertension (5.4%) and increased liver enzymes (5.4%) were recorded (Sharma et al., 2021). The study results showed an increase in the median PFS and OS in patients treated with pazopanib with cyclophosphamide and etoposide in combination therapy, however, the limitation of this study is the definitely small number of qualified patients and the fact that it was a single-center study.

In 2022, the final results of the randomized phase II TAPAZ trial were published, the aim of which was to determine the effectiveness of the combination of paclitaxel and pazopanib at a lower dose than in other studies. The study enrolled 116 patients with recurrent ovarian cancer who had previously been treated with bevacizumab. 79 patients were treated with paclitaxel 65 mg/m2 on days 1, 8 and 15 intravenously together with pazopanib 600 mg/day orally. 37 patients were treated with intravenous paclitaxel alone at a dose of 80 mg/m4 on days 1, 8 and 15 every 28 days. The median PFS was 4 months in the combination group and 68% in the paclitaxel alone group. In turn, the median OS in these groups was 13.6 and 12.9 months, respectively. 47% of patients in the paclitaxel plus pazopanib group discontinued treatment, compared to 11% in the paclitaxel alone group. Patients in the group receiving combination therapy were more likely to experience side effects such as hypertension, diarrhea, anorexia, proteinuria and thrombocytopenia than in patients using paclitaxel alone. Moreover, there was one death due to gastrointestinal perforation and 1 death due to pulmonary embolism, which may have been related to the use of pazopanib (Joly et al., 2022). The results of the

TABLE 4 Clinical trials with pazopanib in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of pazopanib	Results
NCT01610206 (Duska et al., 2020)	2020	II	148, (gemcitabine alone group n = 73, gemcitabine plus pazopanib group n = 75)	800 mg orally daily	Gemcitabine alone group, gemcitabine plus pazopanib group: PFS 2.9, 5.3 months; OS 1.3, 1.1 years, respectively
CTRI/2017/10/010219 (Sharma et al., 2021)	2021	II	75, (etoposide and cyclophosphamide group n = 38, etoposide and cyclophosphamide plus pazopanib group n = 37)	400 mg once daily	Etoposide and cyclophosphamide group, etoposide and cyclophosphamide plus pazopanib group: PFS 3.4, 5.1 months; OS 11.2 months, not reached, respectively
TAPAZ (Joly et al., 2022)	2022	II	116, (paclitaxel plus pazopanib group n = 79, paclitaxel only group n = 37)	600 mg/day orally	Paclitaxel plus pazopanib group, paclitaxel only group: PFS 4.9, 5.8 moths; OS 13.6, 12.9 months, respectively
PAZOFOS (Morgan et al., 2020)	2020	Ib/II	Ib: 12, (pazopanib plus fosbretabulin group n = 12) II: 21, (pazopanib only group n = 10, pazopanib plus fosbretabulin group n = 11)	Ib: 600 mg once daily (level 1), 800 mg once daily (level 2) II: 800 mg once daily (pazopanib only group n = 10), 600 mg once daily (pazopanib plus fosbretabulin group n = 11)	II. Pazopanib only group, pazopanib plus fosbretabulin group: PFS 3.7, 7.6 months, OS 8.4 months, not reached, respectively

PFS, progression-free survival; OS, overall survival.

TAPAZ trial not only showed no improvement in median or OS in patients treated with pazopanib, but also showed an increased risk of adverse events with this therapy. The results of this study appear to be similar to the CHIVA trial evaluating the use of nintedanib on paclitaxel, which we reported above (Ferron et al., 2023). Both of these studies did not show that the addition of a given angiogenesis inhibitor had a beneficial effect on PFS and OS, and in fact showed an increased likelihood of side effects (Joly et al., 2022; Ferron et al., 2023).

The PAZOFOS study also seems worth mentioning. This is a phase 1b and randomized phase 2 trial that assessed the effectiveness of pazopanib with fosbretabulin in patients with recurrent epithelial ovarian cancer with a platinum-free interval (PFI) of 3–12 months. To our knowledge, this is the first study to evaluate fosbretabulin with an angiogenesis inhibitor. In phase 1b, 12 patients received pazopanib at a dose of 600 mg once daily and fosbretabulin at a dose of 54 mg/m2 on days 1, 8 and 15 every 28 days (dose level 1), pazopanib at a dose of 800 mg once daily and fosbretabulin 54 mg/m2 on days 1, 8 and 15 every 28 days (dose level 2), and pazopanib at a dose of 800 mg once daily and fosbretabulin at a dose of 60 mg/m2 on days 1,8 and 15 every 28 days. In turn, in phase II of the study, patients were assigned to two groups. 10 patients received pazopanib at a dose of 600 mg once daily and fosbretabulin at a dose of 54 mg/m2 on days 1, 8 and 15 every 28 days. Eleven patients received pazopanib 800 mg once daily every 28 days until disease progression or adverse events occurred. Adverse events in phase 1B included hypertension, neutropenia, fatigue and vomiting. The median PFS in phase II was 7.6 months in patients receiving the combination of pazopanib and fosbretabulin and 3.7 months in patients receiving pazopanib alone (Morgan et al., 2020). The study results showed that combined treatment with pazopanib and fosbretabulin not only improved the PFS result in patients, but also caused significant cardiac toxicity in the form of increased troponin levels and left ventricular dysfunction in 2 patients. Future research must therefore determine which of these substances is responsible for these side effects.

Although the research results seem to be quite promising in patients with platinum-resistant ovarian cancer, the statistics regarding its toxicity and side effects seem disturbing. Before starting phase III trials or new studies, it is necessary for physicians to better and more effectively recognize and mitigate the adverse effects of pazopanib therapy, especially hypertension. Moreover, in order to minimize the risk of side effects, it may be necessary in the future to identify those patients who benefited most from this therapy, which highlights the role of biomarkers.

The clinical trials describing the efficacy of pazopanib in patients with ovarian cancer are presented in Table 4.

4 PARP inhibitors

Poly(ADP-ribose) polymerase (PARP) inhibitors are another option for targeted therapy in the treatment of ovarian cancer. These anticancer drugs bind PARP1 and PARP2, which in turn are involved in DNA repair (Zaremba and Curtin, 2007). Inhibited PARP proteins cannot dissociate from DNA, which makes them unable to coordinate repair at other sites of DNA damage. The concept of synthetic lethality is important, which means that two genetic mutations occurring separately are not harmful (fda.gov, 2022; Jones et al., 2015; Bryant et al., 2005; Farmer et al., 2005; Liu et al., 2014). However, they can cause cell death when combined. When inhibited, PARP, BRCA and other proteins of the homologous recombination repair pathway repair DNA (Saleh-Gohari et al., 2005; Yap et al., 2011). Homologous recombination deficiency (HRD) will result from inactivation of BRCA1 or BRCA2 in the cancer cell. This mechanism shows that PARP inhibitors lead to DNA damage and thus the death of cancer

cells, which is used in solid tumors. The first PARP inhibitors approved by the US FDA are olaparib, niraparib, and rucaparib, which are maintenance therapy for patients with ovarian cancer (O Malley et al., 2023). In this chapter, we analyzed the latest clinical trials on PARP inhibitors and discussed future challenges and goals for this therapy.

4.1 Olaparib

Olaparib (LYNPARZA®, AstraZeneca Pharmaceuticals LP) - an inhibitor of human PARP-1, PARP-2 and PARP3, is the first PARP inhibitor approved by the FDA in 2014 for the treatment of metastatic ovarian cancer (Zhou et al., 2019; Arora et al., 2021). Olaparib is used in women with advanced-stage epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer when first germline or somatic BRCA1/2 mutations are present or recurrent platinum-sensitive ovarian cancer after complete or partial response to platinum-based chemotherapy (Frampton, 2015; Heo and Dhillon, 2018). The first study to demonstrate the effectiveness of olaparib in ovarian cancer is Study 19, which evaluated the drug *versus* placebo in 136 patients with recurrent, high-grade, sensitive, serous ovarian cancer. to platinum. In patients taking olaparib, the median PFS was 11.2 months, and in the placebo group, the median was 4.3 months. No major difference was observed between the median OS in both groups. Regarding grade 1 and 2 adverse events, patients reported mainly fatigue, nausea, vomiting, taste change and anorexia. Grade ≥3 adverse events were reported more frequently in patients in the olaparib group (40%) than in the placebo group (22%) and included nausea, fatigue, neutropenia, and anemia (Ledermann et al., 2014). The results of this study clearly demonstrated that olaparib is an effective therapy in patients with platinum-sensitive recurrent BRCA-mutated serous ovarian cancer. It was the results of this study that contributed to the approval of this drug by the FDA.

The CLIO/BGOG study, which included 160 patients, compared olaparib monotherapy with chemotherapy in patients with platinum-sensitive or resistant ovarian cancer without BRCA mutations or recurrence. 107 patients were assigned to the olaparib group and 53 to the chemotherapy group, including 89 and 49 patients in these groups who did not have a BRCA mutation. The clinical benefit rate (CBR) was achieved by 58 patients from the olaparib group and 30 patients from the chemotherapy group. The median PFS was 4.8 and 5.7 months in these groups, and the median OS was 12.5 and 14.4 months (Vanderstichele et al., 2022). The study results showed similar effectiveness of treatment with both olaparib and chemotherapy. Moreover, these results are valuable for the treatment of patients with ovarian cancer that is sensitive or resistant to standard chemotherapy treatment. It also seems important that this study assessed the effect of olaparib treatment in patients without BRCA mutations.

SOLO2/ENGOT Ov-21 is a randomized phase III trial that evaluated olaparib in women with recurrent platinum-sensitive BRCA1/2 mutation-positive ovarian cancer (BRCA) after response to platinum-based chemotherapy. A *post hoc* analysis of this study was performed in 2023. 147 patients were assigned to the group receiving olaparib (53%) in the form of tablets at a dose of 300 mg twice daily or the placebo group (47%). In the olaparib group, 24 and 54 patients received platinum-free chemotherapy and platinum-containing chemotherapy, respectively, while in the placebo group, the numbers were exactly 27 and 42 patients. Median OS was 51.1 months in patients taking olaparib compared with 38.8 months in patients in the placebo group, and median PFS was 18.4 months in the placebo group (not achieved for the olaparib group). Time to second subsequent treatment (TTSP) was 12.1 months in the placebo group and 6.9 months in the olaparib group. The results of this question lead to reflection on what treatment would be most optimal in patients with early relapse after treatment with a PARP inhibitor (Poveda et al., 2021).

PAOLA-1/ENGOT-ov25 is a double-blind, phase III trial, the aim of which was to evaluate maintenance treatment with olaparib together with bevacizumab in patients diagnosed with ovarian cancer with response after first-line chemotherapy in the form of platinum compounds with bevacizumab. The final analysis of the study results were published in 2023. 535/537 patients received olaparib 300 mg twice daily for up to 24 months in combination with bevacizumab 15 mg/kg every 3 weeks for a total of 15 months and 267/269 patients received placebo in combination with bevacizumab. The final median OS was 56.5 months in the olaparib group and 51.6 months in the placebo group. Moreover, OS was longer in patients with positive HRD (65.5% vs 48.4%). The median PFS in these groups was 46.1% and 19.2%. In the group receiving olaparib, 9 cases of myelodysplastic syndromes, acute myeloid leukemia, and amyloidosis were recorded, and in the group receiving placebo, 6 cases. New primary malignancies occurred in 22 and 8 patients respectively (olaparib vs placebo), and pneumonia occurred in 7 and 2 patients respectively (Ray-Coquard et al., 2023). This study did not include a group treated with olaparib as monotherapy, which makes it difficult to determine the exact effect of olaparib and bevacizumab *versus* olaparib alone. Therefore, it is necessary to conduct further studies that will assess the impact of both combination therapy and monotherapy with a PARP inhibitor. Moreover, this is another study whose results clearly emphasize the importance of conducting research on biomarker tests, which will help to better and more precisely determine the groups of patients who will respond best to treatment with PARP inhibitors. It is important to analyze the median OS in patients depending on the location or type of BRCA mutation. Taking into account the fact that patients with HRD-positive disease responded best to treatment with PARP inhibitors, a question should be asked about possible treatment options for patients with HRD-negative disease.

In 2023, the results of the double-blind phase III trial SOLO1/GOG 3004 were published after 7 years of follow-up, which included the treatment of patients with newly diagnosed advanced ovarian cancer and BRCA mutation after platinum-based chemotherapy with olaparib. Patients were randomized to olaparib tablets 300 mg twice daily (n = 260) or placebo (n = 131). After 7 years, 67.0% of patients in the olaparib group and 46.5% of patients in the placebo group were alive. The median follow-up in this study was approximately 88 months, which, to our knowledge, is the longest follow-up of any PARP inhibitor in ovarian cancer (DiSilvestro et al., 2023). Moreover, the study results showed improved OS in patients with newly diagnosed ovarian cancer treated supportively with olaparib.

TABLE 5 Clinical trials with olaparib in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of olaparib	Results
NCT00753545 (Ledermann et al., 2014)	2014	II	265, (olaparib group n = 136, placebo group n = 129)	400 mg twice daily, capsules	Olaparib group, placebo group: PFS 11.2, 4.3 months; OS 37.1, 37.6 months, respectively
CLIO/BGOG-ov10 (Vanderstichele et al., 2022)	2022	II	160, (olaparib only group n = 107, standard chemotherapy group n = 53)	Starting dose of 300 mg (2 × 150 mg tablets)	Olaparib only group, standard chemotherapy group: PFS 4.8, 5.7 months; OS 12.5, 14.4 months, respectively
SOLO2/ENGOT Ov-21 (Poveda et al., 2021)	2023	III	295 (olaparib only group n = 195, standard chemotherapy group n = 990)	300 mg in two 150 mg tablets, twice daily	Olaparib only group, standard chemotherapy group: PFS not achieved, 18.4 months; OS 51.1, 38.8 months, respectively
PAOLA-1/ENGOT-ov25 (Ray-Coquard et al., 2023)	2023	III	806, (olaparib plus bevacizumab n = 537, placebo plus bevacizumab n = 269)	300 mg twice daily	Olaparib plus bevacizumab, placebo plus bevacizumab: OS 56.5, 51.6 months, respectively
SOLO1/GOG 3004 (DiSilvestro et al., 2023)	2023	III	391, (olaparib group n = 260, placebo group n = 130)	300 mg twice daily, tablets	Olaparib group, placebo group: TFST 64.0, 15.1 months; OS not reached, 75.2 months, respectively

PFS, progression-free survival; OS, overall survival; TFST, time to first subsequent therapy or death.

The use of olaparib in patients with ovarian cancer represents a significant progress in treatment. In the PAOLA-1 (Ray-Coquard et al., 2023) trial, patient selection was not driven by BRCAm status compared to the SOLO-1 (DiSilvestro et al., 2023) trial, which was based on patients with a germline BRCA mutation. In contrast, the CLIO/BEGOG trial also focused on patients without BRCA mutations (Vanderstichele et al., 2022). It is hypothesized that a germline or somatic BRCA mutation causes HRR deficiency, leading to sensitivity to PARP inhibition (Arora et al., 2021).

The clinical trials describing the efficacy of olaparib in patients with ovarian cancer are presented in Table 5.

4.2 Niraparib

Niraparib (MK4827) is an oral PARP-1 and PARP-2 inhibitor that causes cancer cell death with BRCA1 and BRCA2 deficient cell lines (AlHilli et al., 2016; Caruso et al., 2017) Already in 2012, Wang et al. described the increased effectiveness of radiotherapy in human lung and breast xenografts in combination with niraparib (Wang et al., 2012). Moreover, we find that in patients with recurrent platinum-sensitive ovarian cancer, niraparib improved median PFS regardless of BRCA mutation. Thanks to the results of the ENGOT-OV16/NOVA study on 553 patients, in 2017 the US FDA approved the use of niraparib in patients with relapsed ovarian cancer in the CR or PR phase with platinum-based chemotherapy. The results of this study showed not only a higher median PFS in patients with BRCA mutations (12.9 months in the niraparib group vs 3.8 months in the placebo group), but also in patients without mutations (6.0 months vs 3.9 months) (Mirza et al., 2016).

Although the main PARP inhibitors described in the literature for use in patients with ovarian cancer are olaparib and niraparib, it turns out that research on both of these inhibitors may be contradictory. The double-blind phase III NORA trial analyzed niraparib maintenance in patients with platinum-sensitive relapsed ovarian cancer. There were 177 patients in the niraparib group and 88 in the placebo group. 14 patients with a median weight of 82.5 kg received niraparib or placebo at a dose of 300 mg, and 235 patients with a median weight of 59.0 kg received a dose of 200 mg. The median PFS was 18.3 months in the niraparib group and 5.4 months in the placebo group (Wu et al., 2021). Furthermore, for patients taking niraparib, the median PFS was 11.1 for germline BRCA mutations and 3.9 months for germline BRCA negative patients, consistent with the results of the NOVA trial [Wu XH]. Median OS data is not yet mature. The most frequently reported adverse events were decreased neutrophil counts (20.3% of patients in the niraparib group vs 9.0% of patients in the placebo group) and anemia (14.7% vs 2.3%, respectively). The results of this study not only demonstrated the effectiveness of niraparib in recurrent platinum-sensitive ovarian cancer, but also its effectiveness regardless of the presence or absence of BRCA mutations. Moreover, to our knowledge, this is the first study that established an individual drug dosing regimen. The low number of adverse events may have been due to the fact that a large proportion of patients were initially treated with a lower dose of niraparib (200 mg daily) (Wu et al., 2021).

In 2023, 3.5 years of follow-up results of the randomized phase III trial PRIMA/ENGOT-OV26/GOG-3012 were published. The aim of the study was to evaluate the effectiveness of niraparib in patients with newly diagnosed ovarian cancer after achieving a complete (CR) or partial response (PR) to first-line platinum chemotherapy. The study included 487 patients in the niraparib group and 246 patients in the placebo group. The median INV-PFS was 24.5 months in the group of patients taking niraparib and 11.2 months in the placebo group (hazard ratio, 0.52; 95% confidence interval [CI], 0.40–0.68) in the HRd population and 18.8 and 8.2 months in the entire patient population. As for the OS, it was still immature. Adverse events mainly included thrombocytopenia (39.7%), anemia (31.6%), and neutropenia (21.3%) (González-Martín et al., 2023). The results of the *ad hoc* analysis of this study demonstrated the efficacy of niraparib in female patients. However, despite the favorable results of this study, it is not yet possible to evaluate the long-term role of niraparib due to the lack of accurate median OS data.

TABLE 6 Clinical trials with niraparib in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of niraparib	Results
ENGOT-OV16/NOVA (Mirza et al., 2016)	2016	III	553 patients: 203 patients in the gBRCA cohort (niraparib group n = 138, placebo group n = 65), 350 patients in the non-gBRCA cohort (niraparib group n = 234, placebo group n = 116)	300 mg once daily	Niraparib group, placebo group: gBRCA cohort - PFS 21.0, 5.5 months; non-gBRCA cohort - PFS 12.9, 3.8 months, respectively
NORA trial (NCT03705156) (Wu et al., 2021)	2021	III	265, (niraparib group n = 177, placebo group n = 88)	300 mg/day or 200 mg/day (depending on bodyweight and platelet count)	Niraparib group, placebo group: PFS 18.3, 5.4 months, TFST 16.7, 7.7 months, respectively
PRIMA/ENGOT-OV26/GOG-3012 (González-Martín et al., 2023)	2023	III	733, (niraparib group n = 487, placebo group n = 246)	300 mg/day or 200 mg/day (depending on bodyweight and platelet count)	Niraparib group, placebo group: overall population - PFS 13.8, 8.2 months; HRd population - PFS 24.5, 11.2 months; HRp population - 8.4, 5.4 months, respectively
Li et al. (Li et al., 2023)	2023	III	384, (niraparib group n = 255, placebo group n = 129)	300 mg/day or 200 mg/day (depending on bodyweight and platelet count)	Niraparib group, placebo group: PFS 24.8, 8.3 months, respectively

gBRCA, germline BRCA, mutation; PFS, progression-free survival; TFST, time to first subsequent therapy or death; HR, homologous recombination deficiency status (HRd, deficient; HRp, proficient or not determined).

The results of a phase III randomized clinical trial were published in 2023. Li et al. demonstrated prolonged PFS in patients with newly diagnosed ovarian cancer regardless of biomarker status or residual disease with niraparib maintenance therapy. 255 patients were assigned to the niraparib group and 129 patients to the placebo group. Median PFS was 24.8 months in the niraparib group and 8.3 months in the placebo group in the intention-to-treat population HR, 0.45; 95% CI, 0.34–0.60; $p < .001$}. Moreover, increased median PFS was also demonstrated in patients without germline BRCA variants (19.3 vs. 8.3 months; HR, 0.48; 95% CI, 0.34–0.67) and in homologous recombination deficient (16.6 vs. 5.5 months; HR, 0.44; 95% CI, 0.32–0.61) (Li et al., 2023).

Considering the favorable results of niraparib treatment in patients, the results of studies on its use in combination therapies may also be important. NItCHE trial (MITO 33) is a phase III, multicenter trial, the preliminary results of which are expected to be presented in June 2024. The aim of the study is to evaluate therapy with niraparib plus dostarlimab compared to chemotherapy alone in eligible patients with recurrent ovarian cancer. for treatment with platinum derivatives (Musacchio et al., 2021).

The clinical trials describing the efficacy of niraparib in patients with ovarian cancer are presented in Table 6.

4.3 Rucaparib

Rucaparib is another PARP-1/2/3 inhibitor that has been shown to be effective in the treatment of ovarian cancer (Drew et al., 2011; Coleman et al., 2017b; Yubero et al., 2022). Rucaparib was approved by the FDA in 2016 for the monotherapy treatment of patients with advanced ovarian cancer with BRCA mutations (germinal and/or somatic) who have had ≥2 cycles of chemotherapy (Syed, 2017). In turn, the results of the ARIEL3 trial supported the approval of rucaparib in 2019 by the European Medicines Agency (EMA) for the maintenance treatment of patients with platinum-sensitive recurrent ovarian cancer with a complete or partial response to platinum chemotherapy (Rubraca, 2022).

ARIEL3 is a double-blind, placebo-controlled, phase III trial in which 564 patients with platinum-sensitive ovarian cancer who received ≥2 cycles of platinum-based chemotherapy were randomized to rucaparib (n = 375) in 600 mg twice daily or placebo (n = 189). Median PFS was 8.2 months in the rucaparib group and 4.1 months in the placebo group (n = 224 vs. n = 113; HR 0.39, 95% CI 0.30 to 0.52, $p < 0.0001$) in patients with PFS 6 to ≤12 months and 13.6 months respectively and 5.6 months for patients with PFS >12 months. Moreover, PFS in the rucaparib group was 16.6 months (HR = 0.23, $p < 0.0001$) in the BRCA mutation group and 13.6 months (HR = 0.32, $p < 0.0001$) in the HRD group (including patients with BRCA mutations or wild/high LOH). Adverse events included anemia (18.8% in the rucaparib group and 0.5% in the placebo group) and increased alanine/aspartate aminitransferase activity (10.5% and 0%, respectively), indicating a fairly consistent and similar safety profile in patients in both groups (Clamp et al., 2021). The results of the ARIEL3 trial showed a benefit from rucaparib both in patients who had received 2 or more prior chemotherapy regimens and regardless of biomarker status.

ATHENA (NCT03522246) is a randomized, phase III trial evaluating rucaparib maintenance therapy in patients with stage III-IV advanced ovarian cancer who responded to first-line double platinum chemotherapy. 427 patients were assigned to rucaparib 600 mg twice daily and 111 patients were assigned to placebo. In the HRD population, the median PFS was 28.7 months in the rucaparib group and 11.3 months in the placebo group, in the intention-to-treat population it was 20.2 and 9.2 months, respectively, and in the HRD-negative population it was 12.1 and 9.1 months, respectively. The most common side effect was anemia (28.7% of patients in the

TABLE 7 Clinical trials with rucaparib in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of rucaparib	Results
ARIEL3 (Clamp et al., 2021)	2021	III	564, (rucaparib group n = 375, placebo group n = 189)	Rucaparib 600 mg twice daily	Rucaparib group, placebo group: progression-free interval 6–≤12 months - PFS 8.2, 4.1 months; progression-free interval >12 months - PFS 13.6, 5.6 months, respectively
ATHENA-MONO/GOG-3020/ ENGOT-ov45 (Monk et al., 2022)	2022	III	538, (rucaparib group n = 427, placebo group n = 111)	Rucaparib 600 mg twice daily	Rucaparib group, placebo group: HRD population - PFS 28.7, 11.3 months; HRD-negative population - PFS 9.2, 9.1 months, respectively
ARIEL4 (Kristeleit et al., 2022)	2022	III	349, (rucaparib only group n = 233, chemotherapy group n = 116)	Rucaparib 600 mg twice daily	Rucaparib only group, chemotherapy group: PFS 7.4, 5.7 months, respectively

PFS, progression-free survival; HRD, homologous recombination deficiency.

study group and 0% in the placebo group) (Monk et al., 2022). The study results showed that, regardless of HRD or BRCA status, the median PFS was significantly higher in patients treated with rucaparib. Moreover, patients with HRD-negative tumors also benefited. This is important because patients with BRCA wild-type and HRD-negative tumors constituted 78.6% and 44.2% of the study population, respectively, which can only confirm the use of rucaparib in people who hypothetically benefit less from treatment with PARP inhibitors.

ARIEL4 is an open-label, randomized, controlled, phase 3 study comparing the efficacy of rucaparib *versus* platinum-based and non-platinum-based chemotherapy in 349 eligible patients with BRCA1/BRCA2 mutation-positive ovarian cancer who were receiving 2 or more chemotherapy regimens. This is the first study of its kind to compare any PARP inhibitor with or without platinum chemotherapy in patients with recurrent ovarian cancer and a BRCA1/BRCA2 mutation. 233 patients were assigned to receive rucaparib 600 mg twice daily orally and 116 patients to receive chemotherapy. Median PFS was 7.4 months in the rucaparib group and 5.7 months in the chemotherapy group HR 0·67 [95% CI 0·52–0·86]; p = 0·0017). The most common side effects were anemia or decreased hemoglobin, which is consistent with side effects in previous studies (Kristeleit et al., 2022). This is the first such study to show that patients with BRCA reversion mutations benefit less from treatment with rucaparib than patients without these mutations. Furthermore, it appears that in responding patients, the use of rucaparib may result in a durable response.

The clinical trials describing the efficacy of rucaparib in patients with ovarian cancer are presented in Table 7.

5 Folate receptor alpha inhibitors

FRα is a glycoprotein anchored to glycosylphosphatidylinositol on the cell surface. Folic acid regulates the level of FRα expression, and its deficiency leads to increased FRα expression *in vivo* and *in vitro*. Moreover, FRα has the ability to participate in cell division, proliferation and tissue growth (Yang et al., 2007; Cheung et al., 2018). FRα is encoded by the FOLR1 gene and is expressed in breast and lung cancer, including on the plasma membrane of epithelial cells of the kidneys, placenta, uterus, cervix, and finally - ovary and fallopian tube (Bueno et al., 2001; Shi et al., 2009; O Shannessy et al., 2013). It turns out that FRα overexpression may occur in up to 90% of ovarian cancers (Kalli et al., 2008; Markert et al., 2008). In addition, FRα may be a biomarker for ovarian cancer because it can be detected in a soluble form in serum. Thanks to the possibility of assessing FRα protein expression using immunohistochemical staining, it is possible to qualify patients who may benefit from FRα-targeted therapy (Ebel et al., 2007). The first anti-FRα monoclonal antibody is farletuzumab (MORab003; Morphotek, Inc.), whose antitumor activity is based on the induction of antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and inhibition of the Lyn kinase signaling pathway. However, a 2016 Phase III trial did not demonstrate that farletuzumab plus carboplatin and a taxane improved PFS outcomes in ovarian cancer patients (Vergote et al., 2016). Positive study results with mivetuximab soravtansine (MIRV) led to US FDA approval of MIRV for the treatment of platinum-resistant ovarian cancer in 2022 (Heo, 2023). Thus, an increasing number of studies are focusing on other anti-FRα monoclonal antibodies.

5.1 Mirvetuximab soravtansine

Mirvetuximab soravtansine (MIRV/Elagere/IMGN853) is an antibody-drug conjugate that consists of a humanized anti-FRα monoclonal antibody, a cleavable linker sulfo-SPDB and the cytotoxic maytansinoid effector molecule DM4 (Oroudjev et al., 2010; Ab et al., 2015). MIRV works by decomposing it to produce lysine-Nε-sulfo-SPDB-DM4. Subsequently, the maytansinoid derivatives DM4 and S-methyl-DM4 are formed by reduction and S-methylation of lysine-DM4. These substances suppress microtubule dynamics due to their strong anti-mitotic effect [Mai J]. The phase 1 IMGN853 trial aimed to establish the preliminary safety profile of MIRV in 44 patients. The study included 44 patients with FRα-positive solid tumors who received the drug at doses ranging from 0.15 to 7.0 mg/kg body weight. Of the patient cohort, 2 patients with epithelial ovarian cancer experienced clinical benefit which were confirmed tumor partial responses

TABLE 8 Clinical trials with mirvetuximab soravtansine in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of mirvetuximab soravtansine	Results
IMGN853 (Moore et al., 2017)	2017	I	44	Doses escalating from 0.15 to 7.0 mg/kg, once every 3 weeks	2 patients with epithelial ovarian cancer achieved confirmed tumor responses, according to Response Evaluation Criteria in Solid Tumors 1.1 - partial response
FORWARD II (O Malley et al., 2020)	2020	Ib	66	6 mg/kg, once every 3 weeks	PFS 6.9 months, ORR 39% (including 5 complete responses and 21 partial responses)
SORAYA (Matulonis et al., 2023)	2023	II	106	6 mg/kg, once every 3 weeks	PFS 4.3 months, OS 13.8
FORWARD I (Moore et al., 2021)	2021	III	352, (mirvetuximab soravtansine group n = 243, chemotherapy group n = 109)	6 mg/kg, once every 3 weeks	Mirvetuximab soravtansine group, chemotherapy group: PFS 4.8, 3.3 months, respectively

PFS, progression-free survival; ORR, objective response rate; OS, overall survival.

according to Response Evaluation Criteria in Solid Tumors 1.1. (Moore et al., 2017). The favorable results regarding the safety and tolerability of MIRV in ovarian cancer have become a reason to conduct further research on its use in patients with this cancer.

FORWARD II is a phase I study that aimed to evaluate the safety and tolerability of MIRV in combination with bevacizumab in 66 patients with platinum-resistant FRα-positive ovarian cancer. Patients were administered MIRV at a dose of 6 mg/kg along with bevacizumab at a dose of 15 mg/kg once every 3 weeks. The objective response rate (ORR) was 39%, including 5 complete and 21 partial responses. Median PFS was 6.9 months. The most common side effects were diarrhea, blurred vision, nausea and fatigue. The favorable results of the combination of MIRV and bevacizumab were encouraging to conduct further studies (O Malley et al., 2020).

SORAYA is a single-arm, phase II study that aimed to evaluate the safety and effectiveness of MIRV in 106 patients with platinum-resistant epithelial ovarian cancer. ORR was 32.4%, including 5 complete and 29 partial responses. Moreover, the ORR according to the investigator was 35.3% in patients with 1–2 treatments and 30.2% in patients with 3 treatments. The most common side effects included blurred vision, keratopathy, and nausea. For patients taking PARP inhibitors, the investigator-reported ORR was 38.0% and 27.5% for patients not taking PARP inhibitors (Matulonis et al., 2023).

FORWARD I is a randomized, open-label, phase III study designed to evaluate the efficacy and safety of MIRV compared with investigator's choice of chemotherapy in 112 patients with ovarian cancer. 36 patients were assigned to the MIRV group. The median PFS in this group was 6.7 months (Moore et al., 2018). Given these encouraging results, a few years later the results of FORWARD I appeared, covering a larger population of 366 patients with platinum-resistant ovarian cancer. Patients who had previously received 1 to 3 therapies and had a medium or high level of FRα expression were qualified for the study. 243 patients received MIRV at a dose of 6 mg/kg and 109 received selected chemotherapy. The study results did not show a significant increase in the median PFS in the MIRV group (4.8 months) compared to the chemotherapy group (3.3 months) (Moore et al., 2021).

The clinical trials describing the efficacy of mirvetuximab soravtansine in patients with ovarian cancer are presented in Table 8.

5.2 Farletuzumab

Farletuzumab (MORAb-003; Morphotek, Inc.) is the first humanized anti-FRα monoclonal antibody that has the ability to exert antitumor activity via antibody-dependent cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), tumor cell autophagy, and signaling pathway inhibition Lyn kinases (Ledermann et al., 2015; Sato and Itamochi, 2016).

In a phase I study already in 2010, the safety and good tolerability of farletuzumab was demonstrated in patients with platinum-refractory or platinum-resistant epithelial ovarian cancer. 25 patients received farletuzumab at a dose of 12.5–400 mg/m2 on days 1, 6, 15 and 22 of a 5-day cycle (Konner et al., 2010). Results from a 2013 study showed an increased response rate and duration of response among patients with platinum-sensitive ovarian cancer after treatment with fartletuzumab plus carboplatin and a taxane. Total or partial ORR was 75% (Armstrong et al., 2013).

In 2016, the results of a randomized, double-blind, placebo-controlled, phase III study were published, which assessed the effectiveness of farletuzumab in 1,100 patients with platinum-sensitive ovarian cancer. The median PFS was 9.0 months in the placebo group, 9.5 months in the farletuzumab 1.25 mg/kg group, and 9.7 months in the farletuzumab 2.5 mg/kg group. Side effects included those related to chemotherapy. Interestingly, the study showed that patients with higher exposure to farletuzumab and with CA-125 concentration no more than three times ULN had a better PFS result (Vergote et al., 2016). Therefore, although the study did not achieve final PFS, it likely identified those patients who may benefit from treatment with farletuzumab. Therefore, the aim of another randomized phase II trial was to determine the effectiveness of farletuzumab in improving PFS compared to placebo when added to standard chemotherapy in 214 patients with recurrent platinum-sensitive ovarian cancer with low CA-125 levels and at first

TABLE 9 Clinical trials with farletuzumab in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of farletuzumab	Results
Konner et al. (Konner et al., 2010)	2010	I	25 (at least one infusion of farletuzumab)	Escalating dose of 12.5–400 mg/m2 on days 1, 6, 15 and 22 of a 5-day cycle	Stable disease by Response Evaluation Criteria in Solid Tumors observed in 9 (36%) patients and CA-125 reduction in 4
Armstrong et al. (Armstrong et al., 2013)	2013	Phase II	47, (combination therapy with farletuzumab)	100 mg/m2, once weekly	Total or partial ORR was 75% with combination therapy
Vergote et al. (Vergote et al., 2016)	2016	III	1,091, (placebo group n = 352, farletuzumab 1.25 mg/kg group n = 376, farletuzumab 2.5 mg/kg group n = 363)	1.25 mg/kg or 2.5 mg/kg	Placebo group, farletuzumab 1.25 mg/kg group, farletuzumab 2.5 mg/kg group: PFS 9.0, 9.5, 9.7 months; OS 29.1, 28.7, 32.1 months, respectively
Herzog et al. (Herzog et al., 2023)	2023	II	214, (farletuzumab plus chemotherapy group n = 142, placebo plus chemotherapy group n = 72)	5 mg/kg weekly	Farletuzumab plus chemotherapy group, placebo plus chemotherapy: PFS 11.7, 10.8 months, respectively

ORR, overall response rate; PFS, progression-free survival; OS, overall survival.

recurrence. 142 patients received farletuzumab 5 mg/kg weekly with chemotherapy and 72 patients received chemotherapy with placebo. The study results did not show that the median PFS was significantly different between the farletuzumab and placebo groups. However, such study results may be due to the selection of a patient population with a lower CA-125 marker concentration, which correlates with a smaller disease volume and a potentially better immunological environment, which could affect the effectiveness of farletuzumab (Herzog et al., 2023).

The clinical trials describing the efficacy of farletuzumab in patients with ovarian cancer are presented in Table 9.

5.3 Vintafolide

Vintafolide (MK-8109; EC145) is a water-soluble folate that is conjugated with deacetylvinyl-blastine monohydrase (DAVLBH). DAVLBH destabilizes microtubules, thereby disrupting mitotic division and leading to cell death. Vintafolide is a folate receptor ligand and has potent activity against xenograft tumors expressing FR (Parker et al., 2005; Reddy et al., 2007). Despite the potential use of vintafolide also in ovarian cancer, there are still very few studies in the literature determining its effectiveness.

In a 2012 phase I study, the goal was to determine the effectiveness and safety of EC145 in patients with refractory solid tumors. EC145 was administered as an intravenous bolus or 1-h infusion. The most common side effects were constipation, nausea, fatigue and vomiting. Of the 4 patients with ovarian cancer, 1 patient had one partial response to treatment (LoRusso et al., 2012). Evidence indicating the potential effectiveness of vintafolide in patients with ovarian cancer was the basis for further studies.

PRECEDENT is a randomized, phase II trial whose aim was to compare the effectiveness of vintafolide in combination with pegylated liposomal doxorubicin (PLD) compared to PLD administered alone. Furthermore, the study evaluated an imaging agent targeting FR that would have potential importance in selecting patients who would benefit most from this treatment. 162 patients with recurrent platinum-resistant ovarian cancer were enrolled and assigned in a 2:1 ratio to receive PLD with or without vintafolide 2.5 mg intravenously 3 times per week during weeks 1 and 3. The study results showed that the median PFS was 5.0 months for the group receiving vintafolide plus PLD and 2.7 months for the group receiving PLD alone. Interestingly, in this study, patients with FRα-positive tumors benefited from this combination therapy. Moreover, etharfoliatide has been shown to be helpful for imaging identification (Naumann et al., 2013). However, the phase 3 PRECEDENT trial was stopped due to failure to achieve the primary PFS result (Vergote et al., 2015). In 2016, the results of the phase II PRECEDENT trial were published, which showed that FR status does not matter regarding side effects in combination therapy with vintafolide + PLD or PLD alone in patients with platinum-resistant ovarian cancer (Herzog et al., 2016).

The clinical trials describing the efficacy of vintafolide in patients with ovarian cancer are presented in Table 10.

6 Immunotherapy

Strategies targeting the immune system in case of treating solid tumors, including ovarian cancers gave new hopes for patients, especially those who suffer from recurrences. Except for the evidence that ovarian cancers are immunogenic tumors, they belong to the group, for which immunotherapy did not show positive impact (Zhang et al., 2003; Kandalaft et al., 2012; Disis et al., 2019; Varga et al., 2019; Pujade-Lauraine et al., 2021). The reasons are still not known for sure, although it is speculated that responsible for it are potent immunosuppressive signals, which dominate the tumor microenvironment of ovarian tumors (Chen et al., 2022). Furthermore, it can be caused by the expression of many immune checkpoints, and the coexistence of a low tumor mutational burden with a dearth of neoantigens. (The Cancer Genome Atlas Research Network, 2011; PedBrain et al., 2013; Zamarin et al., 2020a). Nevertheless, there appear more and

TABLE 10 Clinical trials with vintafolide in ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose of vintafolide	Results
Lorusso et al. (LoRusso et al., 2012)	2012	I	32	2.5 mg intravenously on days 1, 3, and 5 and days 15, 17, and 19 of each 28-day cycle	Acceptable safety profile; 1 (out of 4) patient with ovarian cancer had partial response to treatment
PRECEDENT (Naumann et al., 2013)	2013	II	162, (vintafolide plus doxorubicin group n = 109, doxorubicin alone group n = 53)	2.5 mg intravenously 3 times per week during weeks 1 and 3	Vintafolide plus doxorubicin group, doxorubicin alone group: PFS 5.0, 2.7 months, respectively

PFS, progression-free survival.

more new clinical trials that focus on testing different doses or combining standard methods with new ones, which may give better responses. So far researched immunotherapeutic approaches include, among others usage of checkpoint inhibitors, oncolytic viruses, reactive T cells and dendritic cells.

Selected results related to described therapies options are presented in Table 11, Table 12, Table 13. For these ones, which were not included in the table, results are presented in the text.

In the case of checkpoint inhibitors, they work by blocking the inhibitor receptors on the surface of T cells, or their corresponding ligands. Moreover, they prevent exhaustion and promote activation of T cells to enhance tumor detection and destruction (Zamarin et al., 2020a). Although they achieve high effectiveness in the treatment of malignancies, like melanoma or renal clear cell carcinoma (Zamarin et al., 2020b), as for treating ovarian cancers their effectiveness alone induces clinical responses in <10% (Ferrara et al., 2004; Xu et al., 2012). Much more enhanced antitumor activity was demonstrated when testing the simultaneous use of a combination of checkpoint inhibitors targeting PD-1 and CTLA-4, than using them alone (Curran et al., 2010; Duraiswamy et al., 2013; Selby et al., 2016). What is more it was observed that chemotherapy increases tumor responsiveness to checkpoint inhibitors, in the AURELIA trial (research group:361; bevacizumab plus chemotherapy vs. chemotherapy alone: ORR, 30.9 *versus* 12.6% [$p < 0.001$] and median PFS, 6.7 vs. 3.4 months [$p < 0.001$]) (Pfirschke et al., 2016; Pujade-Lauraine et al., 2021). Among them, doxorubicin turned out to be an inducer, which triggers an adaptive immune response (Zitvogel et al., 2013).

Another hope gives usage of viral vectors and dendritic cells. First of them act by selective replication in cancer cells, which leads to local amplification and ultimately to cell death (Chen et al., 2022). In the study by Moreno V. et al. it was showed that usage of tumor selective adenovirus enadenotucirev increased tumor immune-cell infiltration in platinum-resistant ovarian cancer (Moreno et al., 2021). As for autologous dendritic cells, they can be expanded, activated, and loaded with a source of tumor-associated antigens (TAAs) *ex vivo* (Cibula et al., 2021). Loading them with many different of TAAs affects both the reduction of the risk of probability of immune evasion via antigen loss, as well as increasing the potency of immunization. Moreover, it plays a role in generating a polyclonal T-cell response against malignant cells (Keskin et al., 2019). In the II phase study SOV02 it turned out that DC combined with chemotherapy influenced significantly OS prolongation (13,4 months) and enhanced surrogate antigen-specific T-cell activity, but did not improve PFS (Cibula et al., 2021). Research group included 71 patients (39 received 1 mL aliquot of DCVAC/OvC) (Selby et al., 2016).

Research has also been conducted on the use of adoptive T cell immunotherapy. It is based on the use of naturally existing tumor-reactive T cells already present within the tumor, collecting them from the patient, then their activation and expansion *in vitro*, and after that reintroducing them into the patient's body (Andersen et al., 2015). This treatment has extensive clinical experience in patients with metastatic melanoma. Nevertheless, the study by Dobrzański M.J. et al. showed that the best effects were achieved, when treatment was combined with conventional modalities and burden was minimal (Dobrzanski et al., 2012).

Another promising direction is also epigenetics, which, through the use of hypomethylating agents like decitabine and 5-azacitadine (Chen et al., 2022), opens the possibility of increasing the immunogenicity of ovarian cancer and augmenting the activity of immune checkpoint inhibitors (Kershaw et al., 2006; Dobrzanski et al., 2012).

One of the other new possible directions is also usage of the trastuzumab—monoclonal antibody. It has been approved so far for treatment of HER2-expressing breast cancer and HER2-positive gastric or gastroesophageal junction adenocarcinoma in the United States and European Union, and for HER2-mutant non-small cell lung cancer in United States and Japan (Omar et al., 2015). In the second phase open-label DESTINY-PanTumor02 trial (evaluation of the Efficacy and Safety of Trastuzumab Deruxtecan for the Treatment of Selected HER2 Expressing Tumors) in patients with ovarian tumor (n = 40) OS was 13.2 months in the whole group and 20.0 months in the group with HER2 IHC 3+ expression and objective response rate (ORR) was 45% (Meric-Bernstam et al., 2024). These meaningful survival outcomes, which were also demonstrated in endometrial and cervical cohorts, can play role in revolutionizing the treatment of HER2-expressing solid tumors.

What is more, in the study of Yang Y. et al. (Yang et al., 2018) efficacy of trastuzumab alone was evaluated in comparison to combined medication of abraxane (paclitaxel) and trastuzumab amid a group of the 80 patients with recurrent ovarian cancer. Results showed that combination of the two medications vs usage of trastuzumab alone had higher OS (7.3 vs 7 months, $p = 0,63$) and lower incidence of neutropenia (40,5% vs 51,2%).

TABLE 11 Results of the selected studies examining checkpoint inhibitors in treating ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose	Results
NRG GY003 (Zamarin et al., 2020b)	2020	II	100, (treatment 1- nivolumab n = 49, treatment 2 - nivolumab plus ipilimumab n = 51)	Nivolumab 3 mg/kg iv. every 2 weeks or nivolumab 3 mg/kg iv. plus ipilimumab 1 mg/kg iv. every 3 weeks	Treatment 1, treatment 2: OS 21.8, 28.1 months; PFS 2.0, 3.9 months, respectively
Lee et al. (Lee et al., 2020)	2020	II	26 (all were given pembrolizumab)	Pembrolizumab 200 mg iv.) every 3 weeks and PLD 40 mg/m2 iv. every 4 weeks	Median PFS—8.1 (1.7–14.7) months and median OS was 18.3 (9.4–31.5) months
TPIV200 (Zamarin et al., 2020a)	2020	II	27	Durvalumab 750 mg intravenously on days 1 and 15 in cycles 1–12, and TPIV200 (500 μg per peptide; Marker, ref IB) admixed with GM-CSF (125 μg; Sargramostim) via three intradermal injections in the upper extremities on day 1 in cycles 1–6	The median PFS was 2.8 months (2.5–∞), OS was 21 months (13.5–∞)
JAVELIN Ovarian 200 (Pujade-Lauraine et al., 2021)	2021	III	566, (avelumab plus PLD n = 188, PLD n = 190, avelumab n = 188)	Avelumab (10 mg/kg iv. every 2 weeks), avelumab plus PLD (40 mg/m2 iv. every 4 weeks), or PLD	Median PFS (3.7 months combination group, 3.5 months in PLD group and 1.9 months in the avelumab group), overall survival (18.4 months vs. 18.2 months vs. 17.4 months)
JAVELIN Ovarian 100 (Monk et al., 2021)	2021	III	998, (avelumab n = 332, avelumab combination n = 331, and control n = 335)	Chemotherapy (carboplatin plus paclitaxel) followed by avelumab (10 mg/kg iv. every 2 weeks; avelumab maintenance group); chemotherapy plus avelumab (10 mg/kg iv. every 3 weeks) followed by avelumab maintenance (avelumab combination group); or chemotherapy followed by observation (control group)	Median PFS (16.8 months with avelumab maintenance, 18.1 months with avelumab combination treatment, and 18.2 months with control treatment)
NCI-2015–01910 (Konstantinopoulos et al., 2020)	2021	II	70	Gemcitabine iv. (1,000 mg/m2 during 30 min) on day 1 and day 8 of each 21-day cycle, either alone or in combination with intravenous berzosertib (210 mg/m2 during 1 h) on day 2 and day 9 of each 21-day cycle	Median PFS was 22,9 weeks (17.9–72.0) for gemcitabine plus berzosertib and 14.7 weeks for gemcitabine alone
CCR4420 (Papadatos-Pastos et al., 2022)	2022	I	34	Guadecitabine (45 mg/m2 or 30 mg/m2, administered subcutaneously on days 1–4), with pembrolizumab (200 mg administered iv. starting from cycle 2 onwards) every 3 weeks	PFS achieved for ≥24 weeks
CLEE011XUS28T (Coffman et al., 2022)	2022	I	35	Ribociclib, dosing levels groups: (a) 200 mg, (b) 400 mg, (c) 600 mg	Median PFS - 11.4 months
KGOG3046 (Park et al., 2023)	2023	II	23	Three cycles of durvalumab (1,500 mg) and tremelimumab (75 mg) with NAC, followed by IDS; after surgery, three cycles of durvalumab (1,120 mg) and adjuvant chemotherapy followed by durvalumab maintenance (1,120 mg [total 12 cycles]) were administered	The median PFS was 17.5 months, and the median OS was not reached in the modified ITT population

iv. - intravenous; PFS, progression-free survival; OS, overall survival; PLD, pegylated liposomal doxorubicin; GM-CSF, granulocyte-macrophage colony-stimulating factor; NAC, neoadjuvant chemotherapy; IDS, interval debulking surgery; ITT, intent to treat.

TABLE 12 Results of the selected studies examining oncolytic viruses in treating ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose	Results
Galanis et al. (Galanis et al., 2010)	2021	I	21	MV-CEA virus every 4 weeks for up to 6 cycles at seven different dose levels (103–109 TCID50)	Median survival was 12.15 (1.3–38.4 months), best objective response was dose-dependent disease stabilization was observed in 14 of 21 patients and with median duration of 92.5 days (54–277 days)
Cohn et al. (Cohn et al., 2017)	2017	II	108	Paclitaxel (80 mg/m2 intravenously days 1, 8, and 15 every 4 weeks) or the combination of paclitaxel (80 mg/m2 intravenously days 1, 8, and 15) plus reovirus 3 × 1010 TCID50/day intravenously on days 1–5, both every 4 weeks until disease progression or toxicity	Median PFS was 4.3 months for paclitaxel and 4.4 months for paclitaxel plus reovirus
ColoAd1-2001 (Moreno et al., 2021)	2021	I	38	Enadenotucirev iv. (1 × 1,012 viral particles; days 1, 3 and 5 every 28-day for two cycles) plus paclitaxel (80 mg/m2; days 9, 16 and 23 of each cycle)	4-month PFS rate for 20 patients who received intravenous enadenotucirev plus paclitaxel was 64% (median 6.2 months) and 63% of the patients experienced treatment-emergent adverse event - first of all neutropenia (21%)

MV-CEA, carcinoembryonic antigen-expressing oncolytic measles virus derivative; TCID, tissue culture infectious dose; PFS, progression-free survival.

TABLE 13 Results of the selected studies examining T cell immunotherapy in treating ovarian cancer.

Name of the study	Year of the study	Phase of the study	Research group	Dose	Results
Kershaw et al. (Kershaw et al., 2006)	2006	I	14	3 × 109–5 × 1,010 transduced T cells	There were observed some grade 3 and 4 toxicities in the group with high-dose usage of IL-2; in the group, which received T cells without IL-2, patients experienced relatively mild side effects; there was not observed any tumor burden in patients
Dobrzanski et al. (Dobrzanski et al., 2012)	2012	Phase I/II	7	108–109 T cells per infusion (i.e. 1–4 × 108 cells/m2)	There was observed enhanced patient survival in 3 monthly treatment cycles (3->84 months)

IL, interleukin.

Most of the described studies are in the early phases. Nevertheless, results showed that, new ways of approaching microenvironment of ovarian tumors, can be used successfully in coping with its immunosuppressive signals. These directions, especially—epigenetics can be the future of the treatment of the most aggressive ovarian tumors, including recurrences.

7 Discussion

Despite numerous treatment methods available, ovarian cancer is still associated with the risk of recurrence and metastasis. These data raise questions: what changes in the treatment should be made and what should future studies focus on to increase the effectiveness of ovarian cancer treatment?

Bevacizumab remains an important method in the treatment of ovarian cancer. However, it turns out that not only angiogenesis, but also lymphangiogenesis is an important process in the development of cancer. Bevacizumab affects blood vessels, but not lymphatic vessels. Moreover, it turns out that, to our knowledge, there are no studies that would examine the impact of lymph node metastases on the course of ovarian cancer treatment with bevacizumab. Therefore, despite many promising studies using bevacizumab in ovarian cancer, there is a great need to investigate its effect on ovarian cancer (Sopo et al., 2020).

The results of studies confirm the validity of using PARP inhibitors in the treatment of ovarian cancer. Based on the results, PARP inhibitors appear to provide the most favorable efficacy in patients with BRCA1/BRCA2 mutations who test positive for HRD. The results of the SOLO1 study indicate an improvement in OS in patients with a BRCA mutation after receiving olaparib, and the results of the PAOLA-1 study indicate an improvement in OS in HRD-positive patients (DiSilvestro et al., 2023; Ray-Coquard et al., 2023). Therefore, the standard in the diagnosis of patients should be the study of biomarkers, which will allow us to determine the group that will benefit the most from this therapy. There are the AstraZeneca AZ HRR tests for homologous repair mutations, the Myriad MyChoice test for single nucleotide polymorphisms, which can be used to determine whether a patient is HRD-positive or negative (AlHilli et al., 2016).

In terms of side effects, taking into account the results of available studies, the safety profile of PARP inhibitors appears to be similar. In the ARIEL3, ATHENA and ARIEL4 studies, the most common side effects associated with the use of rucaparib were anemia or decreased hemoglobin (Clamp et al., 2021; Kristeleit et al., 2022; Monk et al., 2022). In turn, the low number of adverse

events associated with the use of niraparib was due to the fact that a large proportion of patients were initially treated with niraparib at a lower dose of 200 mg daily. The NORA study is, to our knowledge, the first such study to establish an individual dosing regimen for this drug (Wu et al., 2021). Therefore, individualization of dosage is important to reduce the number of possible side effects and thus improve the quality of life of patients. It seems that the risk of MDS/AML with the use of PARP inhibitors is rather low in newly diagnosed patients. This risk is higher when treating recurrent ovarian cancer. It is therefore important in this case to monitor patients for this side effect to determine exactly which group is actually at risk of MDS/AML.

Currently, research is ongoing on the effectiveness of using PARP inhibitors in combination with other treatment methods. Although the use of PARP inhibitors together with chemotherapy may cause increased toxicity and side effects, there are combinations such as PARP inhibitors with antiangiogenic therapy or immunotherapy that may be optimal in the future.

Taking into account the fact that both angiogenesis inhibitors and PARP inhibitors do not significantly prolong OS in patients with ovarian cancer, it is necessary to conduct further research on new diagnostic and therapeutic strategies for ovarian cancer. The expression of FRα on the surface of ovarian cancer cells is an important premise for conducting research on the effectiveness of folate receptor alpha inhibitors.

The study results indicate a significant benefit from treatment with folate receptor alpha inhibitors in patients whose tumors showed positive FRα expression. In the phase II PRECEDENT trial, the greatest benefit from vintafolide was achieved by patients with 100% positive FRα expression (Naumann et al., 2013). The same is also confirmed by the results of the randomized phase III FORWARD trial comparing chemotherapy with MIRV with IC chemotherapy (Moore et al., 2021).

Mirvetuximab soravtansine seems to have an extremely beneficial effect, and its effect may be greater than that of farletuzumab or vintafolide. MIRV has both an ADC molecule and a cytotoxic agent, which provides both good pharmacokinetic properties and tumor cell death. Moreover, it has an extended half-life, which affects the delivery of the cargo to the site where the tumor is (Kovtun et al., 2006).

Both mirvetuximab, farletuzumab and vintafolid have a good safety and tolerability profile. The most common side effects can be quickly recognized and managed. Such results may constitute a reason to conduct further studies on the combination of folate receptor alpha inhibitors with other treatment methods, e.g., bevacizumab or pembrolizumab. In fact, the FORWARD II study indicates the effectiveness of the combination of MIRV with bevacizumab or pembrolizumab (O Malley et al., 2020). Moreover, it should be noted that folate receptor alpha inhibitors have the ability to disrupt microtubules, and taxanes also have a similar mechanism of action. Perhaps, further research will allow in the future to replace treatment with taxanes in patients with FRα-positive tumors.

Interestingly, it turns out that the use of PARP inhibitors is likely not limited only to patients with mutations in DNA repair pathways, but also to patients with newly diagnosed advanced ovarian cancer. Giannini et al. in their review, they critically assessed the PRIMA, PRIME and ATHENA-mono studies regarding the use of PARP inhibitors in newly diagnosed ovarian cancer (Giannini et al., 2023).

So far, the results of studies using immuno-oncology approaches have brought limited success in the case of treating ovarian cancer. Hopes for enhancing their activity lies in their combination (for example, two checkpoint inhibitors), or in combination with conventional methods, like chemotherapy. Another hopes is increasing immunogenicity of the tumor before using these methods, by epigenetic approaches like the use of hypomethylating agents.

Although our narrative review flexibly reports the latest treatment outcomes for ovarian cancer patients, it has limitations. First, despite the methodology used, the article may lack systematic checking for bias. Secondly, we conducted a review of work articles with no time restriction. Moreover, our review is selective, which may make it difficult to critically evaluate the articles included in our manuscript.

Our review focused on existing and new research related to the treatment of ovarian cancer. The choice of appropriate treatment also involves knowledge of numerous biomarkers responsible for the development and course of the disease. Those responsible for this include, among others: signal transduction pathways, growth factor receptors, angiogenic processes, cell cycle regulators and drug delivery systems. Further research on the molecular changes occurring in ovarian tumors is definitely necessary to develop new therapeutic strategies or improve existing ones.

In summary, the ovarian cancer environment is extremely complicated due to tumor heterogeneity, different histological and molecular types and mutations. For this purpose, a detailed analysis of biomarkers and targeted therapies is extremely important. Future research should aim to investigate biomarker analysis methods in patients with ovarian cancer, which will allow for the selection of the treatment method from which a given patient will benefit the most. Personalized and individualized treatment should be the primary goal of clinicians. While bevacizumab is still an important treatment method in ovarian cancer, its effect on lymph node metastases is still questionable. To determine which group of women is most at risk for side effects associated with PARP inhibitors such as MDS/AML, it is important to monitor patients during and after treatment. When it comes to immunotherapy, hopes are associated with the combination of, for example, two checkpoint inhibitors or their combination with other methods such as chemotherapy. Although none of the current studies have shown that a given treatment method will cure ovarian cancer, great hopes are still associated with new clinical trials on combination therapies, studies of biomarkers or the tumor microenvironment and immunosuppressive pathways.

The standard treatment of the primary ovarian cancer is surgical removal of the tumor and assessment of the cancer's advancement along with possible adjuvant chemotherapy. Nevertheless there are therapies and treatment strategies, which give new hopes for patients, including: antiangiogenic therapy, PARP inhibitors, folate receptor alpha inhibitors, or immunotherapy (checkpoint inhibitors, adoptive T cells, oncolytic viruses) or epigenetics methods like using hypomethylating agents.

Bevacizumab has the ability to bind to all isoforms of vascular endothelial growth factor A (VEGF-A). In this way, activation of the VEGF signaling pathway is blocked, limiting the formation of new vessels in the tumor, which prevents further tumor growth. At the same time, bevacizumab reduces tumor vascular permeability and interstitial fluid pressure (IFP), resulting in greater drug convection within the tumor. Both mechanisms of action of bevacizumab limit local tumor progression and metastasis.

Author contributions

MS: Conceptualization, Data curation, Project administration, Writing–original draft, Writing–review and editing. KK: Conceptualization, Funding acquisition, Supervision, Writing–review and editing. BZ: Data curation, Visualization, Writing–original draft, Writing–review and editing. AG: Data curation, Visualization, Writing–review and editing. PS-S: Writing–review and editing. RT: Funding acquisition, Supervision, Writing–review and editing.

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References

Ab, O., Whiteman, K. R., Bartle, L. M., Sun, X., Singh, R., Tavares, D., et al. (2015). IMGN853, a folate receptor-α (FRα)–Targeting antibody–drug conjugate, exhibits potent targeted antitumor activity against fra-expressing tumors. *Mol. Cancer Ther.* 14 (7), 1605–1613. doi:10.1158/1535-7163.MCT-14-1095

AlHilli, M. M., Becker, M. A., Weroha, S. J., Flatten, K. S., Hurley, R. M., Harrell, M. I., et al. (2016). *In vivo* anti-tumor activity of the PARP inhibitor niraparib in homologous recombination deficient and proficient ovarian carcinoma. *Gynecol. Oncol.* 143 (2), 379–388. doi:10.1016/j.ygyno.2016.08.328

Andersen, R., Donia, M., Westergaard, M. C. W., Pedersen, M., Hansen, M., and Svane, I. M. (2015). Tumor infiltrating lymphocyte therapy for ovarian cancer and renal cell carcinoma. *Hum. Vaccines Immunother.* 11 (12), 2790–2795. doi:10.1080/21645515.2015.1075106

Arend, R. C., Jackson-Fisher, A., Jacobs, I. A., Chou, J., and Monk, B. J. (2021). Ovarian cancer: new strategies and emerging targets for the treatment of patients with advanced disease. *Cancer Biol. Ther.* 22 (2), 89–105. doi:10.1080/15384047.2020.1868937

Armstrong, D. K., Alvarez, R. D., Bakkum-Gamez, J. N., Barroilhet, L., Behbakht, K., Berchuck, A., et al. (2021). Ovarian cancer, version 2.2020, NCCN clinical practice guidelines in oncology. *J. Natl. Compr. Canc Netw.* 19 (2), 191–226. doi:10.6004/jnccn.2021.0007

Armstrong, D. K., White, A. J., Weil, S. C., Phillips, M., and Coleman, R. L. (2013). Farletuzumab (a monoclonal antibody against folate receptor alpha) in relapsed platinum-sensitive ovarian cancer. *Gynecol. Oncol.* 129 (3), 452–458. doi:10.1016/j.ygyno.2013.03.002

Arora, S., Balasubramaniam, S., Zhang, H., Berman, T., Narayan, P., Suzman, D., et al. (2021). FDA approval summary: olaparib monotherapy or in combination with bevacizumab for the maintenance treatment of patients with advanced ovarian cancer. *Oncol.* 26 (1), e164–e172. doi:10.1002/onco.13551

Batchelor, T. T., Duda, D. G., Di Tomaso, E., Ancukiewicz, M., Plotkin, S. R., Gerstner, E., et al. (2010). Phase II study of cediranib, an oral Pan–vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. *J. Clin. Oncol.* 28 (17), 2817–2823. doi:10.1200/JCO.2009.26.3988

Bryant, H. E., Schultz, N., Thomas, H. D., Parker, K. M., Flower, D., Lopez, E., et al. (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434 (7035), 913–917. doi:10.1038/nature03443

Bueno, R., Appasani, K., Mercer, H., Lester, S., and Sugarbaker, D. (2001). The alpha folate receptor is highly activated in malignant pleural mesothelioma. *J. Thorac. Cardiovasc Surg.* 121 (2), 225–233. doi:10.1067/mtc.2001.111176

Burger, R. A., Brady, M. F., Bookman, M. A., Fleming, G. F., Monk, B. J., Huang, H., et al. (2011). Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N. Engl. J. Med.* 365 (26), 2473–2483. doi:10.1056/NEJMoa1104390

Carmeliet, P., and Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature* 407 (6801), 249–257. doi:10.1038/35025220

Caruso, D., Papa, A., Tomao, S., Vici, P., Panici, P. B., and Tomao, F. (2017). Niraparib in ovarian cancer: results to date and clinical potential. *Ther. Adv. Med. Oncol.* 9 (9), 579–588. doi:10.1177/1758834017718775

Chen, S., Xie, P., Cowan, M., Huang, H., Cardenas, H., Keathley, R., et al. (2022). Epigenetic priming enhances antitumor immunity in platinum-resistant ovarian cancer. *J. Clin. Investig.* 132 (14), e158800. doi:10.1172/JCI158800

Cheung, A., Opzoomer, J., Ilieva, K. M., Gazinska, P., Hoffmann, R. M., Mirza, H., et al. (2018). Anti-folate receptor alpha–directed antibody therapies restrict the growth of triple-negative breast cancer. *Clin. Cancer Res.* 24 (20), 5098–5111. doi:10.1158/1078-0432.CCR-18-0652

Cibula, D., Rob, L., Mallmann, P., Knapp, P., Klat, J., Chovanec, J., et al. (2021). Dendritic cell-based immunotherapy (DCVAC/OvCa) combined with second-line chemotherapy in platinum-sensitive ovarian cancer (SOV02): a randomized, open-label, phase 2 trial. *Gynecol. Oncol.* 162 (3), 652–660. doi:10.1016/j.ygyno.2021.07.003

Clamp, A. R., Lorusso, D., Oza, A. M., Aghajanian, C., Oaknin, A., Dean, A., et al. (2021). Rucaparib maintenance treatment for recurrent ovarian carcinoma: the effects of progression-free interval and prior therapies on efficacy and safety in the randomized phase III trial ARIEL3. *Int. J. Gynecol. Cancer* 31 (7), 949–958. doi:10.1136/ijgc-2020-002240

Coffman, L. G., Orellana, T. J., Liu, T., Frisbie, L. G., Normolle, D., Griffith, K., et al. (2022). Phase I trial of ribociclib with platinum chemotherapy in ovarian cancer. *JCI Insight* 7 (18), e160573. doi:10.1172/jci.insight.160573

Cohn, D. E., Sill, M. W., Walker, J. L., O'Malley, D., Nagel, C. I., Rutledge, T. L., et al. (2017). Randomized phase IIB evaluation of weekly paclitaxel versus weekly paclitaxel with oncolytic reovirus (Reolysin®) in recurrent ovarian, tubal, or peritoneal cancer: an NRG Oncology/Gynecologic Oncology Group study. *Gynecol. Oncol.* 146 (3), 477–483. doi:10.1016/j.ygyno.2017.07.135

Coleman, R. L., Brady, M. F., Herzog, T. J., Sabbatini, P., Armstrong, D. K., Walker, J. L., et al. (2017a). Bevacizumab and paclitaxel–carboplatin chemotherapy and secondary cytoreduction in recurrent, platinum-sensitive ovarian cancer (NRG Oncology/Gynecologic Oncology Group study GOG-0213): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* 18 (6), 779–791. doi:10.1016/S1470-2045(17)30279-6

Coleman, R. L., Oza, A. M., Lorusso, D., Aghajanian, C., Oaknin, A., Dean, A., et al. (2017b). Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 390 (10106), 1949–1961. doi:10.1016/S0140-6736(17)32440-6

Curran, M. A., Montalvo, W., Yagita, H., and Allison, J. P. (2010). PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc. Natl. Acad. Sci.* 107 (9), 4275–4280. doi:10.1073/pnas.0915174107

DiSilvestro, P., Banerjee, S., Colombo, N., Scambia, G., Kim, B. G., Oaknin, A., et al. (2023). Overall survival with maintenance olaparib at a 7-year follow-up in patients with

newly diagnosed advanced ovarian cancer and a BRCA mutation: the SOLO1/GOG 3004 trial. *J. Clin. Oncol.* 41 (3), 609–617. doi:10.1200/JCO.22.01549

Disis, M. L., Taylor, M. H., Kelly, K., Beck, J. T., Gordon, M., Moore, K. M., et al. (2019). Efficacy and safety of avelumab for patients with recurrent or refractory ovarian cancer: phase 1b results from the JAVELIN solid tumor trial. *JAMA Oncol.* 5 (3), 393–401. doi:10.1001/jamaoncol.2018.6258

Dobrzanski, M. J., Rewers-Felkins, K. A., Samad, K. A., Quinlin, I. S., Phillips, C. A., Robinson, W., et al. (2012). Immunotherapy with IL-10- and IFN-γ-producing CD4 effector cells modulate "Natural" and "Inducible" CD4 TReg cell subpopulation levels: observations in four cases of patients with ovarian cancer. *Cancer Immunol. Immunother.* 61 (6), 839–854. doi:10.1007/s00262-011-1128-x

Drew, Y., Mulligan, E. A., Vong, W. T., Thomas, H. D., Kahn, S., Kyle, S., et al. (2011). Therapeutic potential of poly(ADP-ribose) polymerase inhibitor AG014699 in human cancers with mutated or methylated BRCA1 or BRCA2. *JNCI J. Natl. Cancer Inst.* 103 (4), 334–346. doi:10.1093/jnci/djq509

Du Bois, A., Floquet, A., Kim, J. W., Rau, J., Del Campo, J. M., Friedlander, M., et al. (2014). Incorporation of pazopanib in maintenance therapy of ovarian cancer. *J. Clin. Oncol.* 32 (30), 3374–3382. doi:10.1200/JCO.2014.55.7348

Duraiswamy, J., Kaluza, K. M., Freeman, G. J., and Coukos, G. (2013). Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. *Cancer Res.* 73 (12), 3591–3603. doi:10.1158/0008-5472.CAN-12-4100

Duska, L. R., Petroni, G. R., Varhegyi, N., Brown, J., Jelovac, D., Moore, K. N., et al. (2020). A randomized phase II evaluation of weekly gemcitabine plus pazopanib versus weekly gemcitabine alone in the treatment of persistent or recurrent epithelial ovarian, fallopian tube or primary peritoneal carcinoma. *Gynecol. Oncol.* 157 (3), 585–592. doi:10.1016/j.ygyno.2019.10.014

Ebel, W., Routhier, E. L., Foley, B., Jacob, S., McDonough, J. M., Patel, R. K., et al. (2007). Preclinical evaluation of MORAb-003, a humanized monoclonal antibody antagonizing folate receptor-alpha. *Cancer Immun.* 7, 6.

Fan, Z., Han, D., Fan, X., and Zhao, L. (2023). Ovarian cancer treatment and natural killer cell-based immunotherapy. *Front. Immunol.* 14, 1308143. doi:10.3389/fimmu.2023.1308143

Farmer, H., McCabe, N., Lord, C. J., Tutt, A. N. J., Johnson, D. A., Richardson, T. B., et al. (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434 (7035), 917–921. doi:10.1038/nature03445

fda.gov (2022). Available at: https://www.fda.gov/drugs/fda-approved-olaparib-lynparza-astrazeneca-pharmaceuticals-lp-maintenance-treatment-adult-patients#:%7E:text=On%20December%2019%2C%202018%2C%20the,ovarian%2C%20fallopian%20tube%20or%20primary.

Ferrara, N., Hillan, K. J., Gerber, H. P., and Novotny, W. (2004). Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat. Rev. Drug Discov.* 3 (5), 391–400. doi:10.1038/nrd1381

Ferron, G., De Rauglaudre, G., Becourt, S., Delanoy, N., Joly, F., Lortholary, A., et al. (2023). Neoadjuvant chemotherapy with or without nintedanib for advanced epithelial ovarian cancer: lessons from the GINECO double-blind randomized phase II CHIVA trial. *Gynecol. Oncol.* 170, 186–194. doi:10.1016/j.ygyno.2023.01.008

Frampton, J. E. (2015). Olaparib: a review of its use as maintenance therapy in patients with ovarian cancer. *BioDrugs* 29 (2), 143–150. doi:10.1007/s40259-015-0125-6

Friedlander, M., Rau, J., Lee, C. K., Meier, W., Lesoin, A., Kim, J. W., et al. (2018). Quality of life in patients with advanced epithelial ovarian cancer (EOC) randomized to maintenance pazopanib or placebo after first-line chemotherapy in the AGO-OVAR 16 trial. Measuring what matters—patient-centered end points in trials of maintenance therapy. *Ann. Oncol.* 29 (3), 737–743. doi:10.1093/annonc/mdx796

Galanis, E., Hartmann, L. C., Cliby, W. A., Long, H. J., Peethambaram, P. P., Barrette, B. A., et al. (2010). Phase I trial of intraperitoneal administration of an oncolytic measles virus strain engineered to express carcinoembryonic antigen for recurrent ovarian cancer. *Cancer Res.* 70 (3), 875–882. doi:10.1158/0008-5472.CAN-09-2762

Garcia, J., Hurwitz, H. I., Sandler, A. B., Miles, D., Coleman, R. L., Deurloo, R., et al. (2020). Bevacizumab (Avastin®) in cancer treatment: a review of 15 years of clinical experience and future outlook. *Cancer Treat. Rev.* 86, 102017. doi:10.1016/j.ctrv.2020.102017

Garcia Garcia, Y., De Juan Ferré, A., Mendiola, C., Barretina-Ginesta, M. P., Gaba Garcia, L., Santaballa Bertrán, A., et al. (2019). Efficacy and safety results from GEICO 1205, a randomized phase II trial of neoadjuvant chemotherapy with or without bevacizumab for advanced epithelial ovarian cancer. *Int. J. Gynecol. Cancer* 29 (6), 1050–1056. doi:10.1136/ijgc-2019-000256

Giannini, A., Di Dio, C., Di Donato, V., D'oria, O., Salerno, M. G., Capalbo, G., et al. (2023). PARP inhibitors in newly diagnosed and recurrent ovarian cancer. *Am. J. Clin. Oncol.* 46 (9), 414–419. doi:10.1097/COC.0000000000001024

Gilbert, L., Oaknin, A., Matulonis, U. A., Mantia-Smaldone, G. M., Lim, P. C., Castro, C. M., et al. (2023). Safety and efficacy of mirvetuximab soravtansine, a folate receptor alpha (FRα)-targeting antibody-drug conjugate (ADC), in combination with bevacizumab in patients with platinum-resistant ovarian cancer. *Gynecol. Oncol.* 170, 241–247. doi:10.1016/j.ygyno.2023.01.020

González-Martín, A., Pothuri, B., Vergote, I., Graybill, W., Lorusso, D., McCormick, C. C., et al. (2023). Progression-free survival and safety at 3.5 years of follow-up: results from the randomised phase 3 PRIMA/ENGOT-OV26/GOG-3012 trial of niraparib maintenance treatment in patients with newly diagnosed ovarian cancer. *Eur. J. Cancer* 189, 112908. doi:10.1016/j.ejca.2023.04.024

Goss, G. D., Arnold, A., Shepherd, F. A., Dediu, M., Ciuleanu, T. E., Fenton, D., et al. (2010). Randomized, double-blind trial of carboplatin and paclitaxel with either daily oral cediranib or placebo in advanced non–small-cell lung cancer: NCIC clinical trials group BR24 study. *J. Clin. Oncol.* 28 (1), 49–55. doi:10.1200/JCO.2009.22.9427

Hall, M. R., Dehbi, H. M., Banerjee, S., Lord, R., Clamp, A., Ledermann, J. A., et al. (2020). A phase II randomised, placebo-controlled trial of low dose (metronomic) cyclophosphamide and nintedanib (BIBF1120) in advanced ovarian, fallopian tube or primary peritoneal cancer. *Gynecol. Oncol.* 159 (3), 692–698. doi:10.1016/j.ygyno.2020.09.048

Hegde, P. S., Wallin, J. J., and Mancao, C. (2018). Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. *Semin. Cancer Biol.* 52, 117–124. doi:10.1016/j.semcancer.2017.12.002

Heo, Y. A. (2023). Mirvetuximab soravtansine: first approval. *Drugs* 83 (3), 265–273. doi:10.1007/s40265-023-01834-3

Heo, Y. A., and Dhillon, S. (2018). Olaparib tablet: a review in ovarian cancer maintenance therapy. *Target Oncol.* 13 (6), 801–808. doi:10.1007/s11523-018-0606-x

Herzog, T. J., Kutarska, E., Bidziński, M., Symanowski, J., Nguyen, B., Rangwala, R. A., et al. (2016). Adverse event profile by folate receptor status for vintafolide and pegylated liposomal doxorubicin in combination, versus pegylated liposomal doxorubicin alone, in platinum-resistant ovarian cancer: exploratory analysis of the phase II PRECEDENT trial. *Int. J. Gynecol. Cancer* 26 (9), 1580–1585. doi:10.1097/IGC.0000000000000806

Herzog, T. J., Pignata, S., Ghamande, S. A., Rubio, M. J., Fujiwara, K., Vulsteke, C., et al. (2023). Randomized phase II trial of farletuzumab plus chemotherapy versus placebo plus chemotherapy in low CA-125 platinum-sensitive ovarian cancer. *Gynecol. Oncol.* 170, 300–308. doi:10.1016/j.ygyno.2023.01.003

Höhn, A. K., Brambs, C. E., Hiller, G. G. R., May, D., Schmoeckel, E., and Horn, L. C. (2020). 2020 WHO classification of female genital tumors. *Geburtshilfe Frauenheilkd* 81 (10), 1145–1153. doi:10.1055/a-1545-4279

Joly, F., Fabbro, M., Berton, D., Lequesne, J., Anota, A., Puszkiel, A., et al. (2022). Paclitaxel with or without pazopanib for ovarian cancer relapsing during bevacizumab maintenance therapy: the GINECO randomized phase II TAPAZ study. *Gynecol. Oncol.* 166 (3), 389–396. doi:10.1016/j.ygyno.2022.06.022

Jones, P., Wilcoxen, K., Rowley, M., and Toniatti, C. (2015). Niraparib: a poly(ADP-ribose) polymerase (PARP) inhibitor for the treatment of tumors with defective homologous recombination. *J. Med. Chem.* 58 (8), 3302–3314. doi:10.1021/jm5018237

Kalli, K. R., Oberg, A. L., Keeney, G. L., Christianson, T. J. H., Low, P. S., Knutson, K. L., et al. (2008). Folate receptor alpha as a tumor target in epithelial ovarian cancer. *Gynecol. Oncol.* 108 (3), 619–626. doi:10.1016/j.ygyno.2007.11.020

Kandalaft, L. E., Powell, D. J., and Coukos, G. (2012). A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer. *J. Transl. Med.* 10 (1), 157. doi:10.1186/1479-5876-10-157

Kaplan, A. R., Gueble, S. E., Liu, Y., Oeck, S., Kim, H., Yun, Z., et al. (2019). Cediranib suppresses homology-directed DNA repair through down-regulation of BRCA1/2 and RAD51. *Sci. Transl. Med.* 11 (492), eaav4508. doi:10.1126/scitranslmed.aav4508

Kershaw, M. H., Westwood, J. A., Parker, L. L., Wang, G., Eshhar, Z., Mavroukakis, S. A., et al. (2006). A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin. Cancer Res.* 12 (20), 6106–6115. doi:10.1158/1078-0432.CCR-06-1183

Keskin, D. B., Anandappa, A. J., Sun, J., Tirosh, I., Mathewson, N. D., Li, S., et al. (2019). Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 565 (7738), 234–239. doi:10.1038/s41586-018-0792-9

Khalique, S., and Banerjee, S. (2017). Nintedanib in ovarian cancer. *Expert Opin. Investig. Drugs* 26 (9), 1073–1081. doi:10.1080/13543784.2017.1353599

Konner, J. A., Bell-McGuinn, K. M., Sabbatini, P., Hensley, M. L., Tew, W. P., Pandit-Taskar, N., et al. (2010). Farletuzumab, a humanized monoclonal antibody against folate receptor alpha, in epithelial ovarian cancer: a phase I study. *Clin. Cancer Res.* 16 (21), 5288–5295. doi:10.1158/1078-0432.CCR-10-0700

Konstantinopoulos, P. A., Cheng, S. C., Wahner Hendrickson, A. E., Penson, R. T., Schumer, S. T., Doyle, L. A., et al. (2020). Berzosertib plus gemcitabine versus gemcitabine alone in platinum-resistant high-grade serous ovarian cancer: a multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol.* 21 (7), 957–968. doi:10.1016/S1470-2045(20)30180-7

Kovtun, Y. V., Audette, C. A., Ye, Y., Xie, H., Ruberti, M. F., Phinney, S. J., et al. (2006). Antibody-drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen. *Cancer Res.* 66 (6), 3214–3221. doi:10.1158/0008-5472.CAN-05-3973

Kristeleit, R., Lisyanskaya, A., Fedenko, A., Dvorkin, M., De Melo, A. C., Shparyk, Y., et al. (2022). Rucaparib versus standard-of-care chemotherapy in patients with relapsed ovarian cancer and a deleterious BRCA1 or BRCA2 mutation (ARIEL4): an international, open-label, randomised, phase 3 trial. *Lancet Oncol.* 23 (4), 465–478. doi:10.1016/S1470-2045(22)00122-X

Ledermann, J., Harter, P., Gourley, C., Friedlander, M., Vergote, I., Rustin, G., et al. (2014). Olaparib maintenance therapy in patients with platinum-sensitive relapsed

serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol.* 15 (8), 852–861. doi:10.1016/S1470-2045(14)70228-1

Ledermann, J. A., Canevari, S., and Thigpen, T. (2015). Targeting the folate receptor: diagnostic and therapeutic approaches to personalize cancer treatments. *Ann. Oncol.* 26 (10), 2034–2043. doi:10.1093/annonc/mdv250

Ledermann, J. A., Embleton-Thirsk, A. C., Perren, T. J., Jayson, G. C., Rustin, G. J. S., Kaye, S. B., et al. (2021). Cediranib in addition to chemotherapy for women with relapsed platinum-sensitive ovarian cancer (ICON6): overall survival results of a phase III randomised trial. *ESMO Open* 6 (2), 100043. doi:10.1016/j.esmoop.2020.100043

Lee, E. K., Xiong, N., Cheng, S. C., Barry, W. T., Penson, R. T., Konstantinopoulos, P. A., et al. (2020). Combined pembrolizumab and pegylated liposomal doxorubicin in platinum resistant ovarian cancer: a phase 2 clinical trial. *Gynecol. Oncol.* 159 (1), 72–78. doi:10.1016/j.ygyno.2020.07.028

Li, N., Zhu, J., Yin, R., Wang, J., Pan, L., Kong, B., et al. (2023). Treatment with niraparib maintenance therapy in patients with newly diagnosed advanced ovarian cancer: a phase 3 randomized clinical trial. *JAMA Oncol.* 9 (9), 1230–1237. doi:10.1001/jamaoncol.2023.2283

Li, Y. L., Zhao, H., Ren, X. B., Li, Y. L., Zhao, H., and Ren, X. B. (2016). Relationship of VEGF/VEGFR with immune and cancer cells: staggering or forward? *Cancer Biol. Med.* 13 (2), 206–214. doi:10.20892/j.issn.2095-3941.2015.0070

Liu, J. F., Barry, W. T., Birrer, M., Lee, J. M., Buckanovich, R. J., Fleming, G. F., et al. (2019). Overall survival and updated progression-free survival outcomes in a randomized phase II study of combination cediranib and olaparib versus olaparib in relapsed platinum-sensitive ovarian cancer. *Ann. Oncol.* 30 (4), 551–557. doi:10.1093/annonc/mdz018

Liu, J. F., Konstantinopoulos, P. A., and Matulonis, U. A. (2014). PARP inhibitors in ovarian cancer: current status and future promise. *Gynecol. Oncol.* 133 (2), 362–369. doi:10.1016/j.ygyno.2014.02.039

LoRusso, P. M., Edelman, M. J., Bever, S. L., Forman, K. M., Pilat, M., Quinn, M. F., et al. (2012). Phase I study of folate conjugate EC145 (vintafolide) in patients with refractory solid tumors. *J. Clin. Oncol.* 30 (32), 4011–4016. doi:10.1200/JCO.2011.41.4946

Madeddu, C., Gramignano, G., Kotsonis, P., Coghe, F., Atzeni, V., Scartozzi, M., et al. (2018). Microenvironmental M1 tumor-associated macrophage polarization influences cancer-related anemia in advanced ovarian cancer: key role of interleukin-6. *Haematologica* 103 (9), e388–e391. doi:10.3324/haematol.2018.191551

Markert, S., Lassmann, S., Gabriel, B., Klar, M., Werner, M., Gitsch, G., et al. (2008). Alpha-folate receptor expression in epithelial ovarian carcinoma and non-neoplastic ovarian tissue. *Anticancer Res.* 28 (6A), 3567–3572.

Matulonis, U. A., Berlin, S., Ivy, P., Tyburski, K., Krasner, C., Zarwan, C., et al. (2009). Cediranib, an oral inhibitor of vascular endothelial growth factor receptor kinases, is an active drug in recurrent epithelial ovarian, fallopian tube, and peritoneal cancer. *J. Clin. Oncol.* 27 (33), 5601–5606. doi:10.1200/JCO.2009.23.2777

Matulonis, U. A., Lorusso, D., Oaknin, A., Pignata, S., Dean, A., Denys, H., et al. (2023). Efficacy and safety of mirvetuximab soravtansine in patients with platinum-resistant ovarian cancer with high folate receptor alpha expression: results from the SORAYA study. *J. Clin. Oncol.* 41 (13), 2436–2445. doi:10.1200/JCO.22.01900

Meric-Bernstam, F., Makker, V., Oaknin, A., Oh, D. Y., Banerjee, S., González-Martín, A., et al. (2024). Efficacy and safety of trastuzumab deruxtecan in patients with HER2-expressing solid tumors: primary results from the DESTINY-PanTumor02 phase II trial. *J. Clin. Oncol.* 42 (1), 47–58. doi:10.1200/JCO.23.02005

Mirza, M. R., Monk, B. J., Herrstedt, J., Oza, A. M., Mahner, S., Redondo, A., et al. (2016). Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N. Engl. J. Med.* 375 (22), 2154–2164. doi:10.1056/NEJMoa1611310

Monk, B. J., Colombo, N., Oza, A. M., Fujiwara, K., Birrer, M. J., Randall, L., et al. (2021). Chemotherapy with or without avelumab followed by avelumab maintenance versus chemotherapy alone in patients with previously untreated epithelial ovarian cancer (JAVELIN Ovarian 100): an open-label, randomised, phase 3 trial. *Lancet Oncol.* 22 (9), 1275–1289. doi:10.1016/S1470-2045(21)00342-9

Monk, B. J., Parkinson, C., Lim, M. C., O'Malley, D. M., Oaknin, A., Wilson, M. K., et al. (2022). A randomized, phase III trial to evaluate rucaparib monotherapy as maintenance treatment in patients with newly diagnosed ovarian cancer (ATHENA–MONO/GOG-3020/ENGOT-ov45). *J. Clin. Oncol.* 40 (34), 3952–3964. doi:10.1200/JCO.22.01003

Moore, K. N., Borghaei, H., O'Malley, D. M., Jeong, W., Seward, S. M., Bauer, T. M., et al. (2017). Phase 1 dose-escalation study of mirvetuximab soravtansine (IMGN853), a folate receptor α-targeting antibody-drug conjugate, in patients with solid tumors. *Cancer* 123 (16), 3080–3087. doi:10.1002/cncr.30736

Moore, K. N., Oza, A. M., Colombo, N., Oaknin, A., Scambia, G., Lorusso, D., et al. (2021). Phase III, randomized trial of mirvetuximab soravtansine versus chemotherapy in patients with platinum-resistant ovarian cancer: primary analysis of FORWARD I. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 32 (6), 757–765. doi:10.1016/j.annonc.2021.02.017

Moore, K. N., Vergote, I., Oaknin, A., Colombo, N., Banerjee, S., Oza, A., et al. (2018). FORWARD I: a Phase III study of mirvetuximab soravtansine versus chemotherapy in platinum-resistant ovarian cancer. *Future Oncol. Lond Engl.* 14 (17), 1669–1678. doi:10.2217/fon-2017-0646

Moreno, V., Barretina-Ginesta, M. P., García-Donas, J., Jayson, G. C., Roxburgh, P., Vázquez, R. M., et al. (2021). Safety and efficacy of the tumor-selective adenovirus enadenotucirev with or without paclitaxel in platinum-resistant ovarian cancer: a phase 1 clinical trial. *J. Immunother. Cancer* 9 (12), e003645. doi:10.1136/jitc-2021-003645

Morgan, R. D., Banerjee, S., Hall, M., Clamp, A. R., Zhou, C., Hasan, J., et al. (2020). Pazopanib and Fosbretabulin in recurrent ovarian cancer (PAZOFOS): a multi-centre, phase 1b and open-label, randomised phase 2 trial. *Gynecol. Oncol.* 156 (3), 545–551. doi:10.1016/j.ygyno.2020.01.005

Mulders, P., Hawkins, R., Nathan, P., De Jong, I., Osanto, S., Porfiri, E., et al. (2012). Cediranib monotherapy in patients with advanced renal cell carcinoma: results of a randomised phase II study. *Eur. J. Cancer* 48 (4), 527–537. doi:10.1016/j.ejca.2011.12.022

Musacchio, L., Salutari, V., Pignata, S., Braicu, E., Cibula, D., Colombo, N., et al. (2021). Randomized phase III trial on niraparib-TSR-042 (dostarlimab) versus physician's choice chemotherapy in recurrent ovarian, fallopian tube, or primary peritoneal cancer patients not candidate for platinum retreatment: NItCHE trial (MITO 33). *Int. J. Gynecol. Cancer* 31 (10), 1369–1373. doi:10.1136/ijgc-2021-002593

Nakai, H., and Matsumura, N. (2022). The roles and limitations of bevacizumab in the treatment of ovarian cancer. *Int. J. Clin. Oncol.* 27 (7), 1120–1126. doi:10.1007/s10147-022-02169-x

Naumann, R. W., Coleman, R. L., Burger, R. A., Sausville, E. A., Kutarska, E., Ghamande, S. A., et al. (2013). PRECEDENT: a randomized phase II trial comparing vintafolide (EC145) and pegylated liposomal doxorubicin (PLD) in combination versus PLD alone in patients with platinum-resistant ovarian cancer. *J. Clin. Oncol.* 31 (35), 4400–4406. doi:10.1200/JCO.2013.49.7685

O Malley, D. M., Krivak, T. C., Kabil, N., Munley, J., and Moore, K. N. (2023). PARP inhibitors in ovarian cancer: a review. *Target Oncol.* 18 (4), 471–503. doi:10.1007/s11523-023-00970-w

O Malley, D. M., Matulonis, U. A., Birrer, M. J., Castro, C. M., Gilbert, L., Vergote, I., et al. (2020). Phase Ib study of mirvetuximab soravtansine, a folate receptor alpha (FRα)-targeting antibody-drug conjugate (ADC), in combination with bevacizumab in patients with platinum-resistant ovarian cancer. *Gynecol. Oncol.* 157 (2), 379–385. doi:10.1016/j.ygyno.2020.01.037

Omar, N., Yan, B., and Salto-Tellez, M. (2015). HER2: an emerging biomarker in non-breast and non-gastric cancers. *Pathogenesis* 2 (3), 1–9. doi:10.1016/j.pathog.2015.05.002

Oroudjev, E., Lopus, M., Wilson, L., Audette, C., Provenzano, C., Erickson, H., et al. (2010). Maytansinoid-antibody conjugates induce mitotic arrest by suppressing microtubule dynamic instability. *Mol. Cancer Ther.* 9 (10), 2700–2713. doi:10.1158/1535-7163.MCT-10-0645

O Shannessy, D. J., Somers, E. B., Palmer, L. M., Thiel, R. P., Oberoi, P., Heath, R., et al. (2013). Serum folate receptor alpha, mesothelin and megakaryocyte potentiating factor in ovarian cancer: association to disease stage and grade and comparison to CA125 and HE4. *J. Ovarian Res.* 6 (1), 29. doi:10.1186/1757-2215-6-29

Oza, A. M., Cook, A. D., Pfisterer, J., Embleton, A., Ledermann, J. A., Pujade-Lauraine, E., et al. (2015). Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. *Lancet Oncol.* 16 (8), 928–936. doi:10.1016/S1470-2045(15)00086-8

Papadatos-Pastos, D., Yuan, W., Pal, A., Crespo, M., Ferreira, A., Gurel, B., et al. (2022). Phase 1, dose-escalation study of guadecitabine (SGI-110) in combination with pembrolizumab in patients with solid tumors. *J. Immunother. Cancer* 10 (6), e004495. doi:10.1136/jitc-2022-004495

Park, J., Lee, J. B., Lim, M. C., Kim, B. G., Kim, J. W., Kim, S., et al. (2023). Phase II study of durvalumab and tremelimumab with front-line neoadjuvant chemotherapy in patients with advanced-stage ovarian cancer: primary analysis in the original cohort of KGOG3046/TRU-D. *J. Immunother. Cancer* 11 (10), e007444. doi:10.1136/jitc-2023-007444

Parker, N., Turk, M. J., Westrick, E., Lewis, J. D., Low, P. S., and Leamon, C. P. (2005). Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal. Biochem.* 338 (2), 284–293. doi:10.1016/j.ab.2004.12.026

PedBrain, ICGC, Alexandrov, L. B., Nik-Zainal, S., Aparicio, S. A. J. R., Behjati, S., Biankin, A. V., et al. (2013). Signatures of mutational processes in human cancer. *Nature* 500 (7463), 415–421. doi:10.1038/nature12477

Pfirschke, C., Engblom, C., Rickelt, S., Cortez-Retamozo, V., Garris, C., Pucci, F., et al. (2016). Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. *Immunity* 44 (2), 343–354. doi:10.1016/j.immuni.2015.11.024

Pfisterer, J., Joly, F., Kristensen, G., Rau, J., Mahner, S., Pautier, P., et al. (2023). Optimal treatment duration of bevacizumab as front-line therapy for advanced ovarian cancer: AGO-OVAR 17 BOOST/GINECO OV118/ENGOT ov-15 open-label randomized phase III trial. *J. Clin. Oncol.* 41 (4), 893–902. doi:10.1200/JCO.22.01010

Poveda, A., Floquet, A., Ledermann, J. A., Asher, R., Penson, R. T., Oza, A. M., et al. (2021). Olaparib tablets as maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a final

analysis of a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 22 (5), 620–631. doi:10.1016/S1470-2045(21)00073-5

Pujade-Lauraine, E., Fujiwara, K., Ledermann, J. A., Oza, A. M., Kristeleit, R., Ray-Coquard, I. L., et al. (2021). Avelumab alone or in combination with chemotherapy versus chemotherapy alone in platinum-resistant or platinum-refractory ovarian cancer (JAVELIN Ovarian 200): an open-label, three-arm, randomised, phase 3 study. *Lancet Oncol.* 22 (7), 1034–1046. doi:10.1016/S1470-2045(21)00216-3

Ray Coquard, I., Cibula, D., Mirza, M. R., Reuss, A., Ricci, C., Colombo, N., et al. (2020). Final results from GCIG/ENGOT/AGO-OVAR 12, a randomised placebo-controlled phase III trial of nintedanib combined with chemotherapy for newly diagnosed advanced ovarian cancer. *Int. J. Cancer* 146 (2), 439–448. doi:10.1002/ijc.32606

Ray-Coquard, I., Leary, A., Pignata, S., Cropet, C., González-Martín, A., Marth, C., et al. (2023). Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. *Ann. Oncol.* 34 (8), 681–692. doi:10.1016/j.annonc.2023.05.005

Reddy, J. A., Dorton, R., Westrick, E., Dawson, A., Smith, T., Xu, L. C., et al. (2007). Preclinical evaluation of EC145, a folate- Vinca alkaloid conjugate. *Cancer Res.* 67 (9), 4434–4442. doi:10.1158/0008-5472.CAN-07-0033

Rubraca (2022). Rubraca (rucaparib) summary of product characteristics. Available at: https://www.ema.europa.eu/en/documents/product-information/rubraca-epar-product-information_en.pdf (Accessed September 21, 2022).

Saleh-Gohari, N., Bryant, H. E., Schultz, N., Parker, K. M., Cassel, T. N., and Helleday, T. (2005). Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks. *Mol. Cell. Biol.* 25 (16), 7158–7169. doi:10.1128/MCB.25.16.7158-7169.2005

Sato, S., and Itamochi, H. (2016). Profile of farletuzumab and its potential in the treatment of solid tumors. *OncoTargets Ther.* 1181, 1181. doi:10.2147/ott.s98242

Selby, M. J., Engelhardt, J. J., Johnston, R. J., Lu, L. S., Han, M., Thudium, K., et al. (2016). Preclinical development of ipilimumab and nivolumab combination immunotherapy: mouse tumor models, *in vitro* functional studies, and cynomolgus macaque toxicology. *PLOS ONE* 11 (9), e0161779. doi:10.1371/journal.pone.0161779

Sharma, A., Singh, M., Chauhan, R., Malik, P. S., Khurana, S., Mathur, S., et al. (2021). Pazopanib based oral metronomic therapy for platinum resistant/refractory epithelial ovarian cancer: a phase II, open label, randomized, controlled trial. *Gynecol. Oncol.* 162 (2), 382–388. doi:10.1016/j.ygyno.2021.05.025

Shi, X., Li, C., Li, Y., Guan, H., Fan, C., Teng, Y., et al. (2009). Circulating lymphocyte subsets and regulatory T cells in patients with postpartum thyroiditis during the first postpartum year. *Clin. Exp. Med.* 9 (4), 263–267. doi:10.1007/s10238-009-0046-0

Slade, D. (2020). PARP and PARG inhibitors in cancer treatment. *Genes. Dev.* 34 (5–6), 360–394. doi:10.1101/gad.334516.119

Sopo, M., Anttila, M., Muukkonen, O. T., Ylä-Herttuala, S., Kosma, V. M., Keski-Nisula, L., et al. (2020). Microvessels in epithelial ovarian tumors: high microvessel density is a significant feature of malignant ovarian tumors. *Anticancer Res.* 40 (12), 6923–6931. doi:10.21873/anticanres.14716

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Syed, Y. Y. (2017). Rucaparib: first global approval. *Drugs* 77 (5), 585–592. doi:10.1007/s40265-017-0716-2

The Cancer Genome Atlas Research Network (2011). Integrated genomic analyses of ovarian carcinoma. *Nature* 474 (7353), 609–615. doi:10.1038/nature10166

Vanderstichele, A., Loverix, L., Busschaert, P., Van Nieuwenhuysen, E., Han, S. N., Concin, N., et al. (2022). Randomized CLIO/BGOG-ov10 trial of olaparib monotherapy versus physician's choice chemotherapy in relapsed ovarian cancer. *Gynecol. Oncol.* 165 (1), 14–22. doi:10.1016/j.ygyno.2022.01.034

Varga, A., Piha-Paul, S., Ott, P. A., Mehnert, J. M., Berton-Rigaud, D., Morosky, A., et al. (2019). Pembrolizumab in patients with programmed death ligand 1–positive advanced ovarian cancer: analysis of KEYNOTE-028. *Gynecol. Oncol.* 152 (2), 243–250. doi:10.1016/j.ygyno.2018.11.017

Vergote, I., Armstrong, D., Scambia, G., Teneriello, M., Sehouli, J., Schweizer, C., et al. (2016). A randomized, double-blind, placebo-controlled, phase III study to assess efficacy and safety of weekly farletuzumab in combination with carboplatin and taxane in patients with ovarian cancer in first platinum-sensitive relapse. *J. Clin. Oncol.* 34 (19), 2271–2278. doi:10.1200/JCO.2015.63.2596

Vergote, I. B., Marth, C., and Coleman, R. L. (2015). Role of the folate receptor in ovarian cancer treatment: evidence, mechanism, and clinical implications. *Cancer Metastasis Rev.* 34 (1), 41–52. doi:10.1007/s10555-014-9539-8

Wang, L., Mason, K. A., Ang, K. K., Buchholz, T., Valdecanas, D., Mathur, A., et al. (2012). MK-4827, a PARP-1/-2 inhibitor, strongly enhances response of human lung and breast cancer xenografts to radiation. *Investig. New Drugs* 30 (6), 2113–2120. doi:10.1007/s10637-011-9770-x

Wind, S., Schmid, U., Freiwald, M., Marzin, K., Lotz, R., Ebner, T., et al. (2019). Clinical pharmacokinetics and pharmacodynamics of nintedanib. *Clin. Pharmacokinet.* 58 (9), 1131–1147. doi:10.1007/s40262-019-00766-0

Wu, X. H., Zhu, J. Q., Yin, R. T., Yang, J. X., Liu, J. H., Wang, J., et al. Niraparib maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer using an individualized starting dose (NORA): a randomized, double-blind, placebo-controlled phase III trial☆. *Ann. Oncol.* 2021;32(4):512–521. doi:10.1016/j.annonc.2020.12.018

Xu, L., Kanasaki, K., Kitada, M., and Koya, D. (2012). Diabetic angiopathy and angiogenic defects. *Fibrogenes. Tissue Repair* 5 (1), 13. doi:10.1186/1755-1536-5-13

Yang, R., Kolb, E. A., Qin, J., Chou, A., Sowers, R., Hoang, B., et al. (2007). The folate receptor alpha is frequently overexpressed in osteosarcoma samples and plays a role in the uptake of the physiologic substrate 5-methyltetrahydrofolate. *Clin. Cancer Res.* 13 (9), 2557–2567. doi:10.1158/1078-0432.CCR-06-1343

Yang, Y., Lu, Z. S., and Zeng, Z. (2018). Clinical efficacy and safety of combination of abraxane and trastuzumab in treatment of recurrent ovarian cancer. *Pak J. Pharm. Sci.* 31 (6(Special)), 2831–2834.

Yap, T. A., Sandhu, S. K., Carden, C. P., and De Bono, J. S. (2011). Poly(ADP-Ribose) polymerase (PARP) inhibitors: exploiting a synthetic lethal strategy in the clinic. *CA Cancer J. Clin.* 61 (1), 31–49. doi:10.3322/caac.20095

Yubero, A., Barquín, A., Estévez, P., Pajares, B., Sánchez, L., Reche, P., et al. (2022). Rucaparib in recurrent ovarian cancer: real-world experience from the rucaparib early access programme in Spain – a GEICO study. *BMC Cancer* 22 (1), 1150. doi:10.1186/s12885-022-10191-5

Zachou, G., El-Khouly, F., and Dilley, J. (2023). Evaluation of follow-up strategies for women with epithelial ovarian cancer following completion of primary treatment. *Cochrane Database Syst. Rev.* 8. doi:10.1002/14651858.CD006119.pub4

Żak, K., Satora, M., Skrabalak, I., Tarkowski, R., Ostrowska-Leśko, M., and Bobiński, M. (2024). The potential influence of residual or recurrent disease on bevacizumab treatment efficacy in ovarian cancer: current evidence and future perspectives. *Cancers* 16 (5), 1063. doi:10.3390/cancers16051063

Zamarin, D., Burger, R. A., Sill, M. W., Powell, D. J., Lankes, H. A., Feldman, M. D., et al. (2020b). Randomized phase II trial of nivolumab versus nivolumab and ipilimumab for recurrent or persistent ovarian cancer: an NRG oncology study. *J. Clin. Oncol.* 38 (16), 1814–1823. doi:10.1200/JCO.19.02059

Zamarin, D., Walderich, S., Holland, A., Zhou, Q., Iasonos, A. E., Torrisi, J. M., et al. (2020a). Safety, immunogenicity, and clinical efficacy of durvalumab in combination with folate receptor alpha vaccine TPIV200 in patients with advanced ovarian cancer: a phase II trial. *J. Immunother. Cancer* 8 (1), e000829. doi:10.1136/jitc-2020-000829

Zaremba, T., and Curtin, N. J. (2007). PARP inhibitor development for systemic cancer targeting. *Anticancer Agents Med. Chem.* 7 (5), 515–523. doi:10.2174/187152007781668715

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., et al. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N. Engl. J. Med.* 348 (3), 203–213. doi:10.1056/NEJMoa020177

Zhou, D., Li, J., Bui, K., Learoyd, M., Berges, A., Milenkova, T., et al. (2019). Bridging olaparib capsule and tablet formulations using population pharmacokinetic meta-analysis in oncology patients. *Clin. Pharmacokinet.* 58 (5), 615–625. doi:10.1007/s40262-018-0714-x

Zitvogel, L., Galluzzi, L., Smyth, M. J., and Kroemer, G. (2013). Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. *Immunity* 39 (1), 74–88. doi:10.1016/j.immuni.2013.06.014

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Real-world TRAE association between niraparib and platinum-based chemotherapy

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Background: Pre-clinical studies showed the anti-tumor mechanisms of PARP inhibitors (PARPi) and platinum have some crossover and overlap in the DNA damage repair pathway, patients who respond to platinum-based chemotherapy are also more likely to be sensitive to PARPi. This real-world study mainly aimed to evaluate whether TRAE (treatment-related adverse event) between platinum based chemotherapy (PBC) and niraparib are also associated.

Methods: Patients received niraparib as maintenance treatment or salvage therapy for advanced ovarian cancer at the First Affiliated Hospital of Gannan Medical University from January 2020 to August 2023 were included. Survival data of niraparib treatment and adverse events occurred during the last platinum-based chemotherapy cycle before starting niraparib treatment and during niraparib treatment are documented. Fisher's exact test were used for correlation analysis.

Results: 1. 40 patients treated with niraparib were included in the analysis, including 31 patients treated with niraparib for 1st-line maintenance therapy, 6 patients for PSR (platinum-sensitive recurrence) maintenance therapy, and 3 patients for salvage therapy. The overall median follow-up time was 15.0 months (ranged from 2.2 months to 32.1 months). 2. Overall grade≥3 TRAE (40% vs 70%, p=0.012) including anemia (20% vs 45%, p=0.041) and neutrophil count decreased (17.5% vs 57.5%, p<0.001) was significantly lower during niraparib treatment compared to during chemotherapy. 3. Any grade TRAE (75% vs 100%, p=0.002) including white blood cell count decreased (47.5% vs 87.5%, p<0.001), red blood cell count decreased (57.5% vs 92.5%, p<0.001), anemia (55% vs 87.5%, p<0.001) and neutrophil count decreased (35% vs 85%, p<0.001) were also significantly lower in niraparib treatment group compared with chemotherapy group. No new safety signals were identified.

Conclusion: 1. In this real-world practice, we observed that patients with advanced ovarian cancer who experienced any grade and grade ≥3 TRAE during chemotherapy were well tolerated when treated with niraparib, particularly the incidence of any grade and grade ≥3 anemia, and neutrophil count decreased during niraparib treatment were significantly lower compared with that during chemotherapy. 2. For patients with ovarian cancer who have

experienced grade ≥3 hematological adverse reactions during prior platinum-based chemotherapy, greater attention should be paid to the monitoring and management of hematological adverse reactions during subsequent treatment with niraparib.

KEYWORDS

ovarian cancer, niraparib, chemotherapy, PARPi, platinum drugs, hematologic adverse reactions

Background

Ovarian cancer is the eighth most common cancer among females. 90% of ovarian cancers are of an epithelial cell type and comprise multiple histologic types, with various specific molecular changes, clinical behaviors, and treatment outcomes. The remaining 10% are non-epithelial ovarian cancers, which include mainly germ cell tumors, sex cord-stromal tumors, and some extremely rare tumors such as small cell carcinomas. Germ cell tumors are the most common ovarian neoplasms in women until 30 years of age and most of the patients are diagnosed with early-stage disease (60–70%) (1). In 2020, 313,959 women worldwide were newly diagnosed with ovarian cancer, and 207,252 women died from the disease (2). The incidence of ovarian cancer in China is increasing and ranks third among malignant tumors of the female reproductive system, with the highest mortality rate. Currently, there is no effective early screening strategy for ovarian cancer, and the early symptoms are often hidden (3). This is also reflected economically and cost-effective strategies for early detection and prevention of ovarian cancer have been investigated over the last decade. The cost of treatment per patient with ovarian cancer remains the highest among all cancer types. As an example, the average initial cost in the first year can amount to around USD 80,000, whereas the final year cost may increase to USD 100,000 (4). Type I epithelial ovarian cancers are suggested to be relatively indolent and genetically stable tumors that typically arise from recognizable precursor lesions, such as endometriosis or borderline tumors with low malignant potential. In contrast, type II epithelial ovarian cancers are proposed to be biologically aggressive tumors from their outset, with a propensity for metastasis from small-volume primary lesions. High-grade serous – the most common type of epithelial ovarian cancers, accounting for approximately 75% of epithelial ovarian cancers – develop according to the type II pathway and present p53 and BRCA mutations (5).the p53 genes involved in DNA repair, the cell cycle, and apoptosis upon irreparable DNA damage (6). The DNA double-strand breaks are repaired by the homologous recombination repair pathway, which is an error-free process requiring a homologous DNA template to function (6). BRCA1, BRCA2, and various other homologous recombination proteins are responsible for the repair of DNA damage that maintains genomic stability and promotes cell survival and replication. Ovarian cancers with BRCA1 alterations (germline and somatic mutations in 12% of cases, DNA hypermethylation in 11% of cases) and BRCA2 alterations (germline and somatic mutations in 11% of cases) (7), are associated with homologous recombination deficiency (HRD). The finding that HRD contributes to approximately 50% of HGSOCs provided a rationale for using cytotoxic platinum-based chemotherapy and exploring the activity of poly (ADPribose) polymerase (PARP) inhibitors in HGSOC (7).Approximately 70% of ovarian cancer patients are diagnosed at an advanced stage, and about 80% of those with advanced stage experience recurrence within 3 years after chemotherapy remission. As the number of treatment lines increases, the platinum-free interval becomes shorter, ultimately leading to platinum resistance. The 5-year survival rate is only 15%-25% (6, 8). As proteomics continues to be studied, such as mass spectrometry and protein array analysis, which have advanced the dissection of the underlying molecular signaling events and the proteomic characterization of ovarian cancer (9). While the over or under expression of certain proteins may indicate reduced sensitivity to chemotherapy, emerging evidence shows that targeted treatment against the pathways conferring resistance may help to overcome it (10).The Cancer Genome Atlas and the International Cancer Genome Consortium have sequenced thousands of ovarian tumor specimens, which has resulted in the identification of novel genomic sequences that could be targets for therapeutic interventions (11), poly (ADP-ribose) polymerase (PARP) inhibitors are one of the two drugs with the best evidence for FDA approval for the treatment of ovarian cancer (12).In recent years, targeted therapy research has advanced, shifting the treatment approach for epithelial ovarian cancer from the traditional 'tumor cytoreductive surgery + platinum-based chemotherapy' mode to a 'tumor cytoreductive surgery + platinum-based chemotherapy + long-term disease management mode of maintenance therapy'. PARP inhibitors have emerged as an important means of maintaining ovarian cancer. However, there is currently a lack of data on the correlation between real-world niraparib use and hematologic adverse reactions (TRAE) that occur during platinum-based chemotherapy in ovarian cancer patients. Therefore, our study aims to analyze the real-world association between niraparib and TRAE during platinum-based chemotherapy.

Methods

Study population

Patients with ovarian cancer who received platinum-based chemotherapy and niraparib successively in the First Affiliated Hospital of Gannan Medical University from January 2020 to May 2023 were enrolled. Including patients with newly treated advanced epithelial ovarian cancer who achieved complete response (CR) or partial response(PR) after platinum-based chemotherapy, patients with platinum-sensitive recurrent ovarian cancer who achieved CR or PR after platinum-based chemotherapy, ovarian cancer achieves CR or PR or stable disease(SD) to multiple lines(≥2 lines and platinum resistance)of platinum-based chemotherapy. Follow-up ended on August 31, 2023. Baseline data of the patients were collected, including the patient's age, weight, family history, clinical stage of the International Federation of Obstetrics and Gynecology (FIGO), pathological type, presence of other comorbidities before chemotherapy, Eastern Cooperative Oncology Group (ECOG) score, Frontline platinum-based chemotherapy cycles,number of front-line chemotherapy lines, The last line of chemotherapy regimen prior to treatment with niraparib. The hematologic adverse reactions during chemotherapy, ≥ grade 3 adverse reactions during chemotherapy, the end time of the last platinum-containing chemotherapy, and the response of previous first-line platinum-based chemotherapy based on RECIST1.1 assessment. Additionally, the baseline number of platelets and CA125 before niraparib treatment, BRCA mutation status, adjuvant therapy, and follow-up after the use of niraparib were documented.

Group standard

Inclusion Criteria: (1) Patients aged ≥18 years and signed an informed consent form related to participation in the study;

(2) Epithelial ovarian, primary peritoneal, or fallopian tube cancer (collectively referred to as ovarian cancer) diagnosed by histological pathology; there is no restriction on whether the patient carries a germline BRCA mutation;

(3) Patients with primary advanced epithelial ovarian cancer who have achieved clinical complete or partial remission as assessed according to RECIST v1.1 with platinum-containing chemotherapy, patients with platinum-sensitive recurrent ovarian cancer who have achieved complete or partial remission with platinum-containing chemotherapy, and patients with ovarian cancer who have achieved complete or partial remission or stable disease with multiple lines of platinum-containing chemotherapy;

(4) No prior treatment with PARP inhibitors and treatment with niraparib for at least 28 days;

Exclusion criteria: (1) ovarian cancer patients under the age of 18;

(2)Use niraparib for <28 days;

(3) Patients with ovarian cancer with histologically confirmed malignant tumors of other origins.

Assessments

Complete response and partial response and stable disease were assessed according to RECIST1.1.

Adverse reactions were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Treatment with niraparib for 28 days as a cycle.

Throughout the treatment period, It is recommended that complete blood counts in niraparib-treated ovarian cancer should be monitored weekly for the first month and monthly thereafter. In case of suspension of treatment due to Grade 3 or 4 hematological adverse events, complete blood counts should be monitored weekly after resumption of the drug until they return to normal levels (13).

Most of the adverse events in patients treated with niraparib occurred in the first 3 months, the incidence of adverse events decreased significantly after 3 months (14). Therefore, all patients in this study were followed up for more than 3 months.

The duration of follow-up was from initiation of niraparib to disease progression or permanent discontinuation or data collection cut-off.

Dosing regimen

The retrospective RADAR analysis of the NOVA trial found that patients with a baseline bodyweight <77 kg or platelet count <150,000/microliter received an average reduced niraparib dose of 200 mg/day, without compromising treatment efficacy (15). Subsequently, the PRIMA trial modified the dosing approach to use an individualized niraparib starting dose (ISD) based on a patient's baseline weight and platelet count base and showed an improved safety profile in 35% of patients who received an ISD compared with 65% who received a fixed starting dose (16).The initial dose of niraparib is administered on an individualized basis. The initial dose is based on basal body weight and platelet count. Patients with a basal weight ≥ 77 kg and a basal platelet count ≥ 150,000/microliter should take 300 mg/d daily, and patients with a basal weight < 77 kg and/or basal platelet count < 150,000/microliter should take 200 mg/d.

Dose reductions were allowed for drug-related adverse effects (300 to 200 mg or 100 mg; 200 mg to 100 mg) or drug interruption. For the management of adverse reactions, refer to the Chinese expert consensus on the management of adverse reactions to PARP inhibitors.

1.Generic management process for hematological adverse reactions

(1) Platelets <100.0 x 10^9/L

First occurrence: suspend niraparib for up to 28d while observing blood counts weekly until platelets return to ≥100.0 x 10^9/L. Restart treatment with the original dose of niraparib or reduce according to the protocol. If platelets are <75.0 × 10^9/L, the dose must be reduced on resumption of dosing.

Second occurrence: suspension of niraparib for up to 28d with weekly observation of blood counts until platelets return to ≥100.0 x 109/L Dose must be reduced on resumption of dosing; discontinue

dosing if platelets do not return to acceptable levels within 28d of consecutive stoppages or if dosage has been reduced to the lowest possible level (100 mg/d).

(2) Neutrophils <1.0 x 10^9/L

Suspend niraparib for up to 28d while observing blood counts weekly until neutrophils return to ≥1.5 × 10^9/L. Dosage must be reduced upon resumption. Discontinue dosing if neutrophils do not return to acceptable levels within 28d of continuous discontinuation or if the dose has been reduced to the lowest possible level (100 mg/d).

(3) Hb <80g/L

Suspend niraparib for up to 28d while observing blood counts weekly until Hb returns to ≥90g/L. Dosage must be reduced upon resumption. Discontinue if Hb does not return to acceptable levels within 28 d of continuous discontinuation or if the dose has been reduced to the lowest possible level (100 mg/d).

(4) The occurrence of an adverse reaction of a lower grade than the one corresponding to the appeal should first be observed or treated symptomatically, and treatment with niraparib should be continued.

(5) Decrease in white blood cell count: the treatment is not clearly defined, and needs to be combined with the neutropenia and the patient's symptoms and other comprehensive decisions.

(6) Definite diagnosis of myelodysplastic syndrome or acute myeloid leukemia

Permanent withdrawal.

2. Generic management process for non-hematological adverse reactions

(1) Grade 1: Continue niraparib therapy and symptomatic management as necessary.

(2) Grade 2: Continue niraparib therapy; consider interrupting therapy if adverse effects are not controlled with symptomatic or prophylactic therapy.

(3) Grade 3–4: Suspend niraparib therapy until it is reduced below Grade 1; if the adverse reaction is nausea, vomiting, or diarrhea, therapy may be continued under pharmacological control; if treatment is interrupted due to an adverse reaction, a reduction in dosage should be considered upon resumption of therapy (especially after a second interruption of dosing due to the same adverse reaction); if Grade 3/4 toxicity persists for more than 28 d after a reduction in the lowest effective therapeutic dose has been made, discontinue the niraparib therapy.

BRCA detection

The detection technology is target region capture + high-throughput sequencing. Detection of exon coding region and exon-intron junction region +/-20bp region of BRCA1/BRCA2 gene.

Statistical methods

SPSS 29.0 software was used for statistical analysis, frequency and percentage descriptions were used for count data, and Fisher exact test were used for correlation analysis.

Results

Patient characteristics

A total of 40 patients diagnosed with ovarian cancer and treated with platinum-based chemotherapy and niraparib were included in this study. The median follow-up time after starting niraparib treatment was 15.0 months (range: 2.2–32.1 months). The median age of the patients was 56 years (range: 24–75 years). There were 6 cases (15%), 31 cases (77.5%) and 3 cases (7.5%) of FIGO stage II, III and IV, respectively. Most patients had serous carcinoma (32 (80%)) and endometrioid carcinoma (4 (10%)). All the patients weighed less than 77KG and 11 of them had a platelet count less than 150*10^9/L. Twenty-eight individuals underwent BRCA testing, including 5 with BRCA1/2 mutations and 23 with BRCA wild-type. The baseline data of the patients are shown in Table 1.

Safety

Forty patients were included in the analysis. There were 40 cases (100%) and 30 cases (75%) of hematologic adverse reactions of any grade during platinum-based chemotherapy and niraparib treatment, including 35 cases (87.5%) and 19 cases (47.5%) of leucopenia, 37 cases (92.5%) and 23 cases (57.5%) of erythropenia, respectively. The incidences of anemia, thrombocytopenia and neutropenia were 35(87.5%) vs 22 (55%), 16(40%) vs 12 (30%), 34(85%) vs 14 (35%).The P values were 0.012, 0.065, 0.625, 0.041, 1.000 and <0.001, respectively. In the two periods, any grade of hematological adverse reactions including leukopenia, erythropenia, anemia and neutropenia were statistically significant. There were 28 cases (70%) and 16 cases (40%) with grade≥ 3 hematological adverse reactions, including 12 cases (30%) and 5 cases (12.5%) with grade ≥3 leukopenia, and 1 case (2.5%) and 3 cases (7.5%) with grade ≥3 erythropenia, respectively. Grade ≥ 3 anemia occurred in 18 cases (45%) versus 8 cases (20%), grade ≥3 thrombocytopenia in 6 cases (15%) versus 5 cases (12.5%), grade ≥3 neutropenia in 23 cases (57.5%) versus 7 cases (17.5%), P values were: 0.012, 0.065, 0.625, 0.041, 1.000, < 0.001. Grade ≥3 hematological adverse reactions including anemia and neutropenia in the two periods were statistically significant. There were 10 cases (25%) and 7 cases (17.5%) of severe hematologic toxicity (grade≥ 4), respectively (p = 0.549). The data are presented in Table 2.

During the use of platinum-based chemotherapy, 40 patients had hematologic adverse effects of any grade and 28 patients had hematologic adverse effects of grade ≥3. During niraparib maintenance therapy, adverse events of any grade occurred in 38 patients (95%), and bone marrow suppression of any grade occurred in 30 patients, of whom 16 patients had grade≥3 bone marrow suppression. There were 28 cases (70%) of non-hematologic adverse reactions, all of which were grade 1–2, and the most common adverse reactions were fatigue, nausea and Vomiting. No new safety signals were found. The data are presented in Table 3.

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TABLE 1 Baseline characteristics in 40 patients.

Characteristic	Number of patients (percent)	Characteristic	Number of patients (percent)
Median age years(range)	56(24–75)	**Front line chemotherapy cycles**	
≤59	25(62.5)	≤5	5(12.5)
>59	15(37.5)	6–9	34(85)
Median baseline CA125(range)	10.02 (2.15–59.20)	10	1(2.5)
Baseline body weight		**Clinical response after platinum-based chemotherapy**	
≥77kg	0	Complete response	36(90)
<77kg	40(100)	Partial response	2(5)
International FIGO stage		Stable disease	2(5)
II	6(15)	**Platelet count**	
III	31(77.5)	≥150*10^9/L	29(72.5)
IV	3(7.5)	<150*10^9/L	11(27.5)
Presence of other comorbidities		**BRCA status**	
Yes	38(95)	BRCA1 mutation	2(5)
No	2(5)	BRCA2 mutation	3(7.5)
ECOG score		BRCA wild-type	23(57.5)
0	39(97.5)	BRCA unknown	12(30)
1	1(2.5)	**Histological type**	
2	0	Serous	32(80)
Surgical outcome		Endometrioid	4(10)
R0	37(92.5)	Other	4(10)
R1	1(2.5)	**Prior use of bevacizumab**	
No surgical	2(5)	Yes	7(17.5)
Type of surgery		No	33(82.5)
NACT+IDS	21	**Niraparib time was used**	
Comprehensive staged surgery	17	<3 months	2(5)
No surgical	2	≥3 months	38(95)
Prior lines of chemotherapy		**Platinum type at the time of frontline chemotherapy**	
1	31(77.5)	carboplatin	34(85)
>1	9(22.5)	cis-platinum+carboplatin	3(7.5)
		Oxaliplatin+carboplatin	3(7.5)

Values are reported as frequency (n [%]) or as mean (range).

TABLE 2 TRAE.

TRAE	PBC	Niraparib	p value
	no. of patients(%)		
Any*	40 (100)	30 (75)	0.002
Grade ≥3*	28 (70)	16 (40)	0.012
Serious*	10 (25)	7 (17.5)	0.549
Any grade white blood cell count decreased	35 (87.5)	19 (47.5)	<0.001
Grade ≥3 white blood cell count decreased	12 (30)	5 (12.5)	0.065
Any grade red blood cell count decreased	37 (92.5)	23 (57.5)	<0.001
Grade ≥3 red blood cell count decreased	1 (2.5)	3 (7.5)	0.625
Any grade anemia	35 (87.5)	22 (55)	<0.001
Grade ≥3 anemia	18 (45)	8 (20)	0.041
Any grade platelet count decreased	16 (40)	12 (30)	0.424
Grade ≥3 platelet count decreased	6 (15)	5 (12.5)	1.000
Any grade neutrophil count decreased	34 (85)	14 (35)	<0.001
Grade ≥3 neutrophil count decreased	23 (57.5)	7 (17.5)	<0.001

*: treatment-related hematologic adverse events.

Discussion

Platinum-based drugs inhibit tumor cell proliferation by interfering with DNA replication and transcription by binding to DNA (17, 18).The PI3K pathway is frequently upregulated in epithelial ovarian cancer and plays an important role in chemoresistance and preservation of genomic stability, as it is implicated in many processes of DNA replication and cell cycle regulation. The inhibition of the PI3K may lead to genomic instability and mitotic catastrophe through a decrease of the activity of the spindle assembly checkpoint protein Aurora kinase B and consequently increase of the occurrence of lagging chromosomes during prometaphase (19). BRCA1/2 mutations are also associated with high sensitivity for platinum groups. Patients with BRCA mutations have improved overall response to platinum-based therapy, which is associated with longer survival in patients with BRCA-mutated ovarian cancer (17, 20).PARP enzymes, especially PARP-1 and PARP-2, play a key role in the repair of DNA single-strand breaks. Inhibition of PARP leads to the accumulation of single-strand breaks, leading to the collapse of the replication strand and the accumulation of double-strand breaks, which are usually repaired by homologous recombinases. There have been six primary pathways of DNA damage repair (DDR) identified, which are variably used to address double-strand DNA breaks (DSB) and single-strand DNA breaks damage from a variety of mechanisms of injury. Homologous recombination (HR) and nonhomologous end joining (NHEJ) recombination are the two major pathways responsible for repairing DSB (21). HR

TABLE 3 Summary of adverse events.

Adverse event	niraparib maintenance therapy	
	Any grade	Grade≥ 3
	number of patients (percent)	
Nausea	14(35%)	
Vomiting	9(22.5%)	
stomachache	5(12.5%)	
Dyspepsia	2(5%)	
Decreased appetite	6(15%)	
Fatigue or asthenia	13(32.5%)	
Abdominal distention	3(7.5%)	
Constipation	7(17.5%)	
Headache	1(2.5%)	
Insomnia	5(12.5%)	
Orbital pain	2(5%)	
A foreign body sensation in the chest	1(2.5%)	
Maculopapular rash	2(5%)	
Dark skin	5(12.5%)	
loss of weight	1(2.5%)	
Elevation of blood pressure	1(2.5%)	
white blood cell count decreased	19(47.5%)	5(12.5%)
red blood cell count decreased	23(57.5%)	3(7.5%)
Thrombocytopenia	12(30%)	5(12.5%)
Neutropenia	14(35%)	7(17.5%)
Anemia	22(55%)	8(20%)
Led to dose reduction	18(45%)	
Led to discontinuation of intervention	18(45%)	
Led to dose interruption	2(5%)	

pathways become active in the S/G2 phase due to the availability of a sister chromatid, whereas NHEJ repairs DSB throughout all cell cycle phases except the M phase. NHEJ is faster than HR and mainly occurs in the G1 phase, Beyond the already-known proteins, such as Ku70/80, DNA-PKcs, Artemis, DNA pol λ/μ, DNA ligase IV-XRCC4, and XLF, new proteins are involved in the NHEJ, namely PAXX, MRI/CYREN, TARDBP of TDP-43, IFFO1, ERCC6L2, and RNase H2 (22, 23). Among them, MRI/CYREN has dual role, as it stimulates NHEJ in the G1 phase of the cell cycle, while it inhibits the pathway in the S and G2 phases (24). Ovarian cancers with BRCA1/BRCA2 mutations or other HRDs are particularly sensitive to PARP inhibitors because the accumulation of unrepaired DNA breaks leads to cell death (25, 26).This is known as "synthetic lethality"。 Niraparib is a highly selective inhibitor of PARP1/2 (a nuclear protein that detects DNA damage and promotes its repair) (27), and the most common adverse effect of niraparib is myelosuppression, with most interruptions of niraparib treatment due to myelosuppressive events (16).

The anti-tumor mechanism of PARP inhibitors (PARPi) overlaps with platinum-based drugs in DNA damage repair pathways. Patients who are effective to platinum-based chemotherapy are also more likely to be sensitive to PARPi. The dose-limiting toxicity of carboplatin is myelosuppression, and its non-hematologic adverse reactions are milder and fewer than those of cisplatin (28–30), and several studies have shown that the most common ≥grade 3 adverse reactions of niraparib are also hematologic adverse reactions (31–33). In this real-world study, we observed a lower rate of hematologic adverse effects with niraparib than with platinum-based chemotherapy in patients with advanced ovarian cancer. Niraparib maintenance therapy is better tolerated than platinum-based chemotherapy in this study. Due to the small sample size, larger sample size is needed for further verification.

In this study, all patients received a starting dose of niraparib of 200mg/d according to their basal body weight and basal platelet count, which was consistent with the Chinese prospective study (31, 32). The most common adverse reactions of any grade were hematologic adverse reactions, nausea, and fatigue, and there were 16 cases of ≥ grade 3 adverse reactions, all of which were hematologic adverse reactions, which were similar to the results of the NORA (31)study. A meta-analysis showed that niraparib adverse effects were significantly dose-related, and most of them could be controlled by suspending therapy, reducing dose, and treating symptomatic therapy (34).In this study, during the maintenance treatment with niraparib, 16 patients experienced grade ≥3 adverse reactions, 18 patients reduced their dose due to adverse drug reactions, 18 patients discontinued their medication due to adverse drug reactions, 1 patient spontaneously terminated the drug due to stomach pain after taking the drug, and 1 patient terminated the drug due to recurrent ≥ grade 3 bone marrow suppression, which is consistent with the results of the meta-analysis (35)of the current clinical trial. In the context of the new crown epidemic, 8 patients stopped taking the drug for 1–4 weeks due to new coronavirus infection, and all patients passed the new coronavirus infection period safely.

Niraparib has a long treatment cycle and is therefore particularly important for the management of adverse effects. Standardized whole-process management, including pre-medication evaluation and adequate doctor-patient communication, standardized detection during medication and timely treatment of AEs, can reduce and reduce the occurrence of AEs, increase the safety of medication, and improve the compliance of patients, so as to further ensure the efficacy of niraparib treatment cycle is long, so the management of adverse reactions is particularly important. Standardized whole-process management, including pre-medication evaluation and adequate doctor-patient communication, standardized detection during medication and timely treatment of AEs, can reduce and reduce the occurrence of AEs, increase the safety of medication, and improve the compliance of patients, so as to further ensure the efficacy. Myelosuppression is

a dose-limiting toxicity of most platinum drugs and niraparib (17, 30).This study evaluates whether there are treatment-related hematological adverse reactions between platinum-based chemotherapy and niraparib treatment, and provides a clinical reference for the whole process of precise and standardized management of ovarian cancer patients.

This study shows that any grade of adverse blood reactions, including (decreased white blood cells, decreased red blood cells, anemia, and neutrophils), occurred during platinum-based chemotherapy and niraparib maintenance therapy in patients with ovarian cancer, and there was a correlation between grade ≥ grade 3 adverse reactions including (anemia, neutrophil decline). There was no statistically significant correlation between any grade of anemia and grade ≥ grade 3 leukocytopenia, grade ≥ grade 3 erythrocyte decline, and grade 3 thrombocytopenia ≥ the two periods. Due to the small sample size, it is not possible to obtain a valid correlation strength analysis, which requires more data for further validation. Based on this study, it is believed that the occurrence of serious hematologic adverse reactions with platinum-based chemotherapy may be a risk factor for patients to develop serious hematologic adverse reactions in maintenance therapy with niraparib. Based on the results of this study, the use of niraparib treatment should take into account whether the patient has experienced ≥ grade 3 hematological adverse reactions, especially anemia and ≥ grade 3 neutropenia during the first-line platinum-based chemotherapy, and the timing of drug administration can be determined according to the patient's condition. More attention should be paid to the monitoring and management of hematologic toxicity in patients with a history of ≥ grade 3 hematologic toxicity during the subsequent treatment with niraparib. Doctors can strengthen the relevant medical education for these patients and inform patients to see a doctor in time when they have symptoms related to blood adverse reactions such as pale complexion, fatigue, fever, gingival bleeding, and skin ecchymosis. Increase the frequency of hematological analysis and testing for this group of patients, as appropriate, and intervene as early as possible to standardize treatment in the event of adverse hematological reactions in patients. In order to better guide the clinic, accumulate clinical medication experience, increase patient compliance, so that patients can better benefit from PARP inhibitors.

Conclusion

1. In this real-world practice, we observed that patients with advanced ovarian cancer who experienced any grade and grade ≥3 TRAE during chemotherapy were well tolerated when treated with niraparib, particularly the incidence of any grade and grade ≥3 anemia, and neutrophil count decreased during niraparib treatment were significantly lower compared with that during chemotherapy.

2. For patients with ovarian cancer who have experienced grade ≥3 hematological adverse reactions during prior platinum-based chemotherapy, greater attention should be paid to the monitoring and management of hematological adverse reactions during subsequent treatment with niraparib.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving humans were approved by Ethics Committee of First Affiliated Hospital of Gannan Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LW: Data curation, Investigation, Writing – original draft, Software. JZ: Project administration, Supervision, Writing – review & editing, Data curation. HW: Investigation, Writing – original draft. WH: Supervision, Writing – review & editing. CF: Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Saani I, Raj N, Sood R, Ansari S, Mandviwala HA, Sanchez E, et al. Clinical challenges in the management of Malignant ovarian germ cell tumours. *Int J Environ Res Public Health*. (2023) 20:6089. doi: 10.3390/ijerph20126089

2. Sung H, Ferlay J, Siegel RJ, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

3. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin Med J (Engl)*. (2021) 134:783–91. doi: 10.1097/CM9.0000000000001474

4. Ghose A, Bolina A, Mahajan I, Raza SA, Clarke M, Pal A, et al. Hereditary ovarian cancer: towards a cost-effective prevention strategy. *Int J Environ Res Public Health*. (2022) 19:12057. doi: 10.3390/ijerph191912057

5. Pavlidis N, Rassy E, Vermorken JB, Assi T, Kattan J, Boussios S, et al. The outcome of patients with serous papillary peritoneal cancer, fallopian tube cancer, and epithelial ovarian cancer by treatment eras: 27 years data from the SEER registry. *Cancer Epidemiol*. (2021) 75:102045. doi: 10.1016/j.canep.2021.102045

6. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. *CA Cancer J Clin*. (2019) 69:280–304. doi: 10.3322/caac.21559

7. Bell D, Berchuck A, Birrer M, Chien J, Cramer DW, Dao F, et al. Integrated genomic analyses of ovarian carcinoma. *Nature*. (2011) 474:609–15. doi: 10.1038/nature10166

8. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. *Lancet*. (2014) 384:1376–88. doi: 10.1016/S0140-6736(13)62146-7

9. Ghose A, Gullapalli S, Chohan N, Bolina A, Moschetta M, Rassy E, et al. Applications of proteomics in ovarian cancer: dawn of a new era. *Proteomes*. (2022) 10:16. doi: 10.3390/proteomes10020016

10. Bradbury M, Borràs E, Pérez-Benavente A, Gil-Moreno A, Santamaria A, Sabidó E. Proteomic studies on the management of high-grade serous ovarian cancer patients: A mini-review. *Cancers (Basel)*. (2021) 13:2067. doi: 10.3390/cancers13092067

11. Hutter C, Zenklusen JC. The cancer genome atlas: creating lasting value beyond its data. *Cell*. (2018) 173:283–5. doi: 10.1016/j.cell.2018.03.042

12. Labrie M, Kendsersky ND, Ma H, Campbell L, Eng J, Chin K, et al. Proteomics advances for precision therapy in ovarian cancer. *Expert Rev Proteomics*. (2019) 16:841–50. doi: 10.1080/14789450.2019.1666004

13. Madariaga A, Bowering V, Ahrari S, Oza AM, Lheureux S. Manage wisely: poly (ADP-ribose) polymerase inhibitor (PARPi) treatment and adverse events. *Int J Gynecol Cancer*. (2020) 30:903–15. doi: 10.1136/ijgc-2020-001288

14. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N Engl J Med*. (2016) 375:2154–64. doi: 10.1056/NEJMoa1611310

15. Berek JS, Matulonis UA, Peen U, Ghatage P, Mahner S, Redondo A, et al. Safety and dose modification for patients receiving niraparib. *Ann Oncol*. (2018) 29:1784–92. doi: 10.1093/annonc/mdy181

16. González-Martín A, Pothuri B, Vergote I, Christensen RD, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med*. (2019) 381:2391–402. doi: 10.1056/NEJMoa1910962

17. Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q, Tian R, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discovery*. (2012) 2:366–75. doi: 10.1158/2159-8290.CD-11-0206

18. Rosenberg B, Van Camp L, Grimley EB, Thomson AJ. The inhibition of growth or cell division in Escherichia coli by different ionic species of platinum(IV) complexes. *J Biol Chem*. (1967) 242:1347–52. doi: 10.1016/S0021-9258(18)96186-7

19. Richardson DL, Eskander RN, O'Malley DM. Advances in ovarian cancer care and unmet treatment needs for patients with platinum resistance: A narrative review. *JAMA Oncol*. (2023) 9:851–9. doi: 10.1001/jamaoncol.2023.0197

20. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol*. (2012) 30:2654–63. doi: 10.1200/JCO.2011.39.8545

21. Boussios S, Mikropoulos C, Samartzis E, Karihtala P, Moschetta M, Sheriff M, et al. Wise management of ovarian cancer: on the cutting edge. *J Pers Med*. (2020) 10:41. doi: 10.3390/jpm10020041

22. Bhattacharjee S, Nandi S. Choices have consequences: the nexus between DNA repair pathways and genomic instability in cancer. *Clin Transl Med*. (2016) 5:45. doi: 10.1186/s40169-016-0128-z

23. Ghosh D, Raghavan SC. Nonhomologous end joining: new accessory factors fine tune the machinery. *Trends Genet*. (2021) 37:582–99. doi: 10.1016/j.tig.2021.03.001

24. Boussios S, Rassy E, Moschetta M, Ghose A, Adeleke S, Sanchez E, et al. BRCA mutations in ovarian and prostate cancer: bench to bedside. *Cancers (Basel)*. (2022) 14:3888. doi: 10.3390/cancers14163888

25. Brown JS, O'Carrigan B, Jackson SP, Yap TA. Targeting DNA repair in cancer: beyond PARP inhibitors. *Cancer Discovery*. (2017) 7:20–37. doi: 10.1158/2159-8290.CD-16-0860

26. Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. (2005) 434:917–21. doi: 10.1038/nature03445

27. Heo YA, Duggan ST. Niraparib: A review in ovarian cancer. *Target Oncol*. (2018) 13:533–9. doi: 10.1007/s11523-018-0582-1

28. Go RS, Adjei AA. Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *J Clin Oncol*. (1999) 17:409–22. doi: 10.1200/JCO.1999.17.1.409

29. Lokich J, Anderson N. Carboplatin versus cisplatin in solid tumors: an analysis of the literature. *Ann Oncol*. (1998) 9:13–21. doi: 10.1023/A:1008215213739

30. Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA , et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol*. (2003) 21:3194–200. doi: 10.1200/JCO.2003.02.153

31. Wu XH, Zhu JQ, Yin RT, Yang JX, Liu JH, Wang J, et al. Niraparib maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer using an individualized starting dose (NORA): a randomized, double-blind, placebo-controlled phase III trial(☆). *Ann Oncol*. (2021) 32:512–21. doi: 10.1016/j.annonc.2020.12.018

32. Li N, Zhu JQ, Yin RT, Wang J, Pan LY, Kong BH, et al. Treatment with niraparib maintenance therapy in patients with newly diagnosed advanced ovarian cancer: A phase 3 randomized clinical trial. *JAMA Oncol*. (2023) 9:1230–7. doi: 10.1001/jamaoncol.2023.2283

33. Oza AM, Matulonis UA, Malander S, Hudgens S, Sehouli J, Campo JMD, et al. Quality of life in patients with recurrent ovarian cancer treated with niraparib versus placebo (ENGOT-OV16/NOVA): results from a double-blind, phase 3, randomised controlled trial. *Lancet Oncol*. (2018) 19:1117–25. doi: 10.1016/S1470-2045(18)30333-4

34. Jiang Y, Zhao J, Zhang L, Tian SJ, Yang T, Wang L, et al. Evaluation of the efficacy and safety of PARP inhibitors in advanced-stage epithelial ovarian cancer. *Front Oncol*. (2020) 10:954. doi: 10.3389/fonc.2020.00954

35. Lorusso D, Guy H, Samyshkin Y, Hawkes C, Estenson K, Coleman RL. Feasibility study of a network meta-analysis and unanchored population-adjusted indirect treatment comparison of niraparib, olaparib, and bevacizumab as maintenance therapies in patients with newly diagnosed advanced ovarian cancer. *Cancers (Basel)*. (2022) 14:1285. doi: 10.3390/cancers14051285

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Lipid droplets: a candidate new research field for epithelial ovarian cancer

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Ovarian clear cell carcinoma (OCCC) is a histological subtype that constitutes approximately 20% of epithelial ovarian cancer cases in Asian countries, but has a relatively low incidence in Western countries. Meanwhile, clear cell renal cell carcinoma (ccRCC) is a major subtype of kidney cancer. OCCC and ccRCC resemble one another histologically and have clear cytoplasmic appearances. Studies have revealed some genetic similarities between OCCC and ccRCC. However, information regarding common biological background factors between these cancers remains scarce. For example, accumulation of cellular lipid droplets was shown to play a crucial role in ccRCC progression, while similar information is lacking for OCCC. In this perspective article, we propose that lipid droplets may be candidates for future exploration to better understand the common biological backgrounds between OCCC and ccRCC, potentially leading to subtype-specific treatment strategies. We further discuss the relationship between poly ADP-ribose polymerase inhibition treatment and lipid metabolism because this therapeutic strategy has attracted considerable attention as a treatment for epithelial ovarian cancer.

KEYWORDS

ovarian clear cell carcinoma, clear cell renal cell carcinoma, lipid droplet, fatty acid oxidation, lipophagy, poly ADP-ribose polymerase inhibition

1 Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecological disease worldwide. Globally, 313,959 new EOC cases were diagnosed and 207,252 deaths were recorded in 2020 (Huang et al., 2022). EOC can be classified into four histological subtypes: serous, endometrioid, mucinous, and clear cell (Torre et al., 2018). Ovarian clear cell carcinoma (OCCC) has an incidence of approximately 20% in Asian countries and limited European countries, but is rare in most Western countries (Sung et al., 2014; Kato, 2020). OCCC cells are glycogen-rich with a clear cytoplasm. Because OCCC is aggressive and exhibits drug resistance (Kato, 2020), it is regarded as an intractable cancer.

The appearance of OCCC resembles that of clear cell renal cell carcinoma (ccRCC) (Ji et al., 2018; Ackroyd et al., 2023), a histological subtype found in approximately 80% of kidney cancer cases. Studies have revealed molecular and genetic similarities and differences between OCCC and ccRCC (Ji et al., 2018; Ackroyd et al., 2023). Further exploration of these factors may lead to not only greater understanding of the similar histological appearances between OCCC and ccRCC but also the generation of common management strategies for cancer types with clear cell histological appearances.

Many studies have revealed the crucial roles of dysregulated lipid metabolism in multiple cancer types (Bian et al., 2020), and new insights continue to be gained,

including the involvement of fatty acid synthesis in breast cancer metastasis (Ferraro et al., 2021), ferroptosis resistance in glioblastoma (Minami et al., 2023), ferroptosis resistance in hepatocellular carcinoma (Li et al., 2024), and citrate transport-driven activation of lipogenesis and fatty acid oxidation (FAO) in pancreatic cancer cells (Zhang et al., 2023). Extensive cellular lipid uptake and synthesis, followed by lipid droplet (LD) formation, can contribute to cancer progression (Koizume and Miyagi, 2016; Cruz et al., 2020). Accumulated LDs play crucial roles in the expression of ccRCC phenotypes, such as cell motility (Chen et al., 2022; Quan et al., 2023), invasiveness (Chen et al., 2022), epithelial-to-mesenchymal transition (Chen et al., 2022), and resistance to cell death (Miess et al., 2018; Zhou et al., 2023). The LDs in ccRCC cells can also be catabolized by neutral lipases to release oleic acid on exposure to serum starvation and hypoxia (SSH), thereby maintaining cellular lipid homeostasis (Ackerman et al., 2018). Thus, both stored and released fatty acids (FAs) can contribute to ccRCC progression.

In contrast to ccRCC, little is known about lipid metabolism in OCCC cells. Indeed, there is scarce information on LD levels in OCCC (Cruz et al., 2020), and it remains unclear whether OCCC has higher LD levels than other histological subtypes of EOC. We recently reported that SSH triggers lipophagy for degradation of LDs in OCCC cells (Koizume et al., 2022). This LD catabolism synergistically activates multiple genes, including *ICAM1* and *CD69*, through activation of transcription factor NFκB binding to their promoter regions (Koizume et al., 2022). The proteins encoded by these genes lead to malignant phenotypes, such as apoptosis resistance (Koizume et al., 2015) and epithelial-to-mesenchymal transition with assistance of extracellular fibronectin (Koizume et al., 2023). Thus, we hypothesized that LDs may play major roles in OCCC progression, similar to the case for ccRCC.

2 Effect of FA oxidation on cancer progression and its correlation with ccRCC and OCCC

Cancer cells can utilize FAs received from their environments, including the bloodstream (Koizume and Miyagi, 2016), cancer-associated fibroblasts (Hwang et al., 2022), reactive astrocytes (Parida et al., 2023), and adipocytes (Nieman et al., 2011), and/or synthesized by themselves (Koizume and Miyagi, 2016; Cruz et al., 2020). FAs are a source of ATP produced through FAO in mitochondria, followed by oxidative phosphorylation. Carnitine palmitoyl transferase 1A (CPT1A) is the rate-limiting enzyme for FA transportation from the cytoplasm into mitochondria via carnitine (Liang, 2023). CPT1A is considered a therapeutic target in cancer (Zeng et al., 2016; Liang, 2023). Indeed, FAO inhibition was shown to block the growth of glioma (Pike et al., 2011), non-clear cell EOC (Nieman et al., 2011; Sawyer et al., 2020), gastric cancer (Wang et al., 2020), and myeloma (Tirado-Vélez et al., 2012) cells. A recent study further revealed that FAO contributes to metastasis of ccRCC through histone acetylation (Shi et al., 2024).

In contrast, FAO blockade can promote cancer cell growth. Studies have provided evidence that FAO can suppress aggressiveness in multiple cancer types (Zhang et al., 2017), hepatocellular carcinoma (Ma et al., 2021), and pancreatic cancer (Kim et al., 2023). This suppressive effect of FAO also functions in some cancer cells exposed to hypoxia, because the supply of molecular oxygen required for oxidative phosphorylation is restricted (Zhang et al., 2017; Kim et al., 2023). Thus, the way in which FAO functions for expression of malignant phenotypes may depend on the cell type and/or context.

3 Effect of LD on cancer progression and its correlation with ccRCC and OCCC

Excess FAs are converted to their esterified form and then incorporated into LDs for storage. FAs are released from LDs by lipophagy (Roy et al., 2017) and neutral lipolysis (Ackerman et al., 2018) when required and subsequently catabolized via FAO in cancer cells. However, LDs are not simply lipid storage compartments. Instead, LDs can contribute to cancer cell progression through multiple mechanisms, including elimination of reactive oxygen species and maintenance of endoplasmic reticulum homeostasis (Qiu et al., 2015; Koizume and Miyagi, 2016; Cruz et al., 2020). Indeed, high levels of cellular LDs (Cruz et al., 2020) and the importance of LDs over FAO has been demonstrated for ccRCC cells (Du et al., 2017; Xu et al., 2020; Zhou et al., 2023). Multiple studies have shown that FAO is suppressed in ccRCC cells to enhance LD generation with augmentation of the Warburg effect (Du et al., 2017; Courtney et al., 2018; Miess et al., 2018). In general, LD anabolism predominantly contributes to ccRCC progression, rather than FAO. However, it remains unclear whether this holds true for OCCC progression because of a lack of published data.

4 Effect of FAO inhibition on OCCC cell growth *in vitro*

To determine whether FAO facilitates or suppresses the growth of OCCC cells, we examined the effect of FAO inhibition on cell viability and LD levels in the presence of exogenous FAs (Figure 1). OCCC cells were cultured in serum-free medium supplemented with albumin and water-soluble fatty acid supplement (FAS) as previously described (Koizume et al., 2015; Koizume et al., 2022) (Figure 1A). The effect of etomoxir, a CPT1A inhibitor, on cell growth under serum starvation and normoxia (SSN) was elucidated by cell counting (trypan blue exclusion assay) and LD staining with fluorescent BODIPY dye as described (Koizume et al., 2022) (Figures 1A, B). We found that CPT1A inhibition increased the cellular LD level (Figures 1B, C), presumably because fatty acid retention in the cytoplasm shifted the equilibrium between LD generation and FAO to the former. This was associated with an increasing trend in OCCC cell growth (Figure 1D). These findings suggest that LDs, rather than FAO, are associated with OCCC cell viability, possibly due to LD-driven elimination of toxic effects, such as the generation of reactive oxygen species and serum deprivation-induced stresses. This is in contrast to the pro-survival effect of FAO associated with NADPH production in gliomas (Pike et al., 2011) and gastric cancer (Wang et al., 2020). The molecular mechanisms defining the relative importance of LD generation and FAO between these cancer cells are currently unclear.

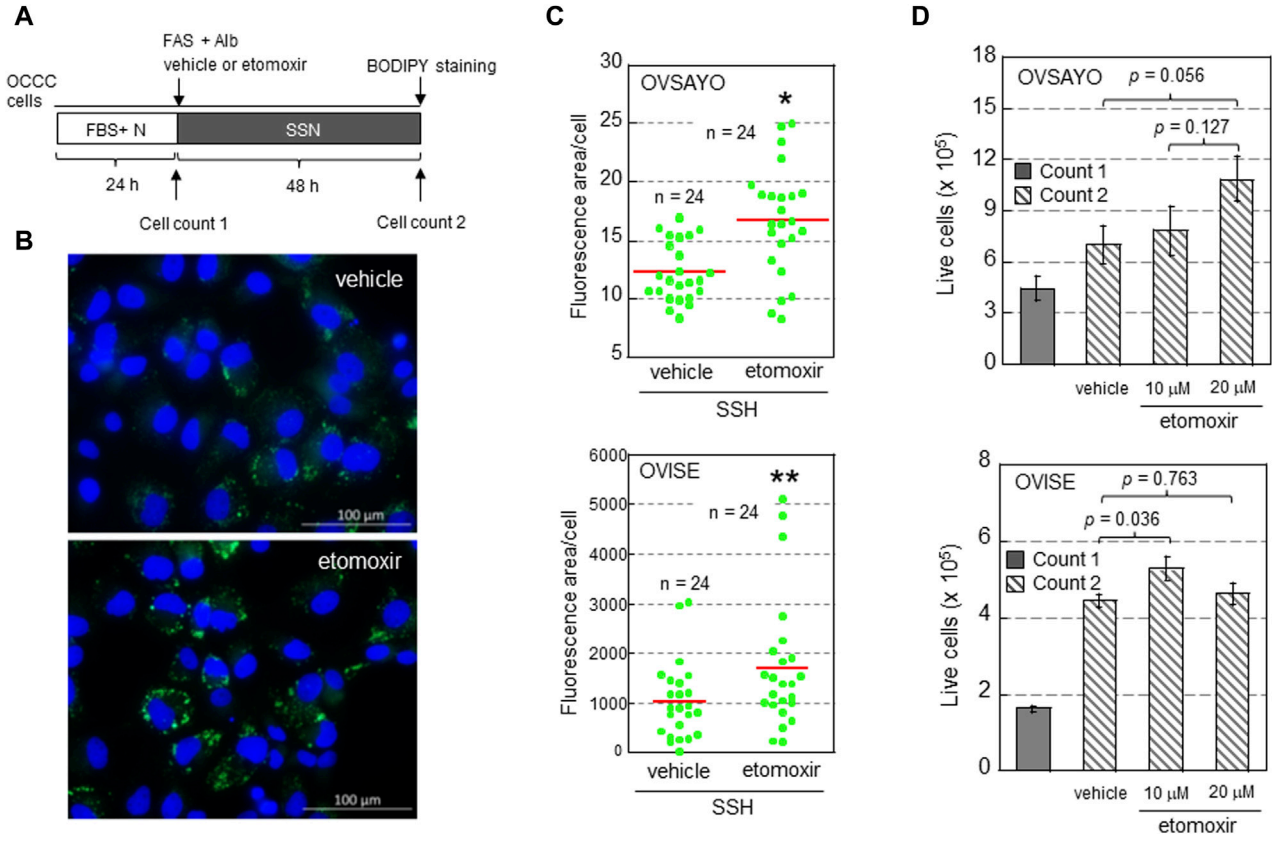

FIGURE 1
(A) Scheme of the assay. Following culture under normoxia (N) in medium supplemented with serum (FBS+), the effect of etomoxir (0, 10, or 20 μg/mL) and fatty acid supplement (FAS) (0.3%)-albumin (Alb) on the viability of OCCC cells cultured under serum starvation and normoxia (SSN) for 48 h was examined. **(B)** Typical Images of LDs (green) in OVISE cells cultured under SSN in medium supplemented with FAS-Alb in the presence of vehicle or etomoxir. The nuclei (blue) were counterstained with DAPI. **(C)** Effect of (+)-etomoxir (Cayman Chemicals) on LD levels in OCCC cells cultured under SSN in medium supplemented with FAS-Alb for 48 h. The LD levels were quantified by ImageJ software. The stained area was evaluated in the indicated number of images (green dots) acquired from three (OVSAYO) and two (OVISE) independent replicates and normalized to the number of cells (nuclei) in each image. Red bars: mean. *$p < 0.001$ versus vehicle, **$p = 0.041$ versus vehicle, by a *t*-test (OVSAYO) or the Mann–Whitney *U*-test (OVISE). **(D)** Effect of etomoxir treatment on the viability of OCCC cells cultured under SSN in medium supplemented with FAS-Alb for 48 h. Data are shown as mean ± SD ($N = 3$). *p* values were calculated by one-way ANOVA using SPSS Statistics 19.

5 Effect of MAP1S depletion on LD levels in OCCC cells

We previously showed that LD catabolism in OCCC cells in response to SSH exposure is mediated by lipophagy (Koizume et al., 2022). This mechanism is similar to the LD catabolism observed in ccRCC cells exposed to SSH to mitigate the toxic effect of excess FAs (Ackerman et al., 2018). Lipophagy is mediated by human sulfatase-1 (HSulf-1) in EOC cells (Roy et al., 2017). Meanwhile, microtubule-associated protein 1S (MAP1S) contributes to lipophagy in ccRCC cells (Xu et al., 2016). MAP1S binds to microtubules to facilitate autophagosomal biogenesis (Xie et al., 2011; Yue et al., 2017). Thus, we examined the effect of these potential lipophagy regulators on SSH-driven LD catabolism in OCCC cells. First, we examined the expression levels of these proteins by western blotting. HSulf-1 showed considerable expression in OCCC cell line OVSAYO cells under various oxygenation and serum supplementation conditions, but its expression was dramatically decreased under SSH (Figure 2A). In contrast, OVSAYO cells expressed MAP1S protein under all cell culture conditions (Figure 2A). Next, we examined the effect of MAP1S on LD degradation under SSH. If MAP1S participates in SSH-driven LD catabolism, the LD level should increase in response to MAP1S knockdown. Thus, we used an RNA interference approach to determine the effect of MAP1S expression on the LD level (Figure 2B). Western blotting (Figure 2C) and immunofluorescence (Figures 2D, F) analyses revealed that MAP1S existed in both the nucleus and the cytoplasm in OVSAYO cells, consistent with the subcellular localization data in a public database (The Human Protein Atlas, https://www.proteinatlas.org). Knockdown of MAP1S expression (Figures 2D, E) did not affect the LD levels in either OVSAYO cells or OVISE cells, another OCCC cell line, under SSH (Figure 2G).

6 Relationship between lipid metabolism and poly ADP-ribose polymerase inhibition in cancer cells

Inhibition of poly ADP-ribose polymerase (PARP) by small molecule inhibitors such as Olaparib is an important therapeutic

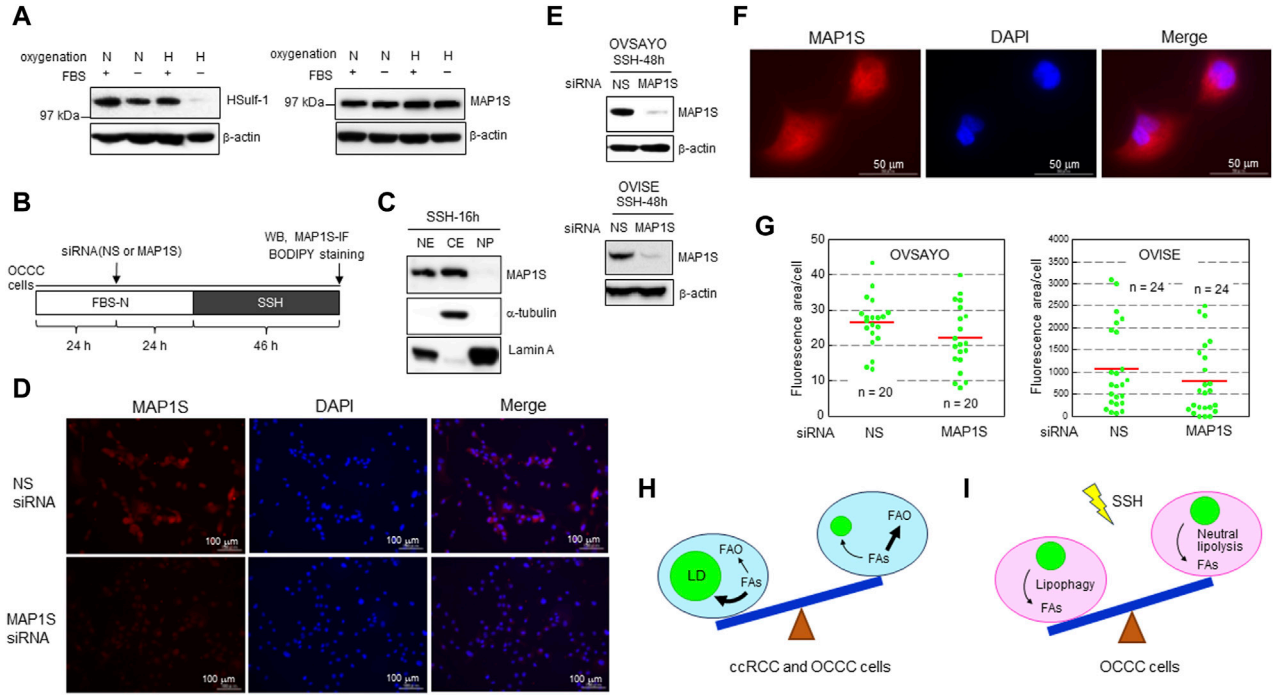

FIGURE 2
(A) Western blot analysis of the indicated proteins in OVSAYO cells exposed to the indicated culture conditions for 48 h. The primary antibodies were an anti-MAP1S rabbit polyclonal antibody (Proteintech) and an anti-HSulf-1 rabbit polyclonal antibody (CUSABIO). FBS: 10% fetal bovine serum; N, normoxia (ambient air); H, hypoxia (1% O_2). β-Actin was examined as a protein loading control. **(B)** Scheme of the assay. Following culture under normoxia (N) in medium supplemented with serum (FBS+), the effect of MAP1S-knockdown on LD levels in OCCC cells cultured under SSH was examined by western blotting (WB), immunofluorescence (IF), and BODIPY staining. A MAP1S siRNA was purchased from Santa Cruz Biotechnology. **(C)** Western blot analysis of MAP1S expression in the cytoplasmic and nuclear fractions of OVSAYO cells cultured under the indicated conditions was performed as previously described (Koizume et al., 2022). NE, nuclear extract; CE, cytoplasmic extract; NP, nuclear pellet. Cytoplasmic (α-tubulin) and nuclear (lamin A) markers are shown. **(D)** Immunofluorescence staining of MAP1S in OVSAYO cells cultured as described for **(B)**. The primary anti-MAP1S antibody was the same as that used in the western blotting. **(E)** Western blotting analysis of MAP1S-knockdown in OCCC cells cultured under the indicated conditions. **(F)** Magnified version of the MAP1S immunofluorescence staining shown in **(D)**. **(G)** Effect of MAP1S knockdown on LD levels in OCCC cells cultured under SSH. The LD levels were quantified by ImageJ software. The stained area was evaluated for the indicated number of images (green dots) acquired from three (OVSAYO) and two (OVISE) independent replicates. Red bars: mean. $p = 0.11$ versus vehicle for OVSAYO, $p = 0.25$ versus vehicle for OVISE, by the Mann–Whitney *U*-test. **(H)** Seesaw model for FA metabolism in clear cell carcinoma cells. Bold arrows indicate predominant pathways. **(I)** Seesaw model for LD catabolism in OCCC cells exposed to SSH.

strategy for EOC based on the synthetic lethality concept for impaired DNA repair machinery in the presence of *BRCA* gene mutations (Maiorano et al., 2023). In this section, we describe the published literature on the relationship between PARP inhibition (PARPi) therapy for cancer and lipid metabolism.

Despite a lack of published data on the relationship between PARPi therapy and lipid metabolism in *BRCA*-mutation-positive cancer types, a few studies have indicated that PARPi is effective in a *BRCA* mutation-independent manner, as described below. Indeed, *BRCA* mutations are rare in glioblastoma cells. However, PARPi functions in this cancer type through BRCAness, a phenotype expressed in sporadic cancers with similar biochemical pathways to familial cancers with *BRCA* mutations (Turner et al., 2004). Glioblastoma cells can evade PARPi-driven tumor suppression by metabolic reprogramming through LD generation followed by FAO (Majuelos-Melguizo et al., 2022). Meanwhile, PARPi can enhance oleic acid treatment-driven LD accumulation in mouse hepatoma cell line Hepa1-6 cells through lipogenic gene activation (Pang et al., 2018). These findings imply that clinical application of PARPi necessitates management of metabolic disease. In contrast, PARPi decreases cholesterol biosynthesis in ccRCC cells to block malignancy (Karpova et al., 2021). Currently, the relationship between lipid metabolism and OCCC cells has not been reported.

7 Discussion

As described previously, LDs play multiple roles in malignancy. In this article, we have presented two OCCC characteristics regarding LDs, namely, cell growth and lipophagy, through experiments using the OCCC cell lines OVSAYO and OVISE. The former is consistent with a reported ccRCC cell phenotype while the latter is not.

ccRCC is a major histological subtype of kidney cancer. Most ccRCC cells lack *VHL* gene function, leading to constitutive expression of hypoxia-inducible factors (HIFs). Consequently, these cells exhibit hypoxia-driven phenotypes associated with FAO suppression and LD generation (Figure 2H). The importance of the hypoxia response is also true for OCCC cells with intact *VHL* function (Ackroyd et al., 2023), because genomic alterations are shared between OCCC and ccRCC and the HIF pathway is more active in OCCC than in other histological subtypes

of EOC (Ji et al., 2018). Our data showing that FAO inhibition can augment OCCC cell growth under normoxia with increased LD levels is consistent with recent studies (Du et al., 2017; Xu et al., 2020; Zhou et al., 2023) showing that lipid storage, rather than lipid consumption, is predominant in ccRCC progression (Figure 2H). It will be interesting to determine whether this metabolic trend can be changed reversibly (Figure 2H) depending on tumor conditions such as hypoxia and poor nutrient supply. Further studies are also needed to clarify the molecular mechanisms that define the relative importance of LD generation and FAO across different cancer types.

Our data further indicated that the autophagy activator MAP1S does not contribute to lipophagy-driven LD catabolism in OCCC cells (Figure 2I). These findings are inconsistent with the importance of MAP1S for autophagy-driven LD clearance in ccRCC cells (Xu et al., 2016). Moreover, unlike our previous data (Koizume et al., 2022), neutral lipolysis via hormone-sensitive lipase is responsible for LD catabolism in ccRCC cells under SSH (Ackerman et al., 2018). It remains unclear how cells utilize these different lipolysis mechanisms and whether SSH-driven LD clearance in OCCC cells can reversibly involve neutral lipolysis depending on the cell culture conditions (Figure 2I). Answers to these issues await future investigations.

Compared with ccRCC, biological information on OCCC is currently scarce, possibly because unlike the serous carcinoma subtype, OCCC is a relatively rare cancer type, especially in Western countries. It is thus currently unclear if OCCC is a lipid-dependent cancer type, similar to ccRCC. OCCC is resistant to standard platinum- and taxane-based chemotherapies, but inhibition of EZH2 histone methyl-transferase has been proposed as an effective synthetic lethal therapy in *ARID1A*-mutated OCCC cases (Bitler et al., 2015). However, information on the relationship between PARPi therapy and lipid metabolism is currently limited for ccRCC and totally unclear for OCCC. Thus, exploration of how LDs contribute to common drug resistance mechanisms between these cancer types may represent another future research direction. Furthermore, ccRCC cells in tumors are under hypoxic mimetic conditions, even under normoxia as described above. Information on the hypoxia status in kidney tissues and ccRCC tumors is currently poor, and limited studies have shown that hypoxic regions exist within the normal renal medulla (Brezis and Rosen, 1995) and renal tumors (Little et al., 2018). The adaptive response mechanisms of ccRCC cells to real-hypoxia conditions and associated drug-resistance thus also remain unclear, but their similarity to OCCC cells warrants further investigation.

The OVSAYO cell line used in our present study and previous studies may have a limitation in its histological origin because genomic analysis revealed that this cell line may be derived from serous carcinoma (Anglesio et al., 2013). However, approved therapeutic strategies for ccRCC have been translated into OCCC treatment (Ji et al., 2018). Investigations into LDs in OCCC cells has just started. We expect that active exploration of OCCC characteristics, including cellular lipid storage, will lead to not only a wealth of information regarding the similarities between OCCC and ccRCC beyond morphology but also the development of common promising treatment options targeting identical metabolic routes.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

SK: Writing–review and editing, Writing–original draft, Supervision, Investigation, Formal Analysis, Conceptualization. TT: Writing–review and editing, Investigation. YM: Writing–review and editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ackerman, D., Tumanov, S., Qiu, B., Michalopoulou, E., Spata, M., Azzam, A., et al. (2018). Triglycerides promote lipid homeostasis during hypoxic stress by balancing fatty acid saturation. *Cell. Rep.* 24, 2596–2605. doi:10.1016/j.celrep.2018.08.015

Ackroyd, S. A., Arguello, D., Ramos, P., Mahdi, H., Einaggar, A., Winer, I., et al. (2023). Molecular portraits of clear cell ovarian and endometrial carcinoma with comparison to clear cell renal cell carcinoma. *Gynecol. Oncol.* 169, 164–171. doi:10.1016/j.ygyno.2022.10.020

Anglesio, M. S., Wiegand, K. C., Melnyk, N., Chow, C., Salamanca, C., Prentice, L. M., et al. (2013). Type-specific cell line models for type-specific ovarian cancer research. *PLOS One* 8, e72162. doi:10.1371/journal.pone.0072162

Bian, X., Liu, R., Meng, Y., Xing, D., Xu, D., and Lu, Z. (2020). Lipid metabolism and cancer. *J. Exp. Med.* 218, e20201606. doi:10.1084/jem.20201606

Bitler, B. G., Aird, K. M., Garipov, A., Li, H., Amatangelo, M., Kossenkov, A. V., et al. (2015). Synthetic lethality by targeting EZH2 methyltransferase activity in *ARID1A*-mutated cancers. *Nat. Med.* 21, 231–238. doi:10.1038/nm.3799

Brezis, M., and Rosen, S. (1995). Hypoxia of the renal medulla-Its implications for disease. *N. En.g J. Med.* 332, 647–655. doi:10.1056/NEJM199503093321006

Chen, C., Zhao, W., Lu, X., Ma, Y., Zhang, P., Wang, Z., et al. (2022). AUP1 regulates lipid metabolism and induces lipid accumulation to accelerate the progression of renal clear cell carcinoma. *Cancer Sci.* 113, 2600–2615. doi:10.1111/cas.15445

Courtney, K. D., Bezwada, D., Mashimo, T., Pichumani, K., Vemireddy, V., Funk, A. M., et al. (2018). Isotope tracing of human clear cell renal cell carcinomas demonstrates suppressed glucose oxidation *in vivo*. *Cell. Metab.* 28, 793–800. doi:10.1016/j.cmet.2018.07.020

Cruz, A. L. S., Barreto, E. D. A., Fazolini, N. P. B., Viola, J. P. B., and Bozza, P. T. (2020). Lipid droplets: platforms with multiple functions in cancer hallmarks. *Cell. Death Dis.* 11, 105. doi:10.1038/s41419-020-2297-3

Du, W., Zhang, L., Brett-Morris, A., Aguila, B., Kerner, J., Hoppel, C. L., et al. (2017). HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism. *Nat. Commun.* 8, 1769. doi:10.1038/s41467-017-01965-8

Ferraro, G. B., Ali, A., Luengo, A., Kodack, D. P., Deik, A., Abbott, K. L., et al. (2021). Fatty acid synthesis is required for breast cancer brain metastasis. *Nat. Cancer* 2, 414–428. doi:10.1038/s43018-021-00183-y

Huang, J., Chan, W. C., Ngai, C. H., Lok, V., Zhang, L., Lucero-Prisno, D. E., III, et al. (2022). Worldwide burden, risk factors, and temporal trends of ovarian cancer: a global study. *Cancers* 14, 2230. doi:10.3390/cancers14092230

Hwang, S.-H., Yang, Y., Jung, J.-H., and Kim, Y. (2022). Oleic acid from cancer-associated fibroblast promotes cancer cell stemness by stearoyl-CoA desaturase under glucose-deficient condition. *Cancer Cell. Int.* 22, 404. doi:10.1186/s12935-022-02824-3

Ji, J. X., Wang, Y. K., Cochrane, D. R., and Hutsman, D. G. (2018). Clear cell carcinomas of the ovary and kidney: clarity through genomics. *J. Pathol.* 244, 550–564. doi:10.1002/path.5037

Karpova, Y., Guo, D., Makhov, P., Haines, A. M., Markov, D. A., Kolenko, V., et al. (2021). Poly(ADP)-ribosylation inhibition: a promising approach for clear cell renal cell carcinoma therapy. *Cancers* 13, 4973. doi:10.3390/cancers13194973

Kato, N. (2020). Pathology of clear cell carcinoma of the ovary: a basic view based on cultured cells and modern view from comprehensive approaches. *Pathol. Int.* 70, 591–601. doi:10.1111/pin.12954

Kim, B., Gwak, J., Kim, M., Yang, S., Hwang, S., Shin, S., et al. (2023). Suppression of fatty acid oxidation supports pancreatic cancer growth and survival under hypoxic conditions through autophagy induction. *Cancer Gene Ther.* 30, 878–889. doi:10.1038/s41417-023-00598-y

Koizume, S., Ito, S., Nakamura, Y., Yoshihara, M., Furuya, M., Yamada, R., et al. (2015). Lipid starvation and hypoxia synergistically activate ICAM1 and multiple genes in an Sp1-dependent manner to promote the growth of ovarian cancer. *Mol. Cancer* 14, 77. doi:10.1186/s12943-015-0351-z

Koizume, S., Kanayama, T., Kimura, Y., Hirano, H., Takahashi, T., Ota, Y., et al. (2023). Cancer cell-derived CD69 induced under lipid and oxygen starvation promotes ovarian cancer progression through fibronectin. *Cancer Sci.* 114, 2485–2498. doi:10.1111/cas.15774

Koizume, S., and Miyagi, Y. (2016). Lipid droplets: a key cellular organelle associated with cancer cell survival under normoxia and hypoxia. *Int. J. Mol. Sci.* 17, 1430. doi:10.3390/ijms17091430

Koizume, S., Takahashi, T., Nakamura, Y., Yoshihara, M., Ota, Y., Sato, S., et al. (2022). Lipophagy-ICAM-1 pathway associated with fatty acid and oxygen deficiencies is involved in poor prognoses of ovarian clear cell carcinoma. *Br. J. Cancer* 127, 462–473. doi:10.1038/s41416-022-01808-4

Li, Y., Ran, Q., Duhan, Q., Jin, J., Wang, Y., Yu, L., et al. (2024). 7-Dehydrocholesterol dictates ferroptosis sensitivity. *Nature* 626, 411–418. doi:10.1038/s41586-023-06983-9

Liang, K. (2023). Mitochondrial CPT1A: insights into structure, function, and basis for drug development. *Front. Pharmacol.* 14, 1160440. doi:10.3389/fphar.2023.1160440

Little, R. A., Jamin, Y., Boult, J. K. R., Naish, J. H., Watson, Y., Cheung, S., et al. (2018). Mapping hypoxia in renal carcinoma with oxygen-enhanced MRI: comparison with intrinsic susceptibility MRI and pathology. *Radiology* 288, 739–747. doi:10.1148/radiol.2018171531

Ma, A. P. Y., Yeung, C. L. S., Tey, S. K., Mao, X., Wong, S. W. K., Ng, T. H., et al. (2021). Suppression of ACADM-mediated fatty acid oxidation promotes hepatocellular carcinoma via aberrant CAV1/SREBP1 signaling. *Cancer Res.* 81, 3679–3692. doi:10.1158/0008-5472.CAN-20-3944

Maiorano, B. A., Maiorano, M. F. P., and Maiello, E. (2023). Olaparib and advanced ovarian cancer: summary of the past and looking into the future. *Front. Pharmacol.* 14, 1162665. doi:10.3389/fphar.2023.1162665

Majuelos-Melguizo, J., Rodriguez-Vargas, J. M., Martinez-Lopez, N., Delgado-Bellido, D., Garcia-Diaz, A., Yuste, V. J., et al. (2022). Glioblastoma cells counteract PARP inhibition through pro-survival induction of lipid droplets synthesis and utilization. *Cancers* 14, 726. doi:10.3390/cancers14030726

Miess, H., Dankworth, B., Gouw, A. M., Rosenfeldt, M., Schmitz, W., Jiang, M., et al. (2018). The glutathione redox system is essential to prevent ferroptosis caused by impaired lipid metabolism in clear cell renal cell carcinoma. *Oncogene* 37, 5435–5450. doi:10.1038/s41388-018-0315-z

Minami, J. K., Morrow, D., Bayley, N. A., Fernandez, E. G., Salinas, J. J., Tse, C., et al. (2023). CDKN2A deletion remodels lipid metabolism to prime glioblastoma for ferroptosis. *Cancer Cell.* 41, 1048–1060.e9. doi:10.1016/j.ccell.2023.05.001

Nieman, K. M., Kenny, H. A., Penicka, C. V., Ladanyi, A., Buell-Gutbrod, R., Zillhardt, M. R., et al. (2011). Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat. Med.* 17, 1498–1503. doi:10.1038/nm.2492

Pang, J., Cui, J., Xi, C., Shen, T., Gong, H., Dou, L., et al. (2018). Inhibition of poly (ADP-ribose) polymerase increased lipid accumulation through SREBP1 modulation. *Cell. Phys. biochem.* 49, 645–652. doi:10.1159/000493028

Parida, P. K., Palencia, M. M., Ghosh, S., Khandelwal, N., Kim, K., Nair, V., et al. (2023). Limiting mitochondrial plasticity by targeting DRP1 induces metabolic reprogramming and reduces breast cancer brain metastases. *Nat. Cancer* 4, 893–907. doi:10.1038/s43018-023-00563-6

Pike, L. S., Smift, A. L., Croteau, N. J., Ferrick, D. A., and Wu, M. (2011). Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells. *Biochim. Biophys. Acta* 1807, 726–734. doi:10.1016/j.bbabio.2010.10.022

Qiu, B., Ackerman, D., Sanchez, D. J., Li, B., Ochocki, J. D., Grazioli, A., et al. (2015). HIF2α-dependent lipid storage promotes endoplasmic reticulum homeostasis in clear-cell renal cell carcinoma. *Cancer Discov.* 5, 652–667. doi:10.1158/2159-8290.CD-14-1507

Quan, Y., Dai, J., Zhou, S., Zhao, L., Jin, L., Long, Y., et al. (2023). HIF2α-induced upregulation of RNASET2 promotes triglyceride synthesis and enhances cell migration in clear cell renal cell carcinoma. *FEBS OpenBio* 13, 638–654. doi:10.1002/2211-5463.13570

Roy, D., Mondal, S., Khurana, A., Jung, D.-B., Hoffmann, R., He, X., et al. (2017). Loss of HSulf-1: the missing link between autophagy and lipid droplets in ovarian cancer. *Sci. Rep.* 7, 41977. doi:10.1038/srep41977

Sawyer, B. T., Qamer, L., Yamamoto, T. M., McMellen, A., Watson, Z. L., Richer, J. K., et al. (2020). Targeting fatty acid oxidation to promote anoikis and inhibit ovarian cancer progression. *Cancer Res.* 18, 1088–1098. doi:10.1158/1541-7786.MCR-19-1057

Shi, J., Lv, Q., Miao, D., Xiong, Z., Wei, Z., Wu, , et al. (2024). HIF2α promotes cancer metastasis through TCF7L2-dependent fatty acid synthesis in ccRCC. *Research* 7, 0322. doi:10.34133/research.0322

Sung, P.-L., Chang, Y.-H., Chao, K.-C., Chuang, C.-M., and Task Force on Systematic Review and Meta-analysis of Ovarian Cancer (2014). Global distribution pattern of histological subtypes of epithelial ovarian cancer: a database analysis and systematic review. *Gynecol. Oncol.* 133, 147–154. doi:10.1016/j.ygyno.2014.02.016

Tirado-Vélez, J. M., Joumady, I., Sáez-Benito, A., and Cózar-Castellano, P. G. (2012). Inhibition of fatty acid metabolism reduces human myeloma cells proliferation. *PLOS ONE* 7, e46484. doi:10.1371/journal.pone.0046484

Torre, L. A., Trabert, B., DeSantis, C. E., Miller, K. D., Samimi, G., Runowicz, C. D., et al. (2018). Ovarian cancer Statistics. *CA.Cancer J. Clin.* 68, 284–296. doi:10.3322/caac.21456

Turner, N., Tutt, A., and Ashworth, A. (2004). Hallmarks of 'BRCAness' in sporadic cancers. *Nat. Rev. Cancer* 4, 814–819. doi:10.1038/nrc1457

Wang, L., Li, C., Song, Y., and Yan, Z. (2020). Inhibition of carnitine palmitoyl transferase 1A-induced fatty acid oxidation suppresses cell progression in gastric cancer. *Arch. Biochem. Biophys.* 696, 108664. doi:10.1016/j.abb.2020.108664

Xie, R., Nguyen, S., McKeehan, K., Wang, F., McKeehan, W. L., and Liu, L. (2011). Microtubule-associated protein 1S (MAP1S) bridges autophagic components with microtubules and mitochondria to affect autophagosomal biogenesis and degradation. *J. Biol. Chem.* 286, 10367–10377. doi:10.1074/jbc.M110.206532

Xu, G., jiang, Y., Xiao, Y., Liu, X.-D., Yue, F., Yue, F., et al. (2016). Fast clearance of lipid droplets through MAP1S-activated autophagy suppresses clear cell renal cell carcinomas and promotes patient survival. *Oncotarget* 7, 6255–6265. doi:10.18632/oncotarget.6669

Xu, Y., Wu, G., Ma, X., Li, J., Ruan, N., Zhang, Z., et al. (2020). Identification of CPT1A as a prognostic biomarker and potential therapeutic target for kidney renal clear cell carcinoma and establishment of a risk signature of CPT1A-related genes. *Int. J. Genom.* 2020, 9493256. doi:10.1155/2020/9493256

Yue, F., Li, W., Zou, J., Jiang, X., Xu, G., Huang, H., et al. (2017). Spermidine prolongs lifespan and prevents liver fibrosis and hepatocellular carcinoma by activating MAP1S-mediated autophagy. *Cancer Res.* 77, 2938–2951. doi:10.1158/0008-5472.CAN-16-3462

Zeng, Q. Q. F., Liu, X., Wang, Q. J., Deng, F., and Luo, X. (2016). Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. *Cell. Death Dis.* 7, e2266. doi:10.1038/cddis.2016.167

Zhang, R., Peng, X., Du, J. X., Boohaker, R., Estevao, I. L., Grajeda, B. I., et al. (2023). Oncogenic $KRAS^{G12D}$ reprograms lipid metabolism by upregulating SLC25A1 to drive pancreatic tumorigenesis. *Cancer Res.* 83, 3739–3752. doi:10.1158/0008-5472.CAN-22-2679

Zhang, X., Saarinen, A. M., Hitosugi, T., Wang, Z., Wang, L., Ho, T. H., et al. (2017). Inhibition of intracellular lipolysis promotes human cancer cell adaptation to hypoxia. *eLIFE* 6, e31132. doi:10.7554/eLife.31132

Zhou, L., Luo, Y., Liu, Y., Zeng, Y., Tong, J., Li, M., et al. (2023). Fatty acid oxidation mediated by malonyl-CoA decarboxylase represses renal cell carcinoma progression. *Cancer Res.* 83, 3920–3939. doi:10.1158/0008-5472.CAN-23-0969

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Past and present: a bibliometric study on the treatment of recurrent ovarian cancer

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Background: Ovarian cancer (OC) is a gynecological malignancy with a high mortality rate worldwide. The unfavorable prognosis of OC is mainly attributed to the recurrent propensity. Recently, mortality from OC has exhibited a downward trend. These favorable patterns are likely to be driven by advancements in novel therapeutic regimens. However, there is a lack of visualize analysis of the application of these new drugs on women with recurrent OC (ROC). Therefore, we aimed to provide a bibliometric analysis of the evolving paradigms in the ROC treatment.

Methods: Documents on ROC treatment were systematically collected from the MEDLINE database and Web of Science Core Collection (WOSCC). The retrieved documents were exported in the plain text file format, and files were named and saved to the paths specified by the Java application. Microsoft Excel (version 2010), Citespace (6.2.R4) and VOSviewer (1.6.19) were used for data analysis, and included the following: 1) annual publication trend; 2) contributions of countries, institutions and authors; 3) co-citation of journals and references; and 4) co-occurrence of keywords.

Results: A total of 914 documents published in the MEDLINE and 9,980 ones in WOSCC were retrieved. There has been an upward trend in the productivity of publications on ROC treatment on by years. The United States was the leading contributor in this field, and the University of Texas System stood out as the most productive institution. Giovanni Scambia and Maurie Markman were the research leaders in the field of ROC treatment. The journal *Gynecologic Oncology* had the highest citation frequency. The reference entitled with "Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer" got highest centrality of 0.14 in the co-citation network. Keyword analysis revealed that the focus of current ROC treatment was on platinum-based anticancer drugs, paclitaxel, angiogenesis inhibitors (AIs), immune checkpoint inhibitors (ICIs) and poly (ADP-ribose) polymerase inhibitors (PARPis).

Conclusion: Scholars from a multitude of countries have been instrumental in the advancement of ROC treatment. The research hotspots and trend in the field of

Abbreviations: OC, ovarian cancer; ROC, recurrent OC; WOSCC, web of science core collection; AIs, angiogenesis inhibitors; PARPis, poly (ADP-ribose) polymerase inhibitors; FDA, food and drug administration; PFS, progression-free survival; IF, impact factor; PFI, platinum-free interval; PSR, platinum-sensitive recurrent; PRR, platinum-resistant recurrent; ICIs, immune checkpoint inhibitors; VEGF, vascular endothelial growth factor; PD-1, programmed cell death 1; *gBRCA*, germline *BRCA*; OS, overall-free survival; HRD, homologous recombination deficiency.

predominantly originated from leading international journals and specialized periodicals focused on gynecologic oncology. Maintenance therapy using AIs or (and) PARPis has emerged as a significant complement to platinum-based chemotherapy for patients with ROC.

KEYWORDS

recurrent ovarian cancer, bibliometric analysis, platinum-based chemotherapy, angiogenesis inhibitors, poly (ADP-ribose) polymerase inhibitors

1 Introduction

Ovarian cancer (OC) is a gynecological malignancy with high mortality. In China, the crude and age-standardized death rates of OC have risen to 9.49/100,000 and 6.02/100,000, and it has become the leading cause of death in the female reproductive tract tumors (Zheng et al., 2023). The unfavorable prognosis of OC is mainly attributed to the advanced disease stage detection and recurrent propensity. The standard therapeutic regimen for patients with advanced ovarian cancer is cytoreductive surgery followed by platinum-based chemotherapy (Moore et al., 2018). Surgical cytoreduction of advanced stage ovarian cancer, also termed "tumor debulking," is defined as an attempt to maximally resect all visible and palpable disease. The procedure includes, but is not limited to, hysterectomy and salpingo-oophorectomy, peritonectomy with or without gastrointestinal surgery, lymph node dissection, omentectomy and upper abdominal surgery (Polcher et al., 2014). Due to the underestimated incidence of hepatobiliary involvement in advanced OC, diaphragms and porta hepatis should be also explored during cytoreductive surgery to identify potentially undetected disease at preoperative instrumental examinations (Di Donato et al., 2021). The Gynecologic Oncology Group defined a maximum tumor diameter of 1 cm or less as an "optimal debulking" status. Approximately 70% of patient will have a relapse within the subsequent few years, despite a complete response to the optimal debulking surgery accompanied by chemotherapy (Richardson et al., 2023). Recurrent ovarian cancer (ROC) is rarely curable, with most patients receiving multiple additional lines of treatment before ultimately dying from the disease (Moore et al., 2018). The dismal destiny of patients with ROC has changed little over the past three decades.

Nevertheless, mortality of ROC has exhibited a downward trend in recent years, especially in the western countries. Accelerated declines of ROC mortality could be observed from 2017 to 2020 (Siegel et al., 2023). The age-standardize death rate of ROC fell by 6% in 2022, reaching 4.3 deaths per 100,000 individuals. This decline is predicted to continue until at least 2025 (Dalmartello et al., 2022; Wojtyła et al., 2023). These favorable patterns likely find their main driving factors for advancements in novel therapeutic regimens (Wojtyła et al., 2023). It is therefore necessary to identify the new drugs that work and to understand their evolving paradigms in the treatment of ROC. Compared to the narrative reviews, the bibliometric review could comprehensively include related studies and provide quantitative results/In comparison to narrative reviews, bibliometric reviews have the capacity to encompass a wide range of relevant studies, and present quantitative and visualized findings in a comprehensive manner (Cai et al., 2023). Therefore, in this study, we aim to perform a bibliometrics analysis to present the evolution and current status of ROC treatment, providing researchers with hotspots and frontiers in the field.

2 Methods

2.1 Data retrieval

We systematically searched for the documents about ROC treatment in the MEDLINE database via the Pubmed website (https://pubmed.ncbi.nlm.nih.gov/) and Web of Science Core Collection (WOSCC) (https://www.webofscience.com/wos/woscc/basic-search). The retrieved publications were required to meet the inclusion criteria: 1) the search terms were determined by the TS ("topic," including title, abstract, and keywords) as TS = ("ovarian cancer*" OR "ovarian neoplasm*" OR "ovarian carcinoma") AND TS = ("recurren*" OR "relapse*") AND TS = ("therap*" OR "treatment*" OR "management"); 2) the period of publication spanned from 1960 to 2023; 3) the article language was limited to English; 4) the following information should be found: publication, authors, countries, institutions, journals, keywords, and citations. The literature obtained was screened based on the following exclusion criteria: publications unrelated to the topic, articles not officially published, meeting summary, repeated articles and incomplete articles. Two authors (Wen-wei Song and Miao-ling Li) independently conducted the data retrieval. Discrepancies were solved through discussion, and when needed a third researcher (Yi Guo) was consulted. Ethics approval and consent to participate were not applicable for the study, since we retrospectively searched the data from public databases.

2.2 Data export

The retrieved documents were exported in the format of plain text file. One file comprised 500 records, and each record included author (s), title, publication year, document source, abstract, addresses, affiliations, document type, keywords, cited references, and total citations. Files were named and saved to the paths specified by the Java application.

2.3 Data analysis

Microsoft Excel (version 2010), Citespace (6.2.R4) and VOSviewer (1.6.19) were used for data analysis. We recorded the

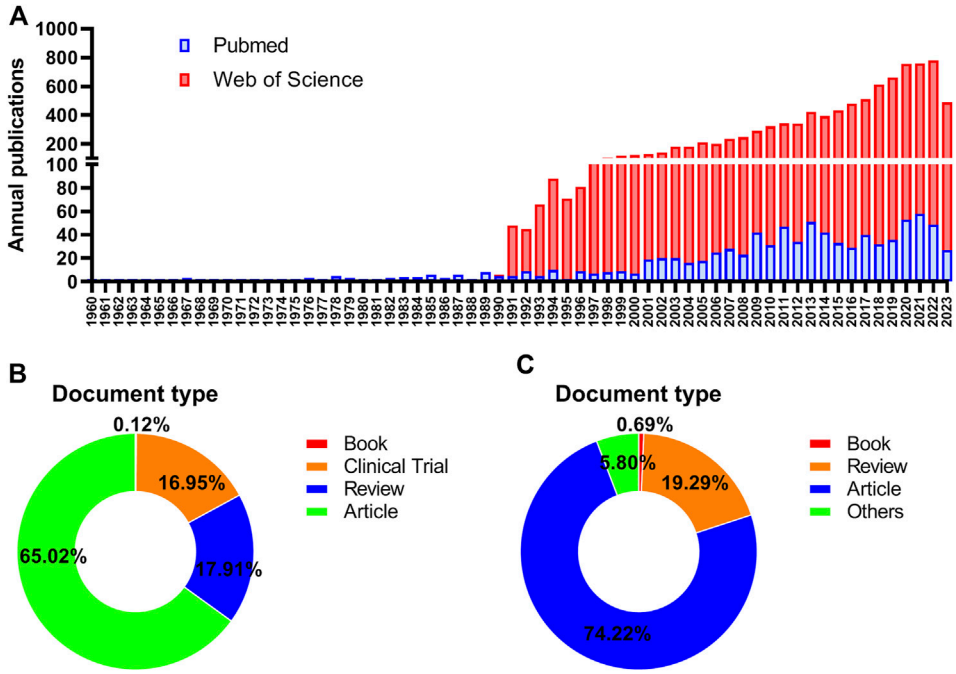

FIGURE 1
Annual publications in the field of ROC treatment **(A)** Annual number trend of publications about recurrent ovarian cancer and therapy in the Pubmed and Web of Science database **(B)** The distribution of document type in the Pubmed database **(C)** The distribution of document type in the Web of Science database. Note: ROC: recurrent ovarian cancer.

numbers of published documents yearly and presented the annual publication trend via Microsoft Excel. Citespace was utilized to evaluate the contributions of countries, institutions and authors to the ROC treatment, as well as the co-citation of journals and references. The co-occurrence of keywords in the field was depicted in the forms of cluster analysis, hotspot distribution and evolution tendency by VOSviewer.

3 Results

3.1 Annual publication trends

In the light of our search strategies, a total of 914 documents pertaining to ROC treatment were collected in the MEDLINE database database spanning the years 1960–2023, while 9,980 ones were retrieved in the WOSCC database for the period between 1977 and 2023. Figure 1A showed the distribution of the related documents over the past few decades. Generally, there has been an upward trend in the productivity of publications by years. The ascent process exhibited two distinct phases: a period of rapid growth from 1990 to 1999, followed by a period of consistent increase from 2000 to 2022. The trend indicated that researches on the ROC treatment looked to usher a favorable turn after a period of frustration. In terms of the document type, original articles accounted for above two-thirds (65.02%) in the MEDLINE and almost three-quarters (74.22%) in the WOSCC. The proportion of other types could be seen in the Figures 1B, C.

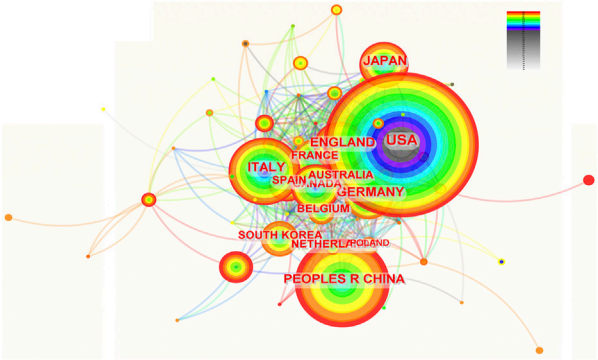

FIGURE 2
The network of countries and institutions involved in ROC treatment. Notes: ROC, recurrent ovarian cancer. Each node represents each country. The size of nodes represents the number of publications. The color of the layer of nodes represents the year of publication. The connection between nodes represents the cooperation between countries. The color of the connecting line represents the cooperation time.

3.2 The contributions of countries/regions in the research of ROC treatment

Scholars from 66 countries/regions have authored at least one academic paper pertaining to ROC. Figure 2 depicted the contributions of these countries and the connections among them. The top 10 countries ranked by the number of publications were the United States, China, Italy, England,

TABLE 1 The top 10 countries contributing to the research of ROC treatment.

Rank	Country	Counts	Centrality	Year
1	The United States	3,499	0.08	1993
2	China	1,120	0.01	2000
3	Italy	1,065	0.08	1993
4	England	785	0.04	1993
5	Germany	785	0.02	1993
6	Japan	677	0.01	1993
7	France	588	0.06	1993
8	Canada	568	0.04	1993
9	Australia	402	0.06	1993
10	Spain	392	0.01	1997

ROC, recurrent ovarian cancer.

Germany, Japan, France, Canada, Australia and Spain (Table 1). It is worth noting that the developed countries have made the major contributions to the publications, though China ranked second with 1,120 records. In addition, the United States and Italy achieved the highest centrality (0.08), followed by France and Australia (centrality = 0.06) (Table 1). These nations were instrumental in advancing research in this field and were seen as conduits for disseminating the innovative ethos to other regions.

3.3 The contributions of institutions in the research of ROC treatment

A total of 109 institutions were involved in the research of ROC treatment independently or by collaboration. Figure 3 portrayed the contributions of these organizations and the relations between each other. The top 10 institutions listed by the productivity of publications were University of Texas System, UT MD Anderson Cancer Center, Harvard University, French Research Universities (UDICE), University of California System, Memorial Sloan Kettering Cancer Center, University of London, Catholic University of the Sacred Heart, University of Toronto and Dana-Farber Cancer Institute (Table 2). Among the top 10 organizations, six were from United States, which reflected its great scientific strength in this area. However, the institution with highest centrality (0.13) was University of London from England, followed by University of California System (centrality = 0.12) and Harvard University (centrality = 0.11) (Table 2).

3.4 The contributions of authors in the research of ROC treatment

The number of authors with more than two papers in the field of ROC treatment was 162. The contributions of these authors and the pattern of interactions among them were delineated in the Figure 4. The top 10 authors with the most

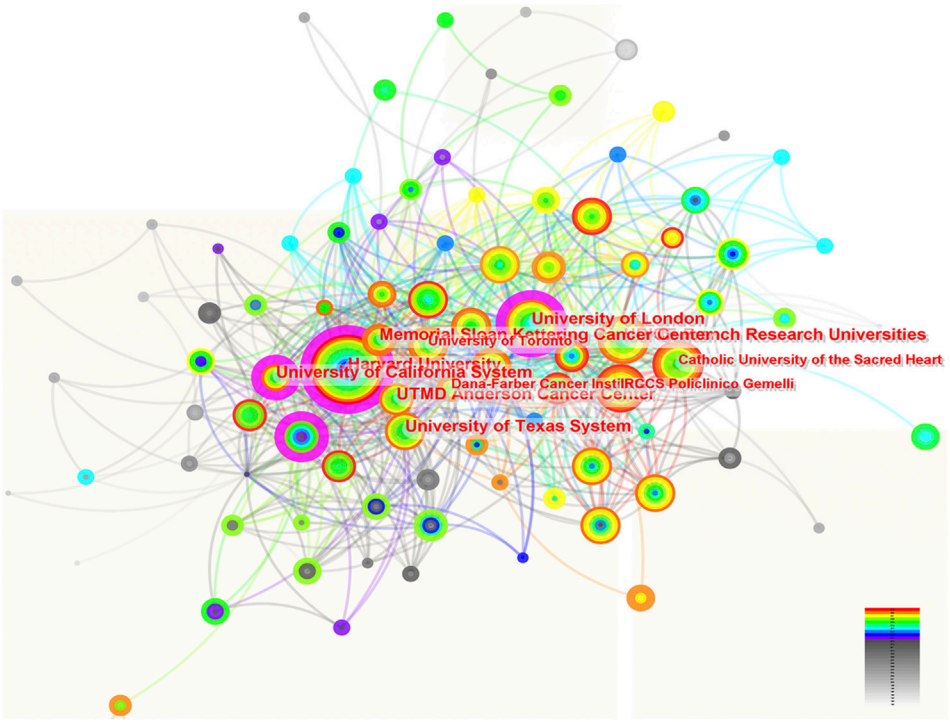

FIGURE 3
The network of institutions involved in ROC treatment. Notes: ROC, recurrent ovarian cancer. Each node represents each institution. The size of nodes represents the number of publications. The color of the layer of nodes represents the year of publication. The connection between nodes represents the cooperation between institutions. The color of the connecting line represents the cooperation time.

TABLE 2 The top 10 institutions contributing to the research of ROC treatment.

Rank	Institution	Counts	Centrality	Year
1	University of Texas System	506	0.09	1994
2	UT MD Anderson Cancer Center	435	0.09	1995
3	Harvard University	426	0.11	1994
4	French Research Universities (UDICE)	302	0.08	1996
5	University of California System	280	0.12	1994
6	Memorial Sloan Kettering Cancer Center	271	0.08	1993
7	University of London	254	0.13	2004
8	Catholic University of the Sacred Heart	241	0.04	2003
9	University of Toronto	228	0.06	2000
10	Dana-Farber Cancer Institute	201	0.08	2001

ROC, recurrent ovarian cancer.

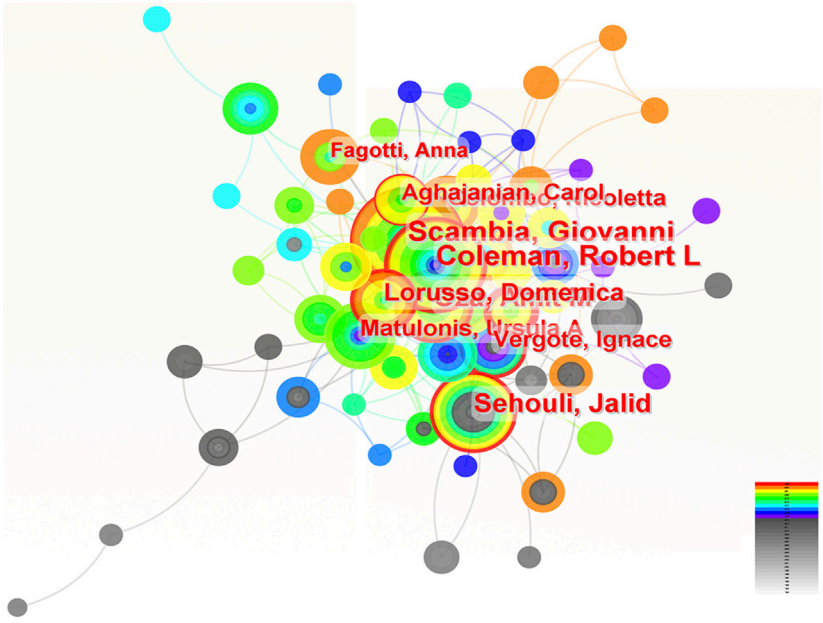

FIGURE 4
The analysis of authors dedicated to ROC treatment. Notes: ROC, recurrent ovarian cancer. Each node represents each author. The size of nodes represents the number of published documents. The color of the layer of nodes represents the year of publication. The connection between nodes represents the cooperation between authors. The color of the connecting line represents the cooperation time.

amounts of publications were Giovanni Scambia, Robert L Coleman, Jalid Sehouli, Amit M Oza, Domenica Lorusso, Ursula A Matulonis, Nicoletta Colombo, Carol Aghajanian, Ignace Vergote and Anna Fagotti (Table 3). Among them, four were from Italy, three from the United States, one from Germany, one from Canada and one from Belgium. Researchers from developed countries were the backbone in the field, and Robert L Coleman from the United States occupied the core position in the network (centrality = 0.07), followed by Giovanni Scambia from Italy (centrality = 0.06) and Ursula A Matulonis from the United States (centrality = 0.05) (Table 3).

3.5 The analysis of co-cited authors in the field of ROC treatment

In total, 253 authors were co-cited by multiple articles due to their excellent research achievements in the field of ROC treatment. The pattern of citation for these authors and their cooperation were showed in the Figure 5. The top 10 authors with the most co-citations were Maurie Markman, Eric Pujade-Lauraine, Robert F Ozols, Andreas du Bois, Robert L Coleman, Robert A Burger, Rebecca L Siegel, Ignace Vergote, William P McGuire and Nicoletta Colombo (Table 4). They were all from developed countries, and six of them were from the United States. Research findings from Maurie Markman were well

TABLE 3 The top 10 authors contributing to the research of ROC treatment.

Rank	Author	Count	Centrality	Year
1	Giovanni Scambia	119	0.06	2011
2	Robert L Coleman	110	0.07	2010
3	Jalid Sehouli	84	0.03	2008
4	Amit M Oza	65	0.02	2015
5	Domenica Lorusso	51	0.01	2017
6	Ursula A Matulonis	45	0.05	2012
7	Nicoletta Colombo	42	0.03	2015
8	Carol Aghajanian	38	0.02	2015
9	Ignace Vergote	35	0.02	2013
10	Anna Fagotti	32	0.00	2018

ROC, recurrent ovarian cancer.

recognized and widely cited by experts in the field, so he got the highest centrality (0.12) (Table 4). Andreas du Bois and Robert A Burger were tied for second (centrality = 0.08), and Ignace Vergote ranked third (centrality = 0.06) (Table 4).

3.6 The analysis of co-cited journals in the field of ROC treatment

Two hundred and eighty-three journals were co-cited by the literature on ROC treatment. The number of these journals cited and when cited can be seen in Figure 6. The top 10 journals ordered by frequency of citation were the *Gynecologic Oncology*, the *Journal of Clinical Oncology*, the *New England Journal of Medicine*, the *Cancer Research*, the *Annals of Oncology*, the *Clinical Cancer Research*, the *International Journal of Gynecological Cancer*, the *British Journal of Cancer*, the *Lancet* and the *Cancer* (Table 5). Two of the 10 journals fell into OBSTETRICS and GYNECOLOGY category, seven belonged to ONCOLOGY category and two were in the category of GENERAL MEDICINE. In addition, there were two journals with impact factors above 100.0 (the *New England Journal of Medicine* and the *Lancet*), 4 with impact factors between 10.0 and 100.0 (the *Annals of Oncology*, the *Journal of Clinical Oncology*, the *Clinical Cancer Research* and the *Cancer Research*) and 4 with impact factors below 10.0 (the *Gynecologic Oncology*, the *International Journal of Gynecological Cancer*, the *British Journal of Cancer* and the *Cancer*). Details could be seen in the Table 6. Remarkably, the focal point journal was the *Cancer Research* with a centrality score of 0.12, followed by the *Gynecological Cancer* (centrality = 0.06) and *England Journal of Medicine* (centrality = 0.04) (Table 5).

3.7 The analysis of co-cited references in the field of ROC treatment

Two hundred and fifteen papers were identified and cited as references in the studies focus on the ROC therapy. In Figure 7, the size of the nodes corresponded to the frequency of citation, the color layer of the nodes signified the year of citation, and the links connecting the nodes indicated that the two references were cited by the same paper. The top 10 references with most citations were listed in the Table 7. Among them, five were published on the *New England Journal of Medicine*, two were on the *Lancet*, two were on the *CA-A Cancer Journal for Clinicians* and one were on the *Journal of Clinical Oncology*. The publication dates of the 10 most cited references spanned from 2011 to 2020. The themes and subjects of these references mainly centered on maintenance therapy based on the poly (ADP-ribose) polymerase inhibitors (PARPis) (e.g., olaparib, niraparib and rucaparib), the angiogenesis inhibitors (AIs) (e.g., bevacizumab), and cancer statistics. The clinical trial titled "Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer" by (Mirza et al., 2016) published in the *New England Journal of Medicine* in 2016 held a prominent position in the co-citation network with a centrality score of 0.14.

3.8 The analysis of co-occurrence keywords about ROC treatment

We totally got 2,777 terms related to the treatment of ROC based on the minimum number of occurrences (10) and the relevance score (60%). In order to remove general terminology and categorize specific terms, a cluster analysis was performed. The specific keywords were sorted into five clusters, as showed in the Figure 8A. The yellow cluster represented combination therapy strategies utilizing the first-generation platinum-containing anticancer drug (cisplatin), the blue cluster served as combined modality therapy involving the second- and third-generation platinum-based chemotherapeutic agents (carboplatin and oxaliplatin) and paclitaxel, the purple cluster mainly meant the hormonal treatment, such as tamoxifen, the red cluster primarily spoke of the induction of immunotherapy, including anti-PD-1, AIs (bevacizumab) and anti-protein kinase receptors (cediranib and pazopanib), and the green cluster stood for the maintenance therapy based on PARPis (niraparib and rucaparib). The density map in Figure 8B indicated that while targeted therapy has gained increasing attention, cytotoxic drugs such as platinum agents and paclitaxel remain essential for the treatment of ROC. The overlay visualization in Figure 8C depicted the evolution trends of keywords in this area over time, suggesting the transitions from cytotoxic agents to targeted therapy drugs. From the timeline view in the Figure 8D, we found that bevacizumab and PARPis have gained popularity in the years 2010 and 2016, respectively. However, their close links with cytotoxic drugs implied the continued value of classical chemotherapy in the treatment of ROC.

4 Discussion

4.1 Main findings of the study

This study represents the first bibliometric analysis to investigate the evolution in the treatment of ROC from the 1960 to 2023. In the study, we visualized current global research landscape on ROC therapy from multiple perspectives, such as involved researchers, countries, institutions, co-cited journals and co-cited keywords. We expected that these findings could offer valuable information for therapeutic decision-making in ROC, and the principal findings included the following:

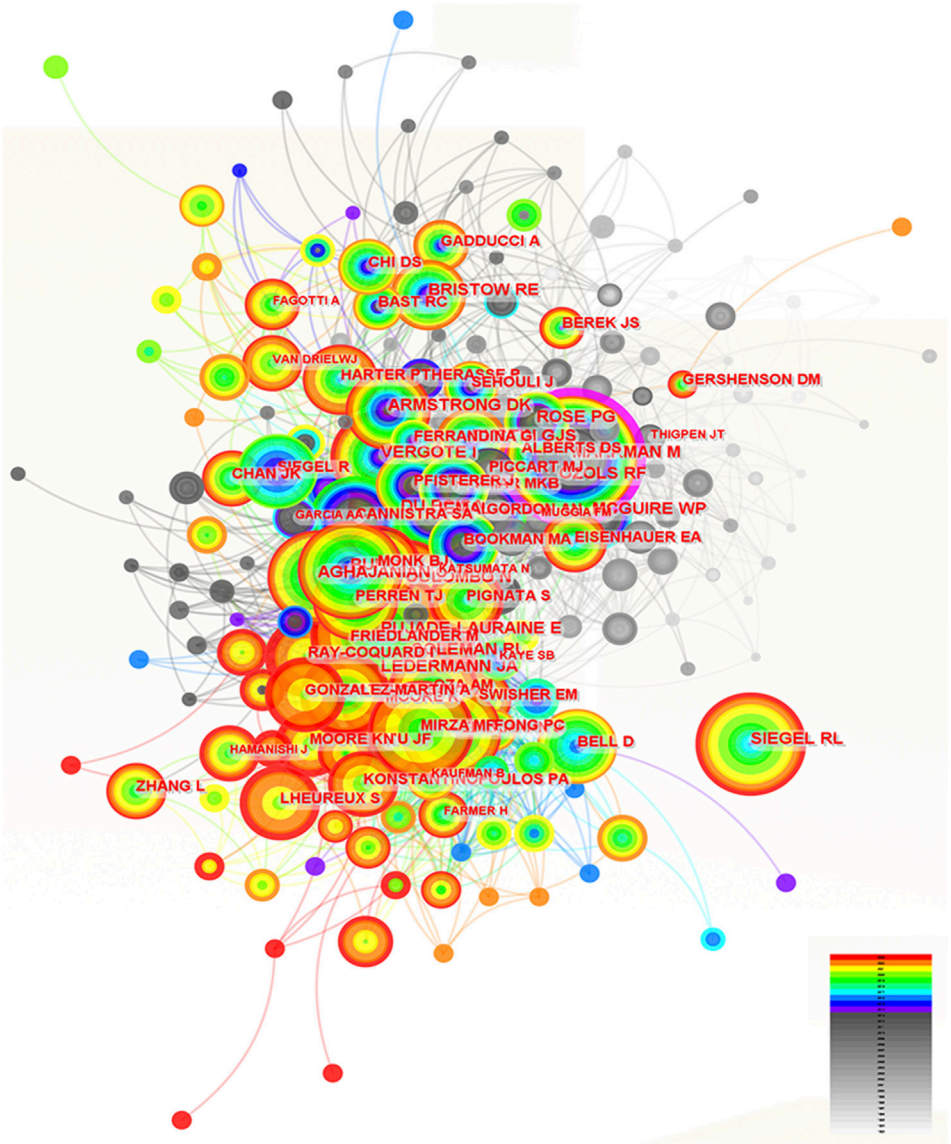

FIGURE 5
The analysis of co-cited authors dedicated to ROC treatment. Notes: ROC, recurrent ovarian cancer. Each node represents each author. The size of nodes represents the number of published documents. The color of the layer of nodes represents the year of publication. The connection between nodes represents the cooperation between authors. The color of the connecting line represents the cooperation time.

(1) Research on the treatment of ROC has shown a consistent upward trend in recent years, presenting the global challenge posed by ROC and effort from worldwide to combat this disease.
(2) Scholars and institutions from developed countries like the United States and Italy have made significant contributions in helping OC patients fight against recurrence; China, the sole developing country on the list, required increased cooperation with other countries.
(3) The co-cited journals in the field of ROC treatment predominantly consisted of prominent international journals and specialized periodicals dedicated to the study of gynecological oncology.
(4) The co-cited references primarily focused on assessing the efficacy of bevacizumab and PARPis as monotherapy or in combination on patients with ROC and newly diagnosed OC.
(5) While chemotherapy still occupied an important position in the treatment of ROC, targeted therapeutic agents like AIs, ICIs and PARPis have emerged as research hotspots and publication trends; traditional chemotherapy and targeted therapy have been closely linked in the field of ROC treatment.

4.2 Implications, comparison with literature and future directions

4.2.1 General information

Based on the annual publication trends, we found that research on ROC treatment has steadily increased over the

TABLE 4 The top 10 co-cited authors in the field of ROC treatment.

Rank	Co-cited author	Count	Centrality	Year
1	Maurie Markman	1,540	0.12	1993
2	Eric Pujade-Lauraine	1,221	0.05	2011
3	Robert F Ozols	1,006	0.04	1993
4	Andreas du Bois	975	0.08	2004
5	Robert L Coleman	967	0.05	2013
6	Robert A Burger	930	0.08	2006
7	Rebecca L Siegel	870	0.00	2010
8	Ignace Vergote	830	0.06	2001
9	William P McGuire	817	0.03	1993
10	Nicoletta Colombo	741	0.02	1999

ROC, recurrent ovarian cancer.

years and is projected to continue growing in 2023. Liu et al. (2023) also observed the upward trend in the number of publications in the past decade, but only in the field of OC and drug resistance. The first surge of studies in the field likely commenced in 1994, following the approval of paclitaxel was approved for the treatment of ROC by the United States Food and Drug Administration (FDA) (Menzin et al., 1994). Since then, numerous trials have been conducted to assess the efficacy of paclitaxel as salvage chemotherapy in patients with platinum-sensitive EOC and those with platinum-resistant disease (Christian and Trimble, 1994; Miglietta et al., 1997; Roland et al., 1998). Meanwhile, more agents like 5-fluorouracil, leucovorin, merbarone and tamoxifen, were being assessed in clinical trials; however, only a small subset of the patients gained benefits (Look et al., 1992; Look et al., 1996; Trope et al., 2000), transiently impeding the progression of the research in this field. It was not until the year 2005 that the growth in the number of publications began to resume. Pfisterer et al. (2005) demonstrated that gemcitabine significantly prolonged progression-free survival (PFS) of patients with platinum-sensitive recurrence (PSR) when used as a second-line combination therapy, which prompted the next accelerated approval by the United States FDA in 2006 (Shea et al., 2013). Subsequently, the availability of targeted therapy for solid tumors like lung, breast, colorectal and renal cancers encouraged gynecological oncologists to incorporate these non-cytotoxic agents into ROC regimen (Palazzo et al., 2010). Therefore, related publications from 2010 to 2020 have been characterized as a steady upward curve, during which the targeted therapy has ushered a new era for ROC treatment.

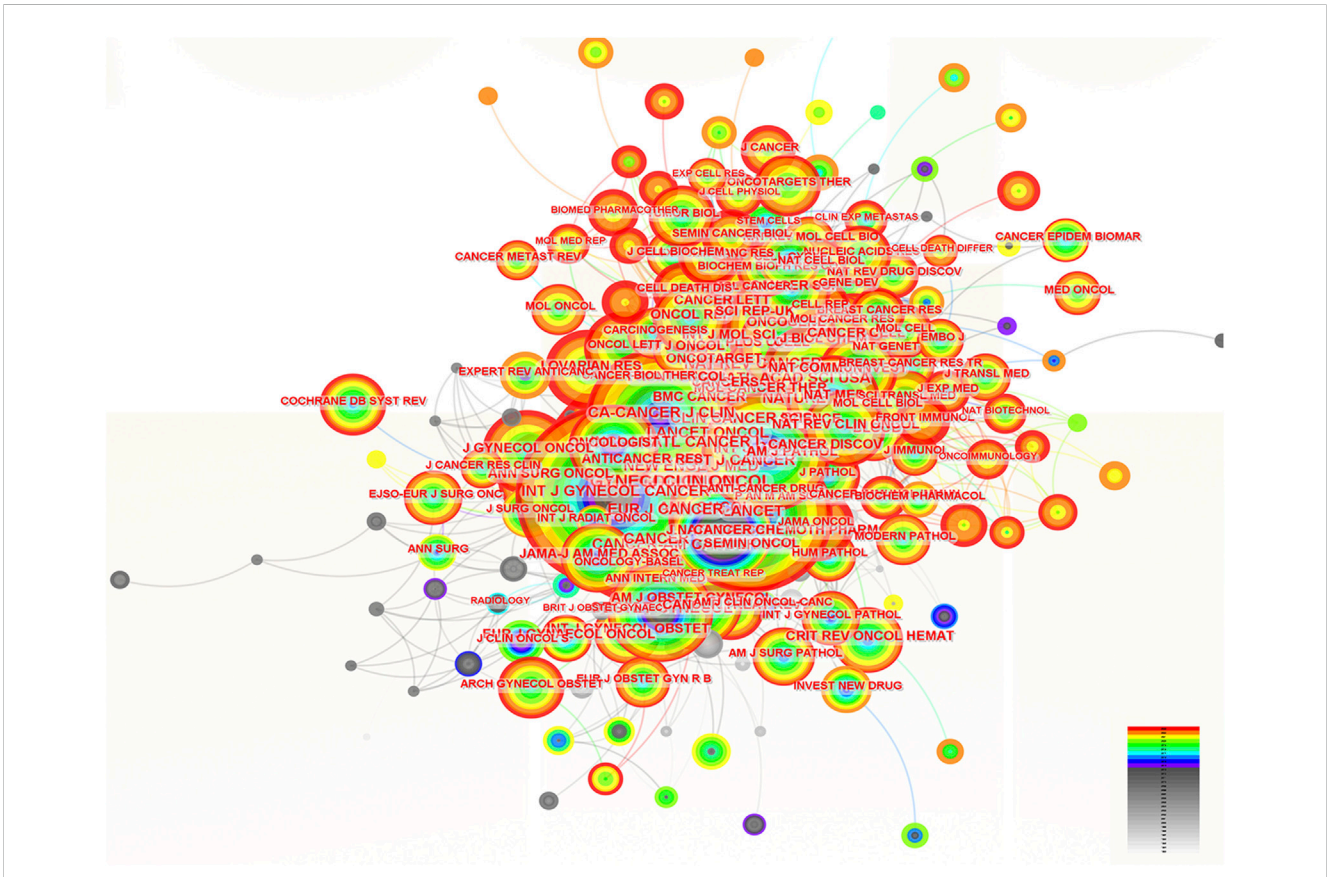

FIGURE 6
The analysis of co-cited journals related to ROC treatment. Notes: ROC, recurrent ovarian cancer. Each node represents each journal. The size of nodes represents the number of published documents. The color of the layer of nodes represents the year of publication.

TABLE 5 The top 10 co-cited journals in the field of ROC treatment.

Rank	Co-cited journal	Count	Centrality	Year
1	Gynecologic Oncology	6,927	0.06	1993
2	Journal of Clinical Oncology	6,579	0.03	1993
3	New England Journal of Medicine	4,606	0.04	1993
4	Cancer Research	4,007	0.12	1993
5	Annals of Oncology	3,974	0.03	1993
6	Clinical Cancer Research	3,859	0.02	1997
7	International Journal of Gynecolo gical Cancer	3,812	0.01	1998
8	British Journal of Cancer	3,543	0.03	1993
9	Lancet	3,021	0.01	1993
10	Cancer	2,995	0.02	1993

ROC, recurrent ovarian cancer.

TABLE 6 The impact factors of the top 10 co-cited journals in the field of ROC.

Rank	Co-cited journal	IF 2022–2023	IF 5 years
1	Gynecologic Oncology	4.7	5.0
2	Journal of Clinical Oncology	45.3	37.6
3	New England Journal of Medicine	158.5	115.7
4	Cancer Research	11.2	13.0
5	Annals of Oncology	50.5	32.4
6	Clinical Cancer Research	11.5	12.5
7	International Journal of Gynecological Cancer	4.8	4.0
8	British Journal of Cancer	8.8	8.4
9	Lancet	168.9	118.1
10	Cancer	6.2	6.8

ROC, recurrent ovarian cancer; IF, impact factors.

4.2.2 Contributions of the countries, the institutions, and the authors

Dozens of countries have dedicated significant effort and resources to improve ROC treatment, underscoring the global challenge posed by managing patients with ROC. The Gynecologic Cancer InterGroup (GCIG) consists of thirty-three clinical research groups that span the globe, and has organized an ovarian cancer consensus conference on clinical research including recurrent disease approximately every 5 years (Vergote et al., 2022). The East Asian Gynecologic Oncology Trial Group (EAGOT) was create to optimize ROC treatment across Japan, Korea, China and Taiwan (Kobayashi et al., 2024). Among them, the United States has become the leading stronghold to help ovarian cancer patients against recurrence: it contributed to the most publications with highest betweenness centrality; six of the top 10 institutions engaged in research on ROC were located in the United States; three authors and six co-cited authors in the ranking lists were American. Italy ranked three among the top 10 countries with the highest number of publications, with the same centrality score as the United States. These results are consistent with the systematic reviews, which have reported that researchers from both the United States and Italy have been actively involved in the majority of significant clinical trials that inform treatment protocols for ROC (Liu et al., 2022; Li et al., 2023). China was the sole developing country on the list, attributed to the government's recognition of the escalating annual mortality rates of ovarian cancer (Feng et al., 2023), leading to increased funding and research efforts in this area. The phase III NORA study has been conducted funded by the National Major Scientific and Technological Special Project for Significant New Drugs Development (grant number: 2018ZX09736019) to evaluate the efficacy and safety of niraparib for the treatment of Chinese patients with platinum-sensitive ROC (Wu et al., 2021). However, the centrality score of China was low, indicating the urgent need for collaboration. Indeed, clinical trials conducted by Chinese scholars mainly were single-center studies (Ni et al., 2021;

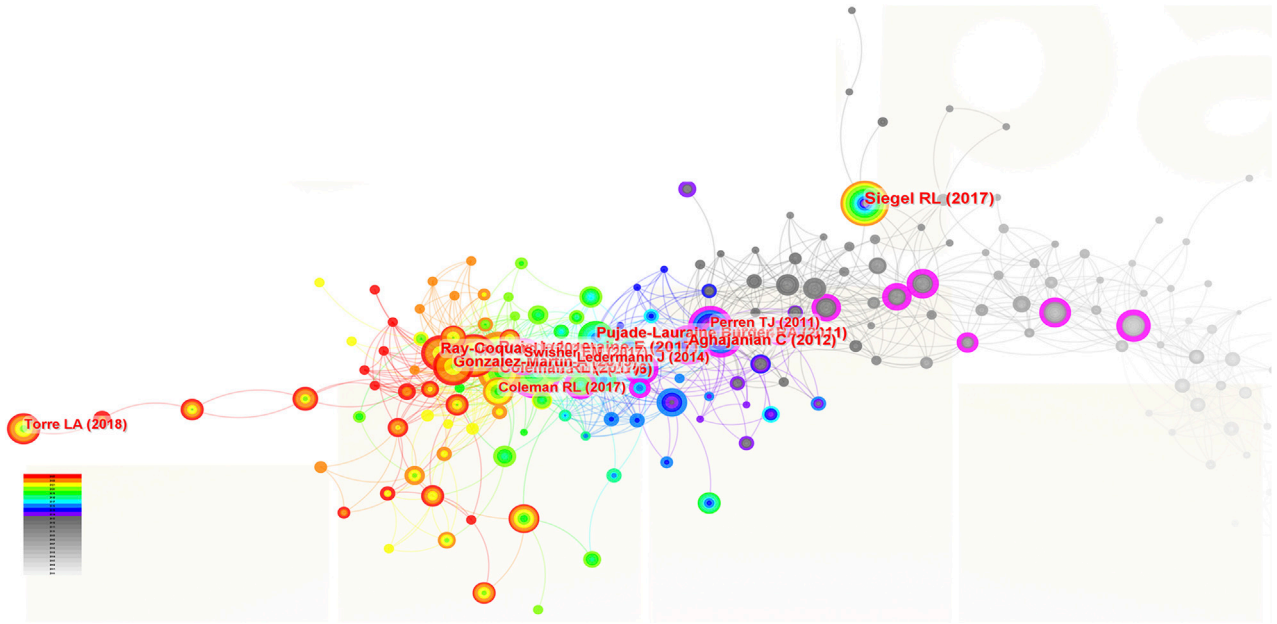

FIGURE 7
The analysis of co-cited references related to ROC treatment. Notes: ROC, recurrent ovarian cancer. Each node represents each reference. The size of nodes represents the number of published documents. The color of the layer of nodes represents the year of publication.

TABLE 7 The top 10 co-cited references in the field of ROC.

Rank	Title	Count	Centrality	Journal	Year	First author
1	Cancer statistics, 2017	571	0.05	CA-A Cancer Journal for Clinicians	2017	Rebecca L Siegel
2	Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer	421	0.04	New England Journal of Medicine	2018	Kathleen Moore
3	Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial	421	0.08	Lancet	2017	Eric Pujade-Lauraine
4	Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer	382	0.14	New England Journal of Medicine	2016	Mansoor R Mirza
5	Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial	368	0.05	Lancet	2017	Robert L Coleman
6	Niraparib in patients with newly diagnosed advanced ovarian cancer	312	0.03	New England Journal of Medicine	2019	Antonio González-Martín
7	Olaparib plus bevacizumab as first-line maintenance in ovarian cancer	286	0.02	New England Journal of Medicine	2020	Isabelle Ray-Coquard
8	Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the aurelia open-label randomized phase III trial	286	0.10	Journal of Clinical Oncology	2014	Eric Pujade-Lauraine
9	Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries	252	0.00	CA-A Cancer Journal for Clinicians	2018	Freddie Bray
10	Incorporation of bevacizumab in the primary treatment of ovarian cancer	251	0.13	New England Journal of Medicine	2011	Robert A Burger

ROC, recurrent ovarian cancer.

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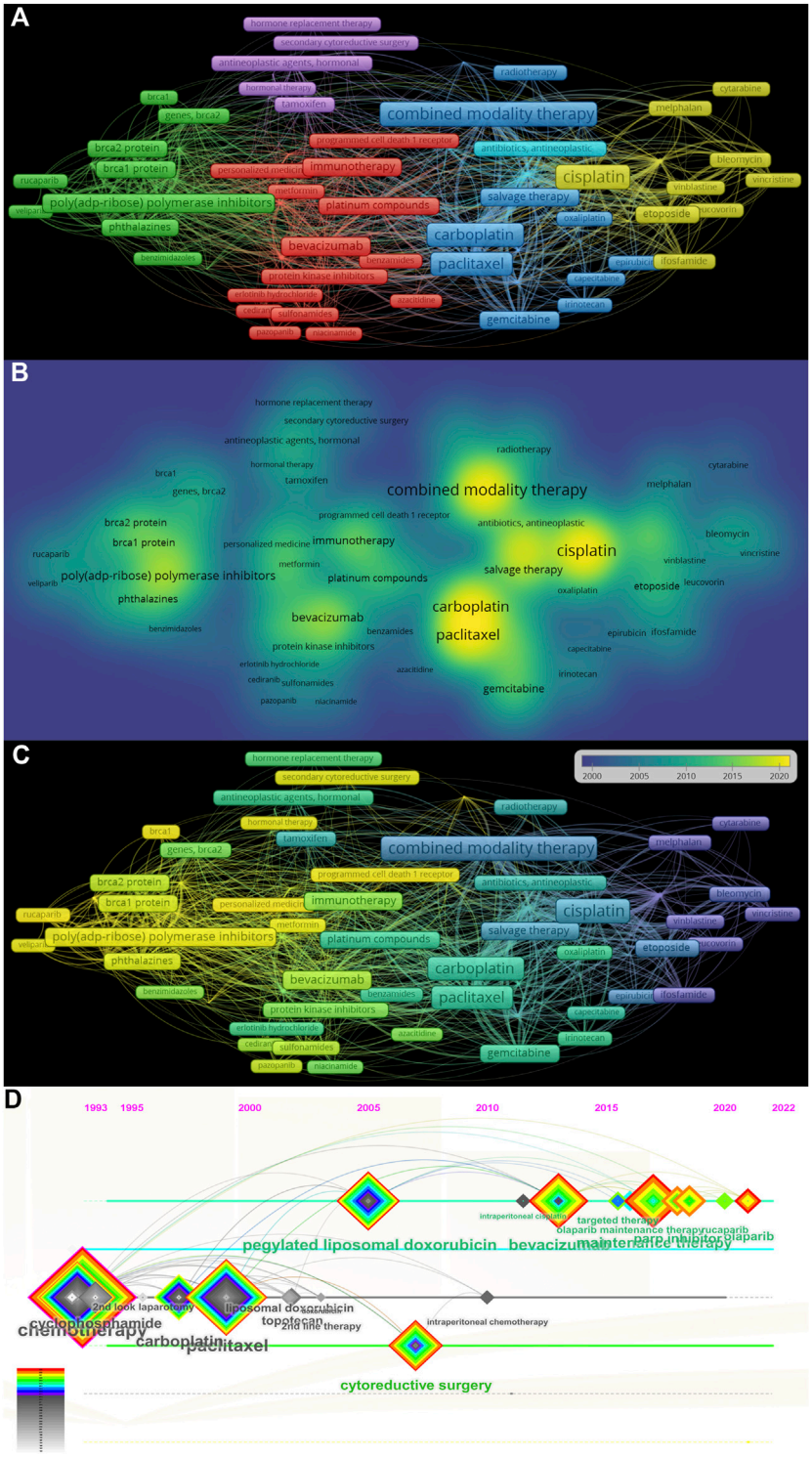

FIGURE 8
The analysis of co-occurrence keywords related to ROC treatment **(A)** The cluster view of co-occurrence keywords in the researches regarding recurrent ovarian cancer treatment **(B)** The density map of co-occurrence keywords **(C)** The evolution of the co-occurrence keywords **(D)** The timeline view of co-occurrence keywords. Note: ROC, recurrent ovarian cancer. The more frequently the keyword co-occur, its background color is closer to yellow in the **(B)**.

Wang et al., 2023). Other developed countries were centered on the United States and Italy, and work closely together. For instance, Amit M Oza from Canada has participated in the ARIEL3 study conducted by Giovanni Scambia (Italy) and Robert L Coleman (the United States) to evaluate the efficacy of rucaparib maintenance treatment for ROC (Coleman et al., 2017).

4.2.3 Analysis of co-cited journals and co-cited references

By analyzing the co-cited journals and co-cited references with high frequency, we could gain an insight into the source of the research trends and highlights within the field. This study identified the top ten most frequently co-cited sources.

Two of the top ten co-cited journals concerned gynecologic tumors (the *Gynecologic Oncology* and the *International Journal of Gynecological Cancer*), six were journals in cancer research and oncology (the *Journal of Clinical Oncology*, the *Cancer Research*, the *Annals of Oncology*, the *Clinical Cancer Research*, the *British Journal of Cancer* and the *Cancer*), and two were comprehensive medical periodicals (the *New England Journal of Medicine* and the *Lancet*). Similarly, Duan et al. (2023) found that the *Gynecologic Oncology* published the most papers about platinum-resistant ovarian cancer research, and the *Journal of Clinical Oncology* received the largest number of co-citations; The *New England Journal of Medicine of Medicine* published numerous studies highlighting significant advancements in the field of oncology (Tu et al., 2022). To be specific, the *New England Journal of Medicine* and the *Lancet* are renowned for publishing top-notch medical research, the *Journal of Clinical Oncology* and the *Annals of Oncology* concentrate on clinical trials evaluating the effectiveness of different anti-cancer medications, the *Clinical Cancer Research* and the *British Journal of Cancer* publish translational cancer research studies that bridge the laboratory and the clinic, the *Cancer Research* and the *Cancer* provide oncological studies on basic, clinical and epidemiological research, the *Gynecologic Oncology* and *International Journal of Gynecological Cancer* are devoted to the publications for topics relevant to the etiology, mechanism, diagnosis, and treatment of gynecologic malignancies. In addition, the journal with the highest impact factor (IF) in 2022–2023 is the *Lancet* (168.9), followed by the *New England Journal of Medicine* (IF = 158.5). There are two journals with IF > 40.0 (the *Annals of Oncology* and the *Journal of Clinical Oncology*), two with IF > 10.0 (the *Cancer Research* and the *Clinical Cancer Research*) and two with IF > 5.0 (the *British Journal of Cancer* and the *Cancer*). For the left two, the *Gynecologic Oncology* is the official publication of the Society of Gynecologic Oncology with the second highest centrality scores, and the *International Journal of Gynecological Cancer* is the official journal of the International Gynecologic Cancer Society and the European Society of Gynecological Oncology. These data indicated that the research hotspots in the field of ROC treatment predominantly originated from leading international journals and specialized periodicals focused on gynecologic oncology.

The references that ranked first and ninth pertained to statistical analysis of global cancer incidence and mortality. Given their relevance to the epidemiological characteristics of ovarian cancer, they were deemed essential for citation in the background section of each manuscript. The earliest published paper among the top 10 co-cited references was titled with "Incorporation of Bevacizumab in the Primary Treatment of Ovarian Cancer" issued on the *New England Journal of Medicine* in 2011. In this study, Burger et al. (2011) integrated bevacizumab into the standard front-line therapy and observed that the combination extended the median progression-free survival by approximately 4 months in patients with newly diagnosed advanced ovarian cancer. Three years later, Pujade-Lauraine et al. (2014) presented evidence from their AURELIA study demonstrating that bevacizumab enhanced the efficacy of chemotherapy for OC patients with platinum-resistant recurrence, which has received 286 citations according to our statistical analysis. These data have facilitated the approval of bevacizumab for the management of OC in 2018. The remaining six co-cited documents within the top ten list were all clinical trials pertaining to PARPis. Mirza et al. (2016) conducted a randomized, double-blind, phase III trial, designated as NOVA, to assess the efficacy of niraparib as a maintenance treatment for women with ROC. Their findings demonstrated that niraparib significantly prolonged the progression-free survival (PFS) duration (Mirza et al., 2016). Their related paper has rapidly garnered widespread attention, with the highest centrality in Table 6. The *New England Journal of Medicine* published an editorial asserting that PARP inhibitors possess the potential to revolutionize OC therapy in a unprecedented manner base on the results from the NOVA study (Spriggs and Longo, 2016). Subsequently, the SOLO-2 and ARIEL-3 studies demonstrated that both olaparib and rucaparib significantly improved progression-free survival (PFS) in patients with relapsed ovarian cancer, particularly among those harboring BRCA mutations (Pujade-Lauraine et al., 2017; Coleman et al., 2017). Articles from these two clinical trials soon achieved global recognition as well, and were ranked third and fifth among the top ten co-cited references, respectively. Given the promising outcomes observed in ROC, researchers have proceeded to incorporate PARPis into first-line therapy regimens. The second and sixth most popular references in Table 6 confirmed that patients with newly diagnosed advanced ovarian cancer could also benefit from olaparib and rucaparib (Moore et al., 2018; Gonzalez-Martin et al., 2019). Besides, there has been an increasing scholarly interest in the synergistic application of AIs and PARPis. Based on the findings of the PAOLA-1 study (Ray-Coquard et al., 2019), which was listed as seventh most cited reference in our analysis, the United States FDA approved the combination of olaparib and bevacizumab as a maintenance therapy for OC in 2022.

4.2.4 Analysis of the co-cited keywords

Through the application of co-occurrence analysis, it is possible to systematically cluster the keywords within the research domain, thereby enabling the observation of the evolution of research trends, identification of prominent research hotspots, and elucidation of the interconnections among various keywords.

4.2.4.1 The platinum-based chemotherapy

The entry "cisplatin" in the yellow cluster and "carboplatin" in the blue cluster, as depicted in Figure 8A, indicates the significance of platinum-based combination chemotherapy in the management of ROC. Based on the duration of the platinum-free interval (PFI), patients with ROC can be categorized into two groups: the PSR and the platinum-resistant recurrence (PRR). The Gynecologic Cancer InterGroup (GCIG) has stated that patients with OC who exhibit PSR are eligible for re-treatment with platinum-based agents (Friedlander et al., 2011). The first-generation platinum-based chemotherapeutic agent, cisplatin, has established the foundational framework for OC chemotherapy since its approval by the United States FDA in 1978. Carboplatin is closely related to cisplatin. However, carboplatin, the newer of the two, was somewhat less toxic than cisplatin, and has been used increasingly as the

front-line agent in clinical practice (Markman, 1994). Oxaliplatin, the third-generation platinum agent, was primarily utilized based on the prior clinical experience due to lack of large-scale clinical trials for OC. Single agents generally yield partial responses; consequently, it has become standard practice to administer multiple agents in combination. Initially, the preferred regimen consisted of either cisplatin or carboplatin along with alkylating agents (Markman, 1994), like cyclophosphamide, ifosfamide and melphalan in the yellow cluster. At present, the combination of platinum with paclitaxel, gemcitabine, or doxorubicin is recommended as the standard chemotherapy regimen for PSR (Baert et al., 2021).

OC patients with PRR have a poor prognosis due to few treatment options with limited efficacy. In the past, it was reasonable to try hormonal therapy, as shown in the purple cluster, when platinum-free chemotherapy would have a limited chance of success and a high likelihood of toxicity; however, the overall response rate is less than 15% (Markman, 1994). Fortunately, the advent of the era of targeted therapy in OC offers renewed hope to individuals affected by PRR OC.

4.2.4.2 The targeted therapy

Nowadays, researchers are increasingly realizing that management of ovarian cancer should be personalized based on the characteristic of the patient. For one thing, systematic lymphadenectomy is not recommended for women with ovarian cancer in early stage, especially for those affected by mucinous and low-grade serous histological subtype (Benedetti Panici et al., 2020). Accumulating evidences showed no survival benefits of lymphadenectomy among early-stage ovarian cancer patients. A multi-center randomized trial assessing the value of systematic lymphadenectomy in early ovarian cancer has revealed that there was no statistically significant difference in 5-year overall survival rates (84.0% versus 81.6%) between the lymphadenectomy group and the control group (Maggioni et al., 2006). Low-grade serous ovarian carcinoma exhibits a unique genetic profile characterized by *KRAS*/*BRAF* mutations compared to high-grade serous carcinoma, thus MEK inhibitors might be appropriate for the treatment of this malignancy (Perrone et al., 2024). For another, a comprehensive assessment is recommended prior to the management of an elderly person with ovarian cancer (Liontos et al., 2021). Elderly patients who are in good performance status should receive standard therapy identical to that of younger patients; In vulnerable elderly patients, the benefit/risk balance of surgery should be assessed, and various adapted chemotherapy modalities could be alternatives (Falandry and Gouy, 2019). Fader et al. compared the toxicities and outcomes of elderly ovarian cancer patients treated with standard-dose (carboplatin AUC 5-6 and paclitaxel 175 mg/m^2) versus reduced-dose chemotherapy (carboplatin AUC 4-5 and paclitaxel 135 mg/m^2), and found that reduced-dose carboplatin/paclitaxel may be better tolerated but equally effective as the standard regimen in elderly ovarian cancer patients (Fader et al., 2008). Similarly, the choice of the appropriate treatment regimen for ROC should also be decided on a case-by-case basis. Platinum-free interval, as a predictor of response to subsequent platinum re-treatment, has long been considered an essential factor to define treatment of recurrent ovarian cancer (Bergamini et al., 2019). Besides, the willingness for further therapy, age, general condition, comorbidities, extent and site of recurrent disease, and residual toxicity from previous treatments must be taken into consideration as well (Glajzer et al., 2020). Moreover, the availability of novel targeted therapies provides increased opportunities for implementing precision medicine in individuals with ROC.

Targeted therapy is characterized by the application of small-molecule drugs or monoclonal antibodies that specifically interact with molecules present on tumor cells or within their micro-environment to block cancer growth or spread, such as AIs, immune checkpoint inhibitors (ICIs) and PARPis.

Due to the strong correlation between vascular endothelial growth factor (VEGF) and OC, studies investigating the effects of AIs have been undertaken. In 2005, a 60-year-old woman with advanced, recurrent and refractory serous carcinoma of ovary firstly received the intravenous infusion of bevacizumab, and benefited an objective durable response lasting at least 5 months (Monk et al., 2005). Soon afterwards, the AURELIA study demonstrated that bevacizumab significantly increased the antitumor efficacy of paclitaxel in OC patients with PRR (Pujade-Lauraine et al., 2014). For those with PRS, bevacizumab combined with chemotherapy also significantly improved their objective response rate (ORR) and PFS from the data in the OCEANS study (Aghajanian et al., 2012). Therefore, bevacizumab has become the only AI approved for ROC treatment by the United States FDA, explaining its central position in our clustering analysis.

In contrast, ICIs now have limited efficacy for OC. Matulonis et al. (2019) concluded that single-agent pembrolizumab, a drug targeting the programmed cell death 1 (PD-1) receptor, showed modest activity in patients with ROC base on the KEYNOTE-100 study. However, our results showed that "immunotherapy" was still presented as a research hotspot in the co-cited keywords, indicating novel immunotherapeutic strategies for ovarian cancer are still an ongoing exploration in the clinical practice. Indeed, Drew et al. (2024) have declared that olaparib combined with durvalumab [a selective monoclonal antibody blocking programmed death-ligand 1 (PD-L1)] showed notable clinical activity in ovarian cancer patients with PSR in 2024. Therefore, combination therapies, particularly with PARPis, might be one of the future directions to enhance the benefit of immunotherapy. Further investigation is necessary to explore the selection of new ICI targets as well as non-immune targets. For the latter one, adoptive cell therapy might be an effective approach. Several clinical trials are currently ongoing in order to investigate the therapeutic efficacy of chimeric antigen receptor (CAR)-T cells targeting MUC16, mesothelin and folate receptor α on patients with ROC (Yang et al., 2020).

The introduction of PARPis has significantly transformed the landscape and paradigm of OC treatment. Clinical trials involving PARPis have made breakthroughs in the field of ROC maintenance treatment as well, holding great promise for the individuals previously considered incurable. The SOLO2 study evaluated olaparib tablet maintenance treatment in platinum-sensitive, relapsed OC patients with a germline *BRCA* (*gBRCA*) mutation who had received at least two lines of previous chemotherapy, and demonstrated that olaparib provided a significant PFS and overall-free survival (OS)

improvement with no detrimental effect on quality of life (Pujade-Lauraine et al., 2017; Poveda et al., 2021). The OPINION analysis further confirmed that ROC patients without a *gBRCA* mutation also could gain clinical benefits from olaparib maintenance treatment (Poveda et al., 2022). Data from the NOVA trial revealed that patients with platinum-sensitive, recurrent ovarian cancer who were treated with niraparib experienced a significantly longer median progression-free survival (PFS) duration (Mirza et al., 2016). Researchers from China found that fuzuloparib maintenance therapy conferred a statistically significant and clinically meaningful improvement in PFS for patients with platinum-sensitive, recurrent ovarian cancer, regardless of g*BRCA 1/2* mutation base on the FZOCUS-2 study (Li et al., 2022). The keywords from aforementioned studies have been incorporated into our findings and visually represented through cluster analysis and timeline, emphasizing the current focus on PARPi and its significance in the realm of ROC therapy. Our findings have implications for clinical practice in the treatment of ROC. Wang et al. (2023) have confirmed that clinical application of PARPi as a maintenance therapy in Chinese patients with ROC was also effective in real world. In the future, research around the applications of PARPis in different scenarios for ROC treatment will be conducted. Recent research has demonstrated that surgery followed by maintenance treatment with PARP inhibitors may offer benefits in cases of recurrent ovarian cancer (Giannini et al., 2023); the NEO trial was performed to evaluate the pharmacodynamic effects of olaparib given prior to surgery for OC patients with PSR (2024 ASCO annual meeting, abstract No.: 5506). Moreover, PARPi resistance has become a problem that cannot be ignored. More than 40% of OC patients with *BRCA* mutation failed to benefit from PARPi, and 25%–50% of patients treated with PARPi will relapse (Li et al., 2020; Giannini et al., 2023). To enhance PARPi sensitivity, the optimal combination of PARPi and other treatment agents, such as oncolytic herpes simplex viruses (oHSVs), cyclin dependent kinases (CDK) inhibitors, ICIs and other DNA damage response-modifying drugs, should be considered (Li et al., 2020; Giannini et al., 2023).

4.2.4.3 Combination of platinum-based chemotherapy and targeted therapy

We observed the evolution of ROC treatment from platinum-based chemotherapy to targeted therapy (Figure 8C). Meanwhile, traditional chemotherapeutic agents like cisplatin, carboplatin and paclitaxel still emerged as high-frequency words as shown in Figure 8B. We also found the constant linkages between traditional chemotherapy and targeted therapy from Figure 8D. In 2022, the United States FDA has withdrawn the approval of olaparib and rucaparib as the mono-therapeutic agents for ROC patients who have been treated with three or more prior lines of chemotherapy (Lee et al., 2023). The action indicated that benefits from PARPis should be built on the response to the platinum-based chemotherapy, which was in line with our results. Hence, PARPi were authorized for maintenance therapy with the goal of extending the benefits associated with chemotherapy, possibly enhancing PFS and OS rates, while ensuring minimal impact on quality of life of patients (Giannini et al., 2023).

4.3 Strengths and limitations of the study

The study utilized software tools, including Citespace and VOSviewer, to quantitatively and visually illustrate the findings, providing a more comprehensive portrayal of the research themes and trends in the field of ROC treatment. We gathered significant clinical trials pertaining to the treatment decision-making process for ROC and shared the latest research findings in the oral presentations at the 2024 ASCO annual conference. In contrast to the study conducted by Liu et al. (2023), our bibliometric analysis not only included the therapeutic strategies for OC patients with PRR, but scrutinized the treatment advancements for those with PSR.

This study surely has several limitations. Firstly, we limited our search to the MEDLINE database and the WOSCC, potentially resulting in the omission of certain articles. Fortunately, our study was deemed adequate for summarizing the research on ROC treatment due to the inclusion of over 10,000 articles. Secondly, lack of literature screen might lead to information redundancy. To mitigate potential shortcomings, extraneous terms were removed prior to conducting program analysis using the Citespace and VOSviewer software. Thirdly, bibliometric tools based on machine learning and natural language processing have the potential to introduce inherent system errors. For instance, it is not uncommon for multiple authors, particularly those of Chinese descent, to have identical names, which might result in the discrepancy of the data of the authors' publications.

5 Conclusion

In summary, the bibliometric analysis revealed a consistent annual increase in the quantity of scholarly articles pertaining to the ROC treatment worldwide, beginning in 1990. Researchers from developed nations such as the United States and Italy, as well as developing countries like China took active part in advancing research on the treatment of ROC. Prominent international journals and professional periodicals focusing on gynecologic cancer have served as primary sources for the latest advancements and trends in the field by publishing large-scale clinical trials. Maintenance therapy using AIs or (and) PARPis has emerged as a significant complement to platinum-based chemotherapy for patients with ROC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

X-yH: Conceptualization, Investigation, Methodology, Project administration, Writing–original draft. W-wS: Project administration, Resources, Supervision, Validation, Writing–review and editing. M-lL: Data curation, Formal Analysis, Software, Visualization, Writing–original draft. YG: Conceptualization, Funding acquisition, Supervision, Validation, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aghajanian, C., Blank, S. V., Goff, B. A., Judson, P. L., Teneriello, M. G., Husain, A., et al. (2012). OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J. Clin. Oncol.* 30 (17), 2039–2045. doi:10.1200/JCO.2012.42.0505

Baert, T., Ferrero, A., Sehouli, J., O'Donnell, D. M., González-Martín, A., Joly, F., et al. (2021). The systemic treatment of recurrent ovarian cancer revisited. *Ann. Oncol.* 32 (6), 710–725. doi:10.1016/j.annonc.2021.02.015

Benedetti Panici, P., Giannini, A., Fischetti, M., Lecce, F., and Di Donato, V. (2020). Lymphadenectomy in ovarian cancer: is it still Justified? *Curr. Oncol. Rep.* 22 (3), 22. doi:10.1007/s11912-020-0883-2

Bergamini, A., Bocciolone, L., Fodor, A., Candiani, M., and Mangili, G. (2019). Management of recurrent ovarian cancer: when platinum-based regimens are not a therapeutic option. *Int. J. Gynecol. Cancer* 29 (9), 1431–1436. doi:10.1136/ijgc-2019-000624

Burger, R. A., Brady, M. F., Bookman, M. A., Fleming, G. F., Monk, B. J., Huang, H., et al. (2011). Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N. Engl. J. Med.* 365 (26), 2473–2483. doi:10.1056/NEJMoa1104390

Cai, M., Ni, Z., Yuan, Z., Yu, J., Zhang, D., Yao, R., et al. (2023). Past and present: a bibliometric study on polycystic ovary syndrome. *J. Ovarian Res.* 16 (1), 42. doi:10.1186/s13048-022-01072-3

Christian, M. C., and Trimble, E. L. (1994). Salvage chemotherapy for epithelial ovarian carcinoma. *Gynecol. Oncol.* 55 (3 Pt 2), S143–S150. doi:10.1006/gyno.1994.1354

Coleman, R. L., Oza, A. M., Lorusso, D., Aghajanian, C., Oaknin, A., Dean, A., et al. (2017). Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 390 (10106), 1949–1961. doi:10.1016/S0140-6736(17)32440-6

Dalmartello, M., La Vecchia, C., Bertuccio, P., Boffetta, P., Levi, F., Negri, E., et al. (2022). European cancer mortality predictions for the year 2022 with focus on ovarian cancer. *Ann. Oncol.* 33 (3), 330–339. doi:10.1016/j.annonc.2021.12.007

Di Donato, V., Giannini, A., D'Oria, O., Schiavi, M. C., Di Pinto, A., Fischetti, M., et al. (2021). Hepatobiliary disease resection in patients with advanced epithelial ovarian cancer: Prognostic Role and optimal cytoreduction. *Ann. Surg. Oncol.* 28 (1), 222–230. doi:10.1245/s10434-020-08989-3

Drew, Y., Kim, J. W., Penson, R. T., O'Malley, D. M., Parkinson, C., Roxburgh, P., et al. (2024). Olaparib plus durvalumab, with or without bevacizumab, as treatment in PARP inhibitor-Naive platinum-sensitive relapsed ovarian cancer: a phase II multi-Cohort study. *Clin. Cancer Res.* 30 (1), 50–62. doi:10.1158/1078-0432.CCR-23-2249

Duan, Y., Zhang, P., Zhang, T., Zhou, L., and Yin, R. (2023). Characterization of global research trends and prospects on platinum-resistant ovarian cancer: a bibliometric analysis. *Front. Oncol.* 13, 1151871. doi:10.3389/fonc.2023.1151871

Fader, A. N., von Gruenigen, V., Gibbons, H., Abushahin, F., Starks, D., Markman, M., et al. (2008). Improved tolerance of primary chemotherapy with reduced-dose carboplatin and paclitaxel in elderly ovarian cancer patients. *Gynecol. Oncol.* 109 (1), 33–38. doi:10.1016/j.ygyno.2008.01.001

Falandry, C., and Gouy, S. (2019). Epithelial ovarian cancer and elderly patients. Article drafted from the French Guidelines in oncology entitled "Initial management of patients with epithelial ovarian cancer" developed by FRANCOGYN, CNGOF, SFOG, GINECO-ARCAGY under the aegis of CNGOF and endorsed by INCa. *Gynecol. Obstet. Fertil. Senol.* 47 (2), 238–249. doi:10.1016/j.gofs.2018.12.008

Feng, J., Xu, L., Chen, Y., Lin, R., Li, H., and He, H. (2023). Trends in incidence and mortality for ovarian cancer in China from 1990 to 2019 and its forecasted levels in 30 years. *J. Ovarian Res.* 16 (1), 139. doi:10.1186/s13048-023-01233-y

Friedlander, M., Trimble, E., Tinker, A., Alberts, D., Avall-Lundqvist, E., Brady, M., et al. (2011). Clinical trials in recurrent ovarian cancer. *Int. J. Gynecol. Cancer* 21 (4), 771–775. doi:10.1097/IGC.0b013e31821bb8aa

Giannini, A., Di Dio, C., Di Donato, V., D'oria, O., Salerno, M. G., Capalbo, G., et al. (2023). PARP inhibitors in newly diagnosed and recurrent ovarian cancer. *Am. J. Clin. Oncol.* 46 (9), 414–419. doi:10.1097/COC.0000000000001024

Glajzer, J., Grabowski, J. P., Sehouli, J., and Pfisterer, J. (2020). Recurrent treatment in ovarian cancer patients: what are the best regimens and the order they should Be given? *Curr. Treat. Options Oncol.* 21 (6), 49. doi:10.1007/s11864-020-00747-7

Gonzalez-Martin, A., Pothuri, B., Vergote, I., DePont Christensen, R., Graybill, W., Mirza, M. R., et al. (2019). Niraparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 381 (25), 2391–2402. doi:10.1056/NEJMoa1910962

Kobayashi, Y., Shimada, M., Tamate, M., Cho, H. W., Zhu, J., Chou, H. H., et al. (2024). Current treatment strategies for ovarian cancer in the East Asian gynecologic oncology trial group (EAGOT). *J. Gynecol. Oncol.* 35 (3), e87. doi:10.3802/jgo.2024.35.e87

Lee, J. Y., Lee, Y. Y., Park, J. Y., Shim, S. H., Kim, S. I., Kong, T. W., et al. (2023). Major clinical research advances in gynecologic cancer in 2022: highlight on late-line PARP inhibitor withdrawal in ovarian cancer, the impact of ARIEL-4, and SOLO-3. *J. Gynecol. Oncol.* 34 (2), e51. doi:10.3802/jgo.2023.34.e51

Li, H., Liu, Z. Y., Wu, N., Chen, Y. C., Cheng, Q., and Wang, J. (2020). PARP inhibitor resistance: the underlying mechanisms and clinical implications. *Mol. Cancer* 19 (1), 107. doi:10.1186/s12943-020-01227-0

Li, J., Zou, G., Wang, W., Yin, C., Yan, H., and Liu, S. (2023). Treatment options for recurrent platinum-resistant ovarian cancer: a systematic review and Bayesian network meta-analysis based on RCTs. *Front. Oncol.* 13, 1114484. doi:10.3389/fonc.2023.1114484

Li, N., Zhang, Y., Wang, J., Zhu, J., Wang, L., Wu, X., et al. (2022). Fuzuloparib maintenance therapy in patients with platinum-sensitive, recurrent ovarian carcinoma (FZOCUS-2): a multicenter, randomized, double-blind, placebo-controlled, phase III trial. *J. Clin. Oncol.* 40 (22), 2436–2446. doi:10.1200/JCO.21.01511

Liontos, M., Papatheodoridi, A., Andrikopoulou, A., Thomakos, N., Haidopoulos, D., Rodolakis, A., et al. (2021). Management of the elderly patients with high-grade serous ovarian cancer in the REAL-WORLD Setting. *Curr. Oncol.* 28 (2), 1143–1152. doi:10.3390/curroncol28020110

Liu, J., Ma, J., Zhang, J., Li, C., Yu, B., Choe, H. C., et al. (2023). Bibliometric and visualized analysis of drug resistance in ovarian cancer from 2013 to 2022. *Front. Oncol.* 13, 1173863. doi:10.3389/fonc.2023.1173863

Liu, Y., Huang, Y., Li, J., Wan, S., Jiang, N., Yang, J., et al. (2022). A comprehensive comparison of medication strategies for platinum-sensitive recurrent ovarian cancer: a Bayesian network meta-analysis. *Front. Pharmacol.* 13, 1010626. doi:10.3389/fphar.2022.1010626

Look, K. Y., Blessing, J. A., Adelson, M. D., Morris, M., and Bookman, M. A. (1996). A phase II trial of merbarone (NSC 336628) in the treatment of recurrent epithelial ovarian carcinoma. A Gynecologic Oncology Group Study. *Am. J. Clin. Oncol.* 19 (1), 7–9. doi:10.1097/00000421-199602000-00002

Look, K. Y., Blessing, J. A., Muss, H. B., and DeGeest, K. (1992). 5-fluorouracil and low-dose leucovorin in the treatment of recurrent epithelial ovarian carcinoma. A phase II trial of the Gynecologic Oncology Group. *Am. J. Clin. Oncol.* 15 (6), 494–496. doi:10.1097/00000421-199212000-00006

Maggioni, A., Benedetti Panici, P., Dell'Anna, T., Landoni, F., Lissoni, A., Pellegrino, A., et al. (2006). Randomised study of systematic lymphadenectomy in patients with epithelial ovarian cancer macroscopically confined to the pelvis. *Br. J. Cancer* 95 (6), 699–704. doi:10.1038/sj.bjc.6603323

Markman, M. (1994). Ovarian cancer update: management challenges and advances. *Cleve Clin. J. Med.* 61 (1), 51–58. quiz 80-2. doi:10.3949/ccjm.61.1.51

Matulonis, U. A., Shapira-Frommer, R., Santin, A. D., Lisyanskaya, A. S., Pignata, S., Vergote, I., et al. (2019). Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. *Ann. Oncol.* 30 (7), 1080–1087. doi:10.1093/annonc/mdz135

Menzin, A. W., King, S. A., Aikins, J. K., Mikuta, J. J., and Rubin, S. C. (1994). Taxol (paclitaxel) was approved by FDA for the treatment of patients with recurrent ovarian cancer. *Gynecol. Oncol.* 54 (1), 103.

Miglietta, L., Amoroso, D., Bruzzone, M., Granetto, C., Catsafados, E., Mammoliti, S., et al. (1997). Paclitaxel plus ifosfamide in advanced ovarian cancer: a multicenter phase II study. *Oncology* 54 (2), 102–107. doi:10.1159/000227671

Mirza, M. R., Monk, B. J., Herrstedt, J., Oza, A. M., Mahner, S., Redondo, A., et al. (2016). Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N. Engl. J. Med.* 375 (22), 2154–2164. doi:10.1056/NEJMoa1611310

Monk, B. J., Choi, D. C., Pugmire, G., and Burger, R. A. (2005). Activity of bevacizumab (rhuMAB VEGF) in advanced refractory epithelial ovarian cancer. *Gynecol. Oncol.* 96 (3), 902–905. doi:10.1016/j.ygyno.2004.12.001

Moore, K., Colombo, N., Scambia, G., Kim, B. G., Oaknin, A., Friedlander, M., et al. (2018). Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 379 (26), 2495–2505. doi:10.1056/NEJMoa1810858

Ni, J., Cheng, X., Zhao, Q., Dai, Z., Xu, X., Guo, W., et al. (2021). The efficacy and safety of niraparib for ovarian cancer: a single-center observational study from China. *J. Ovarian Res.* 14 (1), 68. doi:10.1186/s13048-021-00803-2

Palazzo, A., Iacovelli, R., and Cortesi, E. (2010). Past, present and future of targeted therapy in solid tumors. *Curr. Cancer Drug Targets* 10 (5), 433–461. doi:10.2174/156800910791517145

Perrone, C., Angioli, R., Luvero, D., Giannini, A., Di Donato, V., Cuccu, I., et al. (2024). Targeting BRAF pathway in low-grade serous ovarian cancer. *J. Gynecol. Oncol.* 35. doi:10.3802/jgo.2024.35.e104

Pfisterer, J., Vergote, I., Du Bois, A., Eisenhauer, E., et al. (2005). Combination therapy with gemcitabine and carboplatin in recurrent ovarian cancer. *Int. J. Gynecol. Cancer* 15 (Suppl. 1), 36–41. doi:10.1111/j.1525-1438.2005.15355.x

Polcher, M., Zivanovic, O., and Chi, D. S. (2014). Cytoreductive surgery for advanced ovarian cancer. *Womens Health (Lond)* 10 (2), 179–190. doi:10.2217/whe.14.4

Poveda, A., Floquet, A., Ledermann, J. A., Asher, R., Penson, R. T., Oza, A. M., et al. (2021). Olaparib tablets as maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a final analysis of a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 22 (5), 620–631. doi:10.1016/S1470-2045(21)00073-5

Poveda, A., Lheureux, S., Colombo, N., Cibula, D., Lindemann, K., Weberpals, J., et al. (2022). Olaparib maintenance monotherapy in platinum-sensitive relapsed ovarian cancer patients without a germline BRCA1/BRCA2 mutation: OPINION primary analysis. *Gynecol. Oncol.* 164 (3), 498–504. doi:10.1016/j.ygyno.2021.12.025

Pujade-Lauraine, E., Hilpert, F., Weber, B., Reuss, A., Poveda, A., Kristensen, G., et al. (2014). Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial. *J. Clin. Oncol.* 32 (13), 1302–1308. doi:10.1200/JCO.2013.51.4489

Pujade-Lauraine, E., Ledermann, J. A., Selle, F., Gebski, V., Penson, R. T., Oza, A. M., et al. (2017). Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 18 (9), 1274–1284. doi:10.1016/S1470-2045(17)30469-2

Ray-Coquard, I., Pautier, P., Pignata, S., Pérol, D., González-Martín, A., Berger, R., et al. (2019). Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N. Engl. J. Med.* 381 (25), 2416–2428. doi:10.1056/NEJMoa1911361

Richardson, D. L., Eskander, R. N., and O'Malley, D. M. (2023). Advances in ovarian cancer Care and Unmet treatment needs for patients with platinum resistance: a narrative review. *JAMA Oncol.* 9 (6), 851–859. doi:10.1001/jamaoncol.2023.0197

Roland, P. Y., Barnes, M. N., Niwas, S., Robertson, M. W., Alvarez, R., Austin, J. M., et al. (1998). Response to salvage treatment in recurrent ovarian cancer treated initially with paclitaxel and platinum-based combination regimens. *Gynecol. Oncol.* 68 (2), 178–182. doi:10.1006/gyno.1997.4909

Shea, M. B., Roberts, S. A., Walrath, J. C., Allen, J. D., and Sigal, E. V. (2013). Use of multiple endpoints and approval paths depicts a decade of FDA oncology drug approvals. *Clin. Cancer Res.* 19 (14), 3722–3731. doi:10.1158/1078-0432.CCR-13-0316

Siegel, R. L., Miller, K. D., Wagle, N. S., and Jemal, A. (2023). Cancer statistics, 2023. *CA Cancer J. Clin.* 73 (1), 17–48. doi:10.3322/caac.21763

Spriggs, D. R., and Longo, D. L. (2016). PARP inhibitors in ovarian cancer treatment. *N. Engl. J. Med.* 375 (22), 2197–2198. doi:10.1056/NEJMe1612843

Trope, C., Marth, C., and Kaern, J. (2000). Tamoxifen in the treatment of recurrent ovarian carcinoma. *Eur. J. Cancer* 36 (Suppl. 4), S59–S61. doi:10.1016/s0959-8049(00)00228-8

Tu, J. X., Lin, X. T., Ye, H. Q., Yang, S. L., Deng, L. F., Zhu, R. L., et al. (2022). Global research trends of artificial intelligence applied in esophageal carcinoma: a bibliometric analysis (2000-2022) via CiteSpace and VOSviewer. *Front. Oncol.* 12, 972357. doi:10.3389/fonc.2022.972357

Vergote, I., Gonzalez-Martin, A., Lorusso, D., Gourley, C., Mirza, M. R., Kurtz, J. E., et al. (2022). Clinical research in ovarian cancer: consensus recommendations from the Gynecologic Cancer InterGroup. *Lancet Oncol.* 23 (8), e374–e384. doi:10.1016/S1470-2045(22)00139-5

Wang, D. F., Shi, X. W., Zhang, C., Zhang, J., Liu, H., Huang, J. M., et al. (2023). Real-world applications of PARPi maintenance therapy for recurrent ovarian cancer: a single-center study in China. *Gynecol. Oncol.* 170, 25–31. doi:10.1016/j.ygyno.2022.12.014

Wojtyła, C., Bertuccio, P., Giermaziak, W., Santucci, C., Odone, A., Ciebiera, M., et al. (2023). European trends in ovarian cancer mortality, 1990-2020 and predictions to 2025. *Eur. J. Cancer* 194, 113350. doi:10.1016/j.ejca.2023.113350

Wu, X. H., Zhu, J. Q., Yang, J. X., Liu, J. H., Wang, J., et al. (2021). Niraparib maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer using an individualized starting dose (NORA): a randomized, double-blind, placebo-controlled phase III trial(☆). *Ann. Oncol.* 32 (4), 512–521. doi:10.1016/j.annonc.2020.12.018

Yang, C., Xia, B. R., Zhang, Z. C., Zhang, Y. J., Lou, G., and Jin, W. L. (2020). Immunotherapy for ovarian cancer: Adjuvant, combination, and Neoadjuvant. *Front. Immunol.* 11, 577869. doi:10.3389/fimmu.2020.577869

Zheng, R. S., Zhang, S. W., Sun, K. X., Chen, R., Wang, S. M., Li, L., et al. (2023). Cancer statistics in China, 2016. *Zhonghua Zhong Liu Za Zhi* 45 (3), 212–220. doi:10.3760/cma.j.cn112152-20220922-00647

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The first-in-class pro-apoptotic peptide PEP-010 is effective in monotherapy and in combination with paclitaxel on resistant ovarian adenocarcinoma cell models

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Ovarian adenocarcinoma is the gynecological malignancy with the worst prognosis and the highest mortality rate. In the first stages of treatment, chemotherapy results effective, but its prolonged use and high doses lead to the appearance of resistance to treatments and relapse in most patients, representing a major challenge for clinicians. We developed PEP-010, a cell penetrating proapoptotic peptide disrupting the protein-protein interaction between caspase-9 and protein phosphatase 2A, thereby leading to the recovery of their activity in the apoptotic pathway. MTT assay or Annexin-V/Propidium Iodide staining and flow cytometry analysis were used to assess sensitivity to chemotherapies and apoptosis after treatment with PEP-010 in monotherapy or in combination with paclitaxel in ovarian carcinoma cell lines. DNA damage was assessed by immunofluorescence using γH2AX marker. We show here that PEP-010 effectively induces cell death in monotherapy on in up to 55% of cells from ovarian adenocarcinoma cell models resistant to different chemotherapies. Moreover, when used in combination with paclitaxel, one of the therapeutic options for recurrent ovarian carcinoma, PEP-010 showed a beneficial effect leading to the reduction of the IC_{50} of paclitaxel of 2.2 times and to apoptosis in 87% of cells. The described results suggest the potential therapeutic interest for PEP-010 and lead to the choice of ovarian adenocarcinoma as one of the major indications of the ongoing clinical trial.

KEYWORDS

apoptosis, peptide, monotherapy, combination therapy, ovarian adenocarcinoma

Introduction

Ovarian adenocarcinoma (OA) has the worst prognosis and the highest mortality rate among gynecological malignancies, with less than 50% average 5-year survival (Phung et al., 2023). OA treatment relies on the tumor stage, including surgical removal and platin- or taxane-based chemotherapies. Although chemotherapy is initially effective, prolonged use and high doses lead to the appearance of resistance and relapse in ~70% of patients (Alatise

et al., 2022). Drug resistance is a multi-factorial mechanism. However, the most widely accepted molecular mechanism is the dysregulation of both influx and efflux pumps (multi-drug resistance (MDR) pumps), regulating the transport of compounds in cancer cells (Ortiz et al., 2022).

Strategies to overcome this issue are required. A promising strategy for novel anticancer treatment can be to specifically act on proteins of the apoptotic pathway with the aim of re-establishing the normal ability for a cell to die (Peng et al., 2022). FDA approval of venetoclax in 2016, a pro-apoptotic small molecule inhibitor of Bcl-2, for the treatment of chronic lymphocytic leukemia or acute myeloid leukemia, paved the way for targeting apoptosis in cancer therapy (Carneiro and El-Deiry, 2020). Among the possible therapeutic strategies, peptides are gaining strong interest. Indeed, they allow targeting specifically pathological protein–protein interaction, which is difficult to target with small molecules (Anand et al., 2023). Peptide-based drugs have been FDA-approved both for cancer (as antibody-drug conjugates or labeled peptides for diagnostic use) and other diseases (e.g., diabetes or cardiovascular diseases, among others) (de la Torre and Albericio, 2020; Wang et al., 2022). Other peptide drugs with different downstream targets and mechanisms of action are being investigated in clinical/preclinical trials (Urandur and Sullivan, 2023; Vadevoo et al., 2023). Furthermore, the use of cell-penetrating peptides as carriers for various types of therapeutic molecules is also being explored to overcome the drawbacks of standard chemotherapy (Xie et al., 2020; Matijass and Neundorf, 2021).

We developed PEP-010, a pro-apoptotic, bifunctional peptide with cell-penetrating and interfering peptide capacity. These molecules efficiently penetrate cells and specifically block intracellular protein–protein interactions, leading to the inhibition of key pathological mechanisms without altering physiological mechanisms and re-establishing cellular pathways. Upon cell penetration, PEP-010 disrupts the interaction between caspase-9 and protein phosphatase 2A (PP2A), two key proteins involved in apoptosis, a physiological process frequently altered in cancer.

Previous studies showed a rapid entry of PEP-010 followed by apoptosis induction in several cancer cell lines of different tumor origins (Arrouss et al., 2013; Farhat et al., 2023). In this study, we demonstrated the pharmacological potential of PEP-010 for OA treatment in monotherapy and in combination with paclitaxel (PTX), a chemotherapy drug indicated for platinum-resistant OA treatment. The results obtained in this work paved the way for the ongoing clinical trial (NCT04733027), which thus focuses on platin-resistant OA.

Materials and methods

Cell culture

The ovarian cell lines IGROV1, IGROV1CDDP, and IGROV1VCR were cultivated in RPMI 1640 Medium GlutaMAX with 10% FBS and 1% of pyruvate and glutamine. They were maintained at 5% CO_2 and 37°C in a humidified atmosphere. All reagents are from Gibco® (Thermo Fisher Scientific, Carlsbad, CA, United States).

Peptides

PEP-010 sequence is VKKKKIKAEIKIYVETLDDIFEQWAHSEDL, where VKKKKIKAEIKI is the cell-penetrating part of the peptide and YVETLDDIFEQWAHSEDL is the interfering peptide part. PEP-010 was produced by PolyPeptide (Strasbourg, France).

Cell treatment

PEP-010 was first dissolved in 0.1% formic acid (10 mM) and then diluted in a cell culture medium at pH9 (to allow a full dissolution of the peptide). Experiments in similar conditions, but without the peptide, were conducted as negative controls. Staurosporine (Selleckchem, Houston, TX, United States) was used as a positive control at a concentration of 1 µM. Cells were collected or analyzed at the indicated time points. For combination conditions, the cells were treated with paclitaxel (Accord, London, United Kingdom) at the indicated concentrations for 72 h.

MTT assay

Here, 10,000 cells were seeded on 96-well plates in 100 µL of medium. The day after, the medium was replaced with fresh medium containing the appropriate drugs at the indicated concentrations: cisplatin (Mylan, Canonsburg, PA, United States), paclitaxel (Accord, London, United Kingdom), doxorubicin (Pfizer, New York, NY, United States), vincristine [Pfizer, New York, NY, United States)], and/or PEP-010. The plates were incubated at 37° in humidified air with 5% CO_2 for 72 h. The MTT reagent (20 µL) was added at the end of the indicated time points, and plates were incubated for 2 h at 37°C. In addition, 100 µL of lysis buffer was added to each well. Subsequently, the plates were incubated for 2 h at 37°C, and the absorbance was measured at 570 nm (Tecan, Männedorf, CH).

Apoptosis measurement

Cell death was assessed using Annexin V-APC and propidium iodide (PI) (BioLegend, San Diego, CA, United States) staining following the manufacturer's instructions. Results were analyzed as previously described (Debernardi et al., 2018). A total of 10,000 events were analyzed using a C6 Accuri Cytometer (Becton-Dickinson, Franklin Lakes, NJ, United States), analysis was performed using integrated software, and Annexin V positive cells (PI positive and negative) were counted as apoptotic.

Immunofluorescence

Cells were seeded on 12 mm coverslips placed in a 24-well plate (24 h, 37°C, and 5% CO_2). Cells were treated with PEP-010 as previously described. At the indicated time point, cells were washed with 1X PBS and fixed in 4% paraformaldehyde (Euromedex, Souffelweyersheim, FR) in 1X PBS (10 min, RT), followed by three washes in 1X PBS. Cells were then permeabilized with 2%

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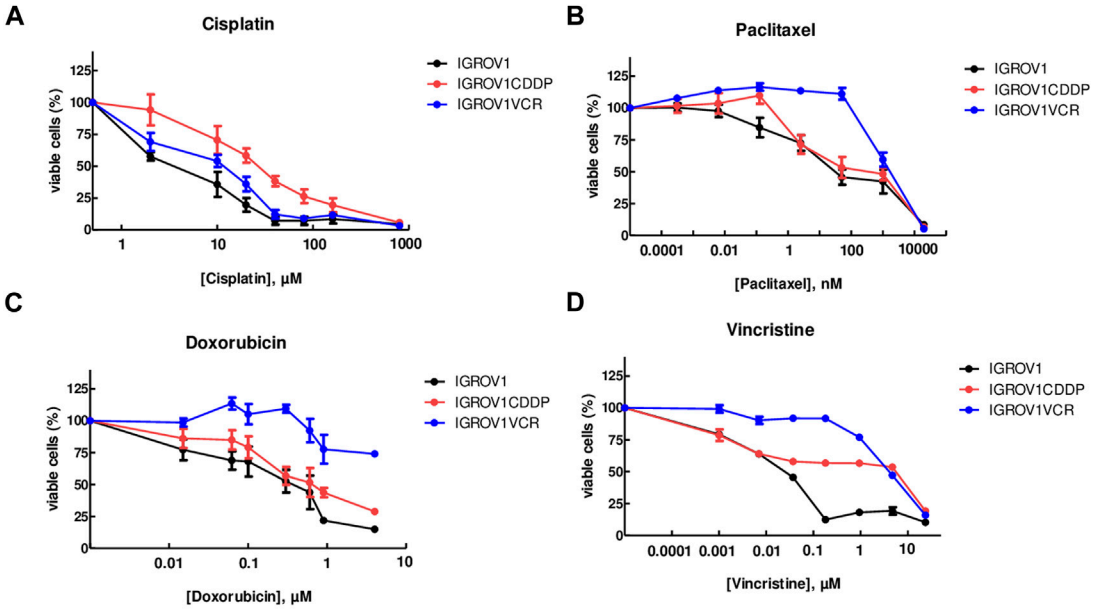

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	Cisplatin	Paclitaxel	Doxorubicin	Vincristine
IGROV1	2.3 µM	0.75 µM	0.4 µM	0.034 µM
IGROV1 CDDP	25.7 µM	0.5 µM	0.5 µM	0.9 µM
IGROV1 VCR	15 µM	2.9 µM	>10µM	3.3 µM

FIGURE 1
OA cell lines used are resistant to different chemotherapies. **(A–D)** Calculation of IC_{50} values using the MTT assay upon treatment with cisplatin **(A)**, paclitaxel **(B)**, doxorubicin **(C)**, or vincristine **(D)** for 72 h. IGROV1 survival percentages are represented by black dots connected by a black line, IGROV1CDDP is represented in red, and IGROV1VCR is represented in blue. All experiments have been performed on three to six independent biological replicates. For each point, the mean ± SEM is represented. **(E)** IC_{50} values obtained in the different models and with the indicated conditions. The table shows the values obtained in IGROV1, IGROV1CDDP, and IGROV1VCR with cisplatin, paclitaxel, doxorubicin, or vincristine.

Triton X-100 (Euromedex, Souffelweyersheim, FR) in 1X PBS (10 min, RT) and then washed in 1X PBS. Cells were saturated with 0.5% BSA (Euromedex, Souffelweyersheim, FR) in 1X PBS (40 min, RT) and then stained with an anti-γH2AX Ser139 antibody (mouse, 1:200, from BioLegend #613402). Incubation with primary antibody (2 h, RT) was followed by three washes in 1X PBS and by incubation (1 h, RT) with a secondary antibody conjugated to Alexa Fluor 588 goat anti-mouse (Thermo Fisher Scientific, Carlsbad, CA, United States), diluted 1:200. Coverslips were mounted on microscopy glass slides using a mounting medium containing DAPI (Sigma-Aldrich, Saint-Quentin-Fallavier, Cedex, France). Images were acquired using a fluorescence microscope (Zeiss Observer Z1). Images were analyzed using ImageJ software.

Statistics

For each time point, different treatments are compared with the control analyzed at the same time point. Data are presented as the mean ± standard error of the mean (SEM). A student's t-test (two-tail distribution) was used to compare the means of the two groups (treated vs. untreated or monotherapies between each other or vs. combination). All tests were performed using GraphPad Prism 5 software (GraphPad Prism, Boston, MA, United States).

Results

To evaluate the efficacy of PEP-010 for OA treatment, we used three OA cell lines, the parental IGROV1 established from a stage III ovarian primary tumor and two *in vitro* derived drug-resistant cell lines: IGROV1CDDP, resistant to cisplatin, and IGROV1VCR, resistant to vincristine. The latter expresses MDR pumps (Supplementary Figure 1A, B).

The resistance of IGROV1CDDP and IGROV1VCR to widely used chemotherapies, cisplatin (CDDP), paclitaxel (PTX), doxorubicin (DOX), and vincristine (VCR), was assessed by IC_{50} determination using the MTT test and compared to the parental line

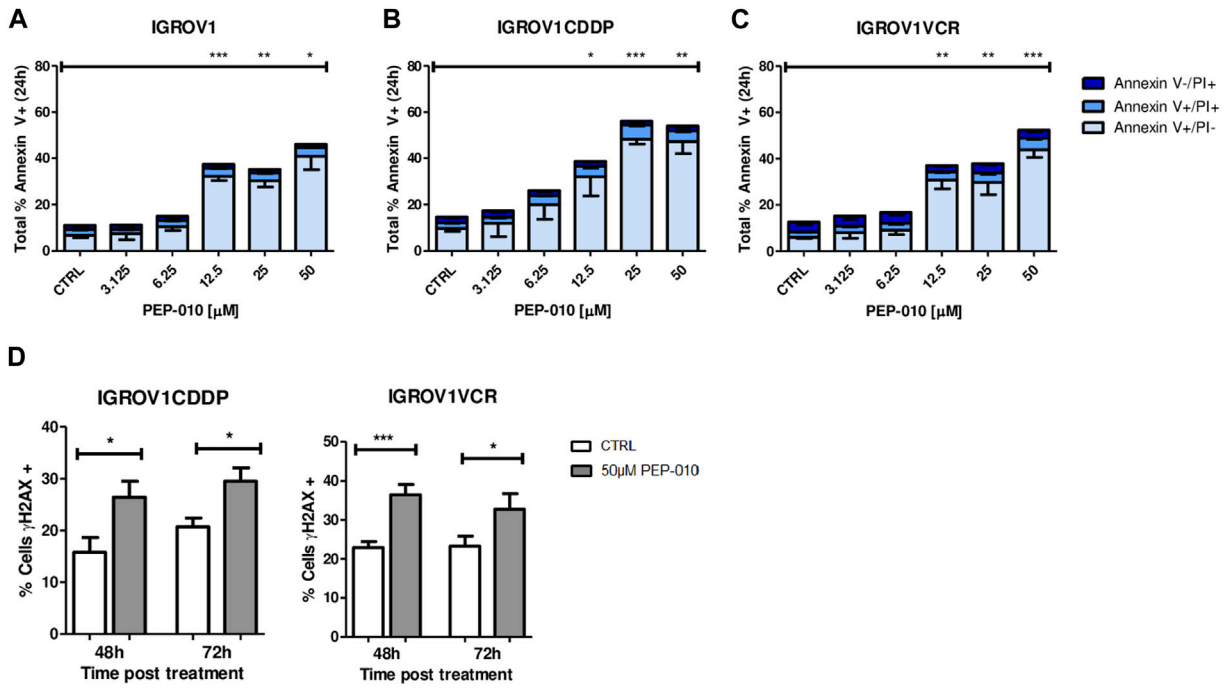

FIGURE 2
PEP-010 induces apoptosis in OA cells resistant or not to chemotherapies. **(A–C)** Cells were treated with PEP-010 at the indicated doses for 24 h. Results of treated cells were always compared to those of the untreated control. Analysis of cell death was performed by Annexin V/PI staining and FACS analysis. The results are represented as the total percentage of cells expressing Annexin V. All experiments have been performed in three independent biological replicates. Early apoptosis (Annexin V+/PI-) is represented in light blue; late apoptosis (Annexin V+/PI+) is represented in blue; and necrosis (Annexin V-/PI+) is represented in dark blue. **(D)** PEP-010 induces DNA damage, as shown by γH2aX expression. Fixed cells were immunostained using an antibody specific for γH2aX as an early marker of DNA damage. The percentage of cells expressing γH2aX was calculated over the total number of cells. Three biological and nine technical replicates and at least 300 cells were analyzed for each biological replicate. Graphs represent the means ± SEM. *, <0.05; **, 0.001 < p < 0.05; and ***, p < 0.001.

IGROV1. As expected, IGROV1CDDP were highly resistant to CDDP (IC_{50} = 25.7 µM ± 4.6; fold change (FC) vs. IC_{50} IGROV1 = 11.1, Figure 1A), while IGROV1VCR showed resistance to all tested chemotherapies (IC_{50} CDDP = 15 µM ± 4, FC = 6.5; IC_{50} PTX = 2.9 µM ± 0.6, FC = 3.9; IC_{50} DOX>10µM; and IC_{50} VCR = 3.3 µM ± 1.5, FC = 97, Figures 1A–D). IC_{50} values are reported in Figure 1E.

Then, we assessed whether PEP-010 could induce apoptosis in these cell models by Annexin V/PI staining and FACS analysis. Starting from 12.5 µM, total Annexin V levels were significantly higher in all three models at 24 h post-treatment than in the untreated control (total % of Annexin V positive cells: 37.4 ± 1.9 vs. 11 ± 1.3 for IGROV1, p = 0.0004, Figure 2A; 38.7 ± 8.5 vs. 14.6 ± 1.2 for IGROV1CDDP, p = 0.046; Figure 2B; and 37 ± 4 vs. 12.6 ± 0.9 for IGROV1VCR, p = 0; 007; Figure 2C). Thus, PEP-010 shows an antitumor effect on these cells independently of their drug resistance status and MDR pump expression.

As previously mentioned, the appearance of resistance is a major cause of failure of OA treatment; therefore, we focused our attention on resistant models. As a further confirmation of the PEP-010 apoptosis induction, we assessed the presence of DNA damage (DD) by γH2AX staining followed by immunofluorescence analysis. γH2AX expression was increased upon treatment with PEP-010 as compared to untreated controls in all tested models (% of γH2AX positive cells 48 h post-treatment = 26.5 ± 3.1 vs. 15.8 ± 2.8 for IGROV1CDDP and 29.5 ± 2.6 vs. 20.7 ± 1.7 for IGROV1VCR, Figure 2D). Taken together, our data establish that PEP-010 effectively induces apoptotic cell death in different OA models independently of potential resistance mechanism of the cell lines.

A combination of drugs targeting various molecular pathways could decrease the therapeutic dose of each chemotherapeutic agent and thereby delay or avoid resistance acquisition (Kuosmanen et al., 2021). We thus assessed, by MTT assay, whether PEP-010 could improve PTX efficacy (i.e., the current chemotherapy indicated for platinum-resistant OA treatment) by monitoring the simultaneous use of PEP-010 with PTX on IGROV1VCR. Cells were treated for 72 h with increasing doses of PTX alone or in combination with PEP-010 used at a single concentration (25 µM). We observed that the treatment with PEP-010 was advantageous as it significantly decreased the IC_{50} value of PTX (PTX alone = 2.9 µM ± 0.6 vs. PTX + PEP-010 = 1.3 µM ± 0.5, FC = 2.2 (Figure 3A,B). These data were further confirmed by Annexin V/PI staining, where an additive effect and a dose-effect correlation on apoptosis were visible starting from 500 nM PTX (combination effect = +4.9% vs. monotherapy with PTX 500 nM, +9.4% at 1 µM, +19.5% at 5 µM, and +18% at 20 µM) (Figure 3C). The combination index is < 1, confirming a synergic effect of the two drugs. The beneficial combination of paclitaxel with pro-apoptotic drugs is consistent with previous

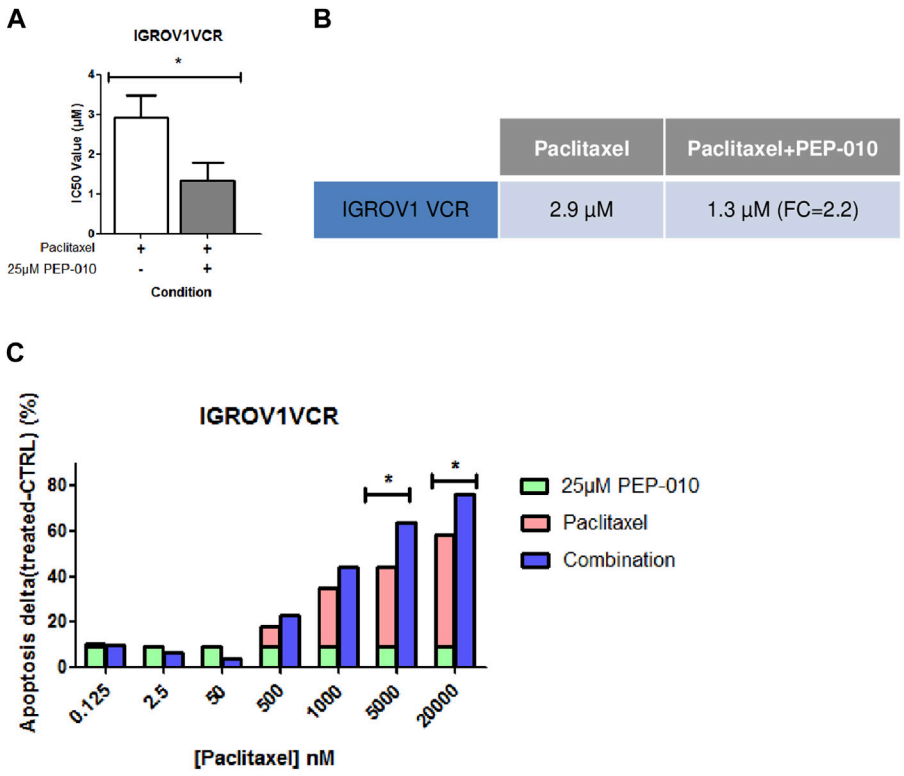

FIGURE 3
PEP-010 combination with paclitaxel shows a beneficial effect on OA cell lines resistant to chemotherapies. **(A, B)** IC_{50} values obtained by calculation using the MTT assay upon treatment of IGROV1VCR with the indicated drugs for 72 h. The IC_{50} value decreases when PEP-010 is added to paclitaxel. Four independent biological replicates have been analyzed. **(C)** The combination effect was also analyzed by Annexin V/PI staining in IGROV1VCR treated with increasing doses of paclitaxel in monotherapy or with the addition of PEP-010 (25 µM) for 72 h. Three independent biological replicates have been analyzed. *, <0.05; **, 0.001 < p < 0.05; and ***, p < 0.001.

observations in other cancer models. This is notably the case of navitoclax (an inhibitor of BCL-2, BCL-xL, and BCL-W), which had a high synergy rate with paclitaxel in some subtypes of breast cancer (Jaaks et al., 2022).

Discussion

Ovarian cancer is the eighth most common cancer in women worldwide and remains the leading cause of death among gynecological cancers. The standard of care is a debulking strategy followed by platin- or taxane-based chemotherapy (Arora et al., 2024). Unfortunately, a majority of patients relapse, and among them, two subpopulations could be distinguished: platinum-sensitive patients, who could receive platinum-based chemotherapies again, often followed by PARP inhibitor treatments, or platinum-resistant patients, for whom cancer recurs within 6 months after the end of chemotherapy, with a median survival rate of 9–12 months. Resistance to cisplatin could pre-exist (approximately 20% of patients do not respond to cisplatin) or, in 70%–80% of the cases, be acquired during treatment (Baert et al., 2021; Awada et al., 2022; Blagden and Nicum, 2021). These patients are therefore in great need of new therapeutic tools, but most of the recently developed approaches have the same strategy as the treatments already approved (immunotherapies, VEGF inhibitors, PARP inhibitors, and folate alpha inhibitors) (Satora et al., 2024). Therefore, the development of novel therapies with novel targets is required.

Dysregulation of influx/efflux pump functionality is one of the most widely accepted platinum resistance mechanisms (Ortiz et al., 2022). At the molecular level, the ability of cancer cells to escape apoptosis is one of the most common ways to resist treatments (Neophytou et al., 2021). For this reason, re-teaching the cell to die is a promising strategy to overcome the resistance issue.

A novel therapeutic opportunity is represented by peptides for their specific targeting of protein–protein interactions. In this regard, we show in this study that PEP-010, a pro-apoptotic peptide targeting and disrupting the interaction between PP2A and caspase-9, efficiently induces apoptosis in OA cell lines resistant to different chemotherapeutic drugs *in vitro*, independently of MDR pump expression. PEP-010 efficacy relies on the restoration of the physiological roles of PP2A and caspase-9 when they are released. On one side, PP2A can regulate and inactivate a large amount of apoptosis/pro-survival-related downstream targets (e.g., Bcl-2 family members and Akt) (Farrel et al., 2014), and on the other side, caspase-9, once activated, can trigger the caspase cascade leading to cell death.

A strategy to reduce or delay the onset of resistance and minimize the toxicity of chemotherapies is to combine them with other drugs. To this aim, we combined PEP-010 with PTX

and demonstrated a beneficial effect of this combination, leading to a reduction in the IC_{50} value of PTX and an increased apoptotic effect compared to monotherapy, thereby revealing a potential therapeutic interest. These results obtained on cell lines could be strengthened in the future by performing *in vivo* studies using PEP-010 in combination with paclitaxel or with other therapies (e.g., PARP inhibitors, which would allow targeting the apoptosis pathway at different levels).

However, it must be mentioned that PEP-010 safety has been evaluated in monotherapy and in combination with chemotherapy during a Phase 1a study, where patients with solid tumors, including OA, were enrolled.

Finally, the data described in this paper served as the basis for the choice of the therapeutic indication for Phase 1b of the PEP-010 ongoing clinical trial (NCT04733027), which thus focuses on platin-resistant OA and pancreatic adenocarcinoma (Rusquec et al., 2023).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

AL: Data curation, Investigation, Writing–original draft, Writing–review and editing. RF: Data curation, Investigation, Writing–original draft, Writing–review and editing. AR: Data curation, Investigation, Writing–original draft, Writing–review and editing. CB: Validation, Writing–review and editing, Writing–original draft. JW: Validation, Writing–review and editing, Writing–original draft. DG: Conceptualization, Project administration, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

Authors AL, RF, and DG were employed by PEP-Therapy.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary material

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References

Alatise, K. L., Gardner, S., and Alexander-Bryant, A. (2022). Mechanisms of drug resistance in ovarian cancer and associated gene targets. *Cancers (Basel)* 14, 6246. doi:10.3390/CANCERS14246246

Anand, U., Bandyopadhyay, A., Jha, N. K., Pérez de la Lastra, J. M., and Dey, A. (2023). Translational aspect in peptide drug discovery and development: an emerging therapeutic candidate. *Biofactors* 49, 251–269. doi:10.1002/BIOF.1913

Arora, T., Mullangi, S., Vadakekut, E. S., and Lekkala, M. R. (2024). “Epithelial Ovarian Cancer,” in *StatPearls* (Treasure Island (FL): StatPearls Publishing). Available at: http://www.ncbi.nlm.nih.gov/books/NBK567760/ (Accessed July 25, 2024).

Arrouss, I., Nemati, F., Roncal, F., Wislez, M., Dorgham, K., Vallerand, D., et al. (2013). Specific targeting of caspase-9/PP2A interaction as potential new anti-cancer therapy. *PLoS One* 8, e60816. doi:10.1371/JOURNAL.PONE.0060816

Awada, A., Ahmad, S., McKenzie, N. D., and Holloway, R. W. (2022). Immunotherapy in the treatment of platinum-resistant ovarian cancer: current perspectives. *Onco. Targets Ther.* 15, 853–866. doi:10.2147/OTT.S335936

Baert, T., Ferrero, A., Sehouli, J., O’Donnell, D. M., González-Martín, A., Joly, F., et al. (2021). The systemic treatment of recurrent ovarian cancer revisited. *Ann. Oncol.* 32, 710–725. doi:10.1016/j.annonc.2021.02.015

Blagden, S. P., and Nicum, S. (2021). A source of hope for platinum-resistant ovarian cancer? *Lancet* 397, 254–256. doi:10.1016/S0140-6736(21)00128-8

Carneiro, B. A., and El-Deiry, W. S. (2020). Targeting apoptosis in cancer therapy. *Nat. Rev. Clin. Oncol.* 17, 395–417. doi:10.1038/S41571-020-0341-Y

Debernardi, J., Hollville, E., Lipinski, M., Wiels, J., and Robert, A. (2018). Differential role of FL-BID and t-BID during verotoxin-1-induced apoptosis in Burkitt’s lymphoma cells. *Oncogene* 37, 2410–2421. doi:10.1038/s41388-018-0123-5

de la Torre, B. G., and Albericio, F. (2020). Peptide therapeutics 2.0. *Molecules* 25, 2293. doi:10.3390/molecules25102293

Farhat, R., Dadon, L., Nemati, F., Rebollo, A., Decaudin, D., Wiels, J., et al. (2023). Abstract 6129: the proapoptotic peptide PEP-010 is efficient on several models of different tumor origins and it can be monitored by pharmacodynamic biomarker candidates in clinical practice. *Cancer Res.* 83, 6129. doi:10.1158/1538-7445.AM2023-6129

Farrell, A. S., Allen-Petersen, B., Daniel, C. J., Wang, X., Wang, Z., Rodriguez, S., et al. (2014). Targeting inhibitors of the tumor suppressor PP2A for the treatment of pancreatic cancer. *Mol. Cancer Res.* 12, 924–939. doi:10.1158/1541-7786.MCR-13-0542

Jaaks, P., Coker, E. A., Vis, D. J., Edwards, O., Carpenter, E. F., Leto, S. M., et al. (2022). Effective drug combinations in breast, colon and pancreatic cancer cells. *Nature* 603, 166–173. doi:10.1038/s41586-022-04437-2

Kuosmanen, T., Cairns, J., Noble, R., Beerenwinkel, N., Mononen, T., and Mustonen, V. (2021). Drug-induced resistance evolution necessitates less aggressive treatment. *PLoS Comput. Biol.* 17, e1009418. doi:10.1371/JOURNAL.PCBI.1009418

Matijass, M., and Neundorf, I. (2021). Cell-penetrating peptides as part of therapeutics used in cancer research. *Med. Drug Discov.* 10, 100092. doi:10.1016/J.MEDIDD.2021.100092

Neophytou, C. M., Trougakos, I. P., Erin, N., and Papageorgis, P. (2021). Apoptosis Deregulation and the Development of Cancer Multi-Drug Resistance. *Cancers* 13, 4363. doi:10.3390/cancers13174363

Ortiz, M., Wabel, E., Mitchell, K., and Horibata, S. (2022). Mechanisms of chemotherapy resistance in ovarian cancer. *Cancer Durg Resist* 5, 304–316. doi:10.20517/cdr.2021.147

Peng, F., Liao, M., Qin, R., Zhu, S., Peng, C., Fu, L., et al. (2022). Regulated cell death (RCD) in cancer: key pathways and targeted therapies. *Signal Transduct. Target. Ther.* 7, 286–366. doi:10.1038/s41392-022-01110-y

Phung, M. T., Pearce, C. L., Meza, R., and Jeon, J. (2023). Trends of ovarian cancer incidence by histotype and race/ethnicity in the United States 1992–2019. *Cancer Res. Commun.* 3, 1–8. doi:10.1158/2767-9764.crc-22-0410

Rusquec, P.Du, Coquan, E., Berger, F., Rezai, K., Brachet, P.-E., Postel-Vinay, S., et al. (2023). Abstract B046: a first-in-human Phase 1a/b of PEP-010, a proapoptotic bifunctional peptide, administered as single agent and in combination with paclitaxel in patients with recurrent and/or metastatic solid cancer: results from the dose escalation study. *Mol. Cancer Ther.* 22, B046. doi:10.1158/1535-7163.TARG-23-B046

Satora, M., Kułak, K., Zaremba, B., Grunwald, A., Świechowska-Starek, P., and Tarkowski, R. (2024). New hopes and promises in the treatment of ovarian cancer focusing on targeted treatment—a narrative review. *Front. Pharmacol.* 15, 1416555. doi:10.3389/FPHAR.2024.1416555

Urandur, S., and Sullivan, M. O. (2023). Peptide-based vectors: a biomolecular engineering strategy for gene delivery. *Annu. Rev. Chem. Biomol. Eng.* 14, 243–264. doi:10.1146/annurev-chembioeng-101121-070232

Vadevoo, S. M. P., Gurung, S., Lee, H.-S., Gunassekaran, G. R., Lee, S.-M., Yoon, J.-W., et al. (2023). Peptides as multifunctional players in cancer therapy. *Exp. Mol. Med.* 55, 1099–1109. doi:10.1038/s12276-023-01016-x

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., et al. (2022). Therapeutic peptides: current applications and future directions. *Signal Transduct. Target Ther.* 7, 48. doi:10.1038/s41392-022-00904-4

Xie, J., Bi, Y., Zhang, H., Dong, S., Teng, L., Lee, R. J., et al. (2020). Cell-penetrating peptides in diagnosis and treatment of human diseases: from preclinical research to clinical application. *Front. Pharmacol.* 11, 697. doi:10.3389/fphar.2020.00697

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Mapping and visualization of global research progress on deubiquitinases in ovarian cancer: a bibliometric analysis

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Background: Ovarian cancer is a highly aggressive malignancy with limited therapeutic options and a poor prognosis. Deubiquitinating enzymes (DUBs) have emerged as critical regulators of protein ubiquitination and proteasomal degradation, influencing various cellular processes relevant to cancer pathogenesis. In this study, the research progress between ovarian cancer and DUBs was mapped and visualized using bibliometrics, and the expression patterns and biological roles of DUBs in ovarian cancer were summarized.

Methods: Studies related to DUBs in ovarian cancer were extracted from the Web of Science Core Collection (WoSCC) database. VOSviewer 1.6.20, CiteSpace 6.3.R1, and R4.3.3 were used for bibliometric analysis and visualization.

Results: For analysis 243 articles were included in this study. The number of publications on DUBs in ovarian cancer has gradually increased each year. China, the United States, and the United Kingdom are at the center of this field of research. The Johns Hopkins University, Genentech, and Roche Holding are the main research institutions. David Komander, Zhihua Liu, and Richard Roden are the top authors in this field. The top five journals with the largest publication volumes in this field are *Biochemical and Biophysical Research Communications*, *Journal of Biological Chemistry*, *PLOS One*, *Nature Communications*, and *Oncotarget*. Keyword burst analysis identified five research areas: "deubiquitinating enzyme," "expression," "activation," "degradation," and "ubiquitin." In addition, we summarized the expression profiles and biological roles of DUBs in ovarian cancer, highlighting their roles in tumor initiation, growth, chemoresistance, and metastasis.

Conclusion: An overview of the research progress is provided in this study on DUBs in ovarian cancer over the last three decades. It offers insight into the most cited papers and authors, core journals, and identified new trends.

KEYWORDS

ovarian cancer, deubiquitinating enzyme, bibliometric analysis, biologic role, systematic review

Introduction

Ovarian cancer, which is the fifth most prevalent cancer among women, significantly contributes to global cancer-related mortalities in women (Siegel et al., 2023). Due to the non-specific or subtle symptoms associated with this disease, early detection and diagnosis remain challenging. Consequently, it is frequently diagnosed at advanced stages, leading to undesirable outcomes. Previous studies have identified various risk factors for ovarian cancer, including family history, age, obesity, genetic mutations, and early onset of menstruation (Wang et al., 2023a; Sung et al., 2023; Sandvei et al., 2023; Matan et al., 2022; Fortner et al., 2019; Arora et al., 2024). However,

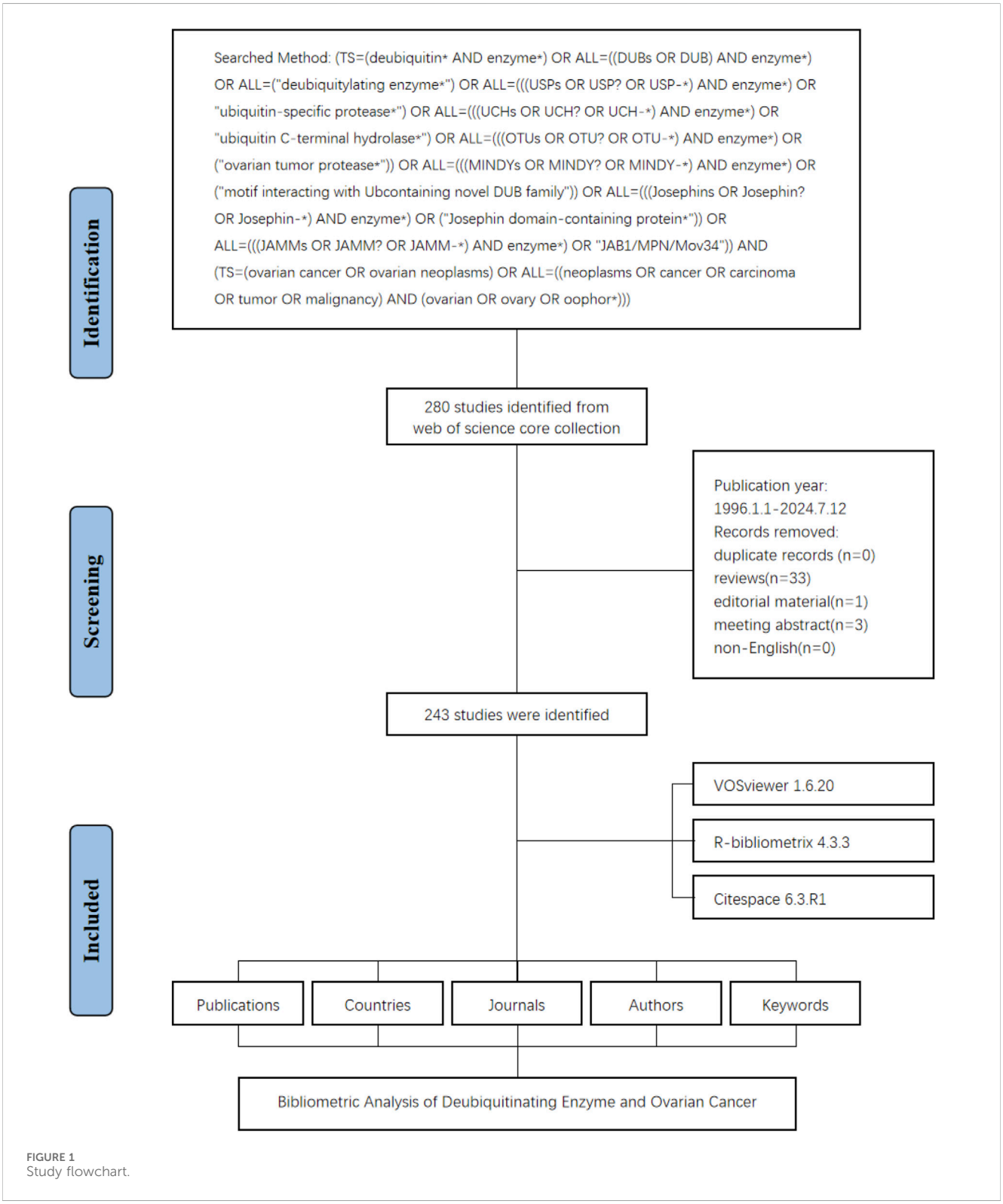

FIGURE 1
Study flowchart.

FIGURE 2
Overview of the main information.

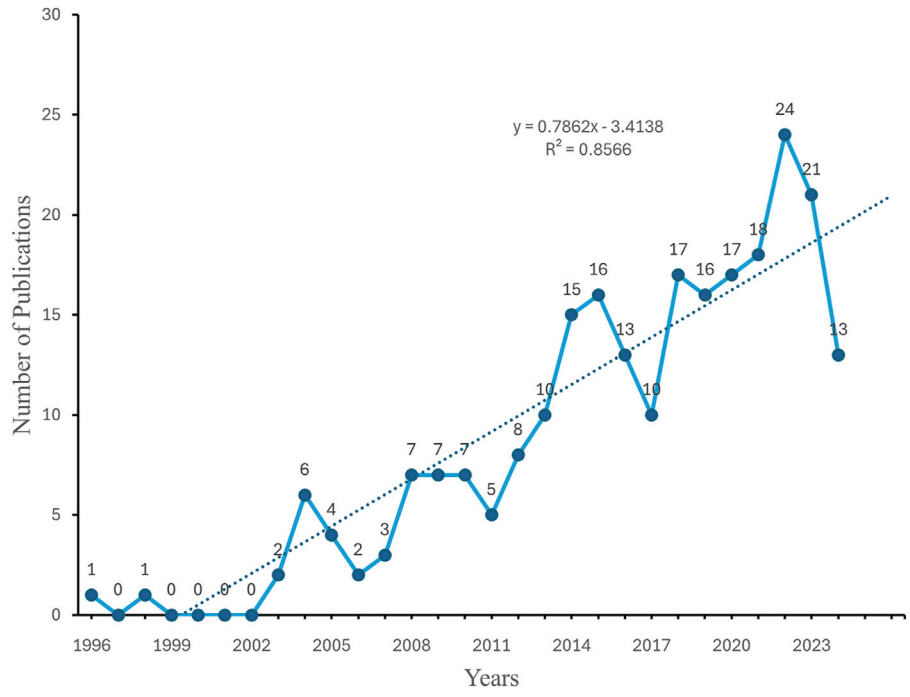

FIGURE 3
Annual number of publications on deubiquitinating enzymes and ovarian cancer.

more efforts are still required to establish effective screening strategies, such as protein biomarkers, for the early diagnosis of ovarian cancer.

Post-translational modification plays an important role in regulating target protein activity, stability, interaction, and/or localization (Singh and Ostwal, 2019; Lee et al., 2023; Wang et al., 2022a; Li et al., 2023). Acetylation, sumoylation, ubiquitination, and phosphorylation are the most common types of protein post-translational modification (Wang et al., 2014a). Specifically, ubiquitination is a process in which an ubiquitin (Ub) protein, or a chain of Ub proteins, is covalently attached to the target substrate, ultimately leading to the proteasomal degradation or localization alteration of the target protein (Damgaard, 2021). This process can be reversed by deubiquitinases (DUBs), which cleave ubiquitin from targeted proteins (Snyder and Silva, 2021). The dynamic balance between ubiquitination and deubiquitination plays critical roles in biological activities, such as cell-signaling transduction, apoptosis, and drug resistance. To date, six classes of DUBs have been identified, namely, ovarian tumor proteases (OTUs), ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), and Josephin domain-containing proteins, MINDYs, and JAMMs (Harrigan et al., 2018). Among them, USPs form the largest family of DUBs. Accumulating evidence suggests that the dysregulation of USPs is involved in various diseases, including cancer. For example, we previously found that targeting USP47 could decrease tyrosine kinase inhibitor resistance and eradicate leukemia stem/progenitor cells in chronic myelogenous leukemia (Lei et al., 2021a). We and others have suggested that USP7 plays essential biological roles in the pathogenesis of multiple myelomas (Jing et al., 2018; Wang et al., 2022b; Chauhan

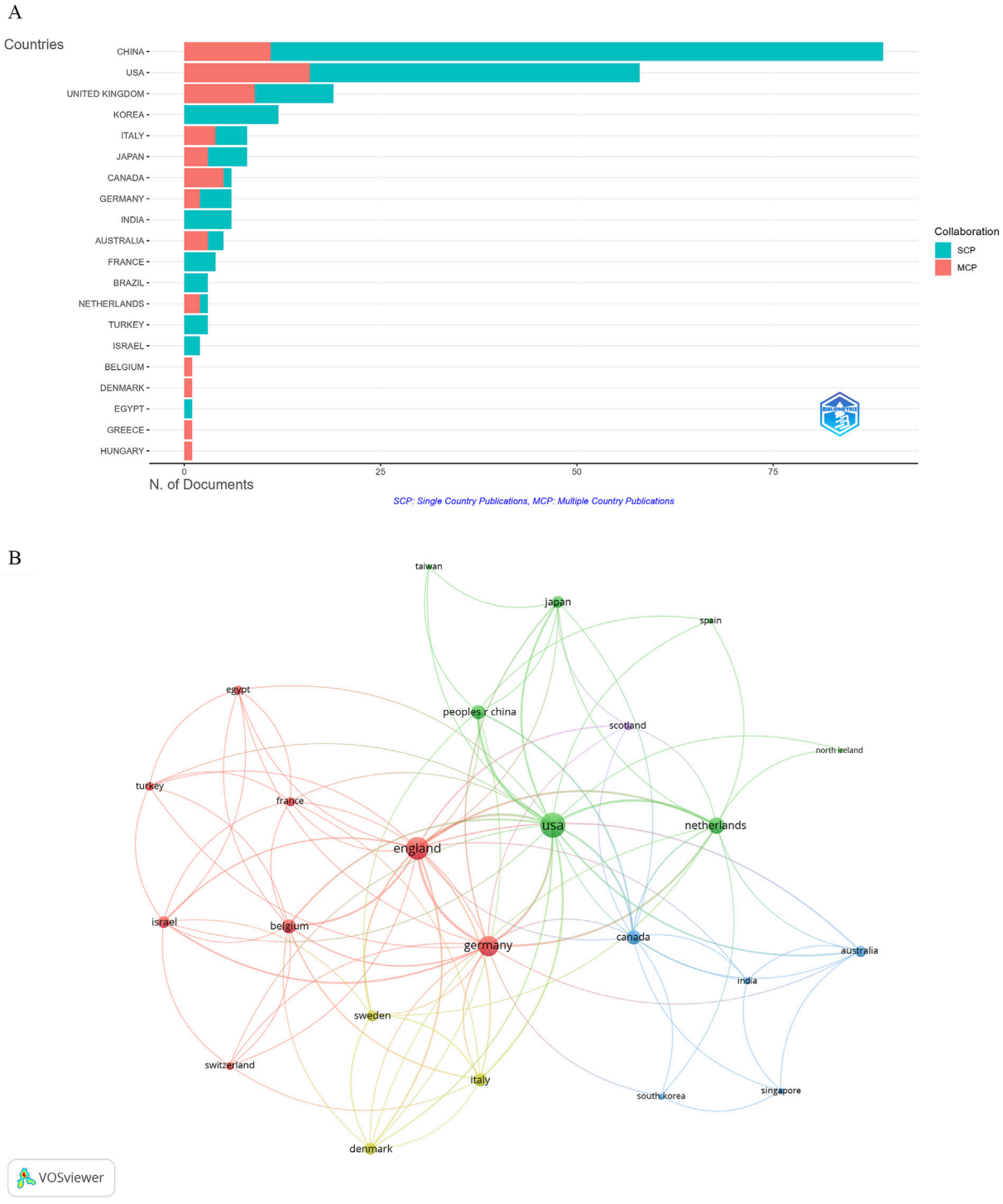

FIGURE 4
Visualization of countries. **(A)** Publications by country. **(B)** International collaboration network.

et al., 2012). Importantly, USP7 has also been revealed as a promising target for ovarian cancer treatment (Ma and Yu, 2016; Zhang et al., 2016; Qin et al., 2016). Thus, DUBs, especially USPs, may serve as promising biomarkers for the early detection and diagnosis of ovarian cancer.

In this study, we performed a bibliometric analysis of the scientific articles published on DUBs in ovarian cancer to evaluate the study trends on this topic. Although several bibliometric analyses have been published on various topics in ovarian cancer (Song et al., 2024; Lin et al., 2024; Meng et al., 2024; Wang et al., 2024; Leng et al., 2023; Giles et al., 2023; Duan et al., 2023; Liu et al., 2023a), this is the first study to identify the most influential literature in this field. We also summarized the expression and biological roles of DUBs in ovarian cancer and explored their potential as biomarkers.

TABLE 1 Publication and citation profiles of the top 10 countries.

Country	Articles	Freq	MCP_Ratio	TP	TP_rank	TC	TC_rank	Average citations
China	89	0.366	0.124	302	1	1,595	3	17.9
United States of America	58	0.239	0.276	283	2	5,619	1	96.9
United Kingdom	19	0.078	0.474	40	4	1,664	2	87.6
Korea	12	0.049	0.000	36	5	179	9	14.9
Italy	8	0.033	0.500	46	3	157	10	19.6
Japan	8	0.033	0.375	33	6	234	8	29.2
Canada	6	0.025	0.833	26	9	237	7	39.5
Germany	6	0.025	0.333	30	7	70	13	11.7
India	6	0.025	0.000	22	10	86	12	14.3
Australia	5	0.021	0.600	29	8	284	6	56.8

Note(s): Articles, publications of corresponding authors only; Freq, frequency of total publications; MCP_Ratio, proportion of multiple country publications; TP, total publications; TP_rank, rank of total publications; TC, total citations; TC_rank, rank of total citations; Average citations, average number of citations per publication.

Methods

Data sources and search strategy

The literature search was conducted to retrieve related articles from inception to May 2024 from the Web of Science Core Collection (WoSCC). The search strategy is presented in Supplementary Table S1. This study included only "articles" and considered only documents written in English. As all data were obtained from a public database, ethical declarations or approvals are not applicable.

Data analysis and visualization

We extracted relevant data from the retrieved literature titles and used Microsoft Excel 16.0 to identify and calculate bibliometric parameters. These metrics cover key aspects of publications, including the number of publications per year, citation frequency, average citation frequency, journal title, journal impact factor, country/region of publication, publishing organization, and authors.

The visualization and analysis process involved the use of three powerful bibliometric analysis tools to fully analyze the academic data: VOSviewer (version 1.6.20), CiteSpace (version 6.3.R1), and R4.3.3. VOSviewer is a versatile software tool that plays a key role in mapping institutional collaborations, co-authorships, citations, and co-citations (van Eck and Waltman, 2010). It was used for keyword co-occurrence analysis. CiteSpace 6.3.R1 was used for keyword emergence detection and co-occurrence analysis, with the parameters set to time slicing: from January 1996 to May 2024 (research in this field was originally published in 1996). The time slicing was set to 1 year, and the node types were set to keywords. When nodes are keywords, the threshold (top N per segment) was set to 5, and pruning was set to the pathfinder + pruning merged network. Based on the parameter settings for each node, a visual analysis was performed to generate a timeline graph of deubiquitinating enzymes with keywords in the field of ovarian cancer research.

Results

Overview of the main information

The study flowchart is presented in Figure 1. A total of 243 articles were identified in this study on DUBs in ovarian cancer over the last three decades. Our investigation showed that 1,895 authors from 926 institutions across 135 countries contributed to the production of these 243 manuscripts. These works were published in 152 journals, citing 8,428 references, with an average of 46.44 citations per article (Figure 2).

Annual publication trend

To gain insight into the evolution of related research in this field, we examined the annual publication trends. The study period exhibited a discernible upward trajectory in annual publications, particularly since 2003. The change in cumulative publications over time follows the trend line equation $y = 0.7{,}862 \times -3.4{,}138$, with a correlation coefficient of 0.8566 and an annual growth rate of 9.59%. Additionally, 2022 witnessed the highest number of publications, accounting for 9.88% of the total (Figures 2, 3).

Analysis of countries

The identified publications came from 135 countries, with China leading in the number of studies (89 publications), constituting 36.62% of all documents. Other top contributors included the United States of America (58 publications), the United Kingdom (19 publications), Korea (12 publications), Japan (8 publications), and Italy (8 publications) (Figure 4A; Table 1). Despite China having the highest number of articles, the United States of America, France, and the United Kingdom had the highest average citations, that is, 96.9, 89.8, and 87.6, respectively. In addition, the collaboration among countries was visualized using VOSviewer. As shown in Figure 4B, the United States, the United Kingdom, and Germany were the top three countries with the strongest international collaboration network.

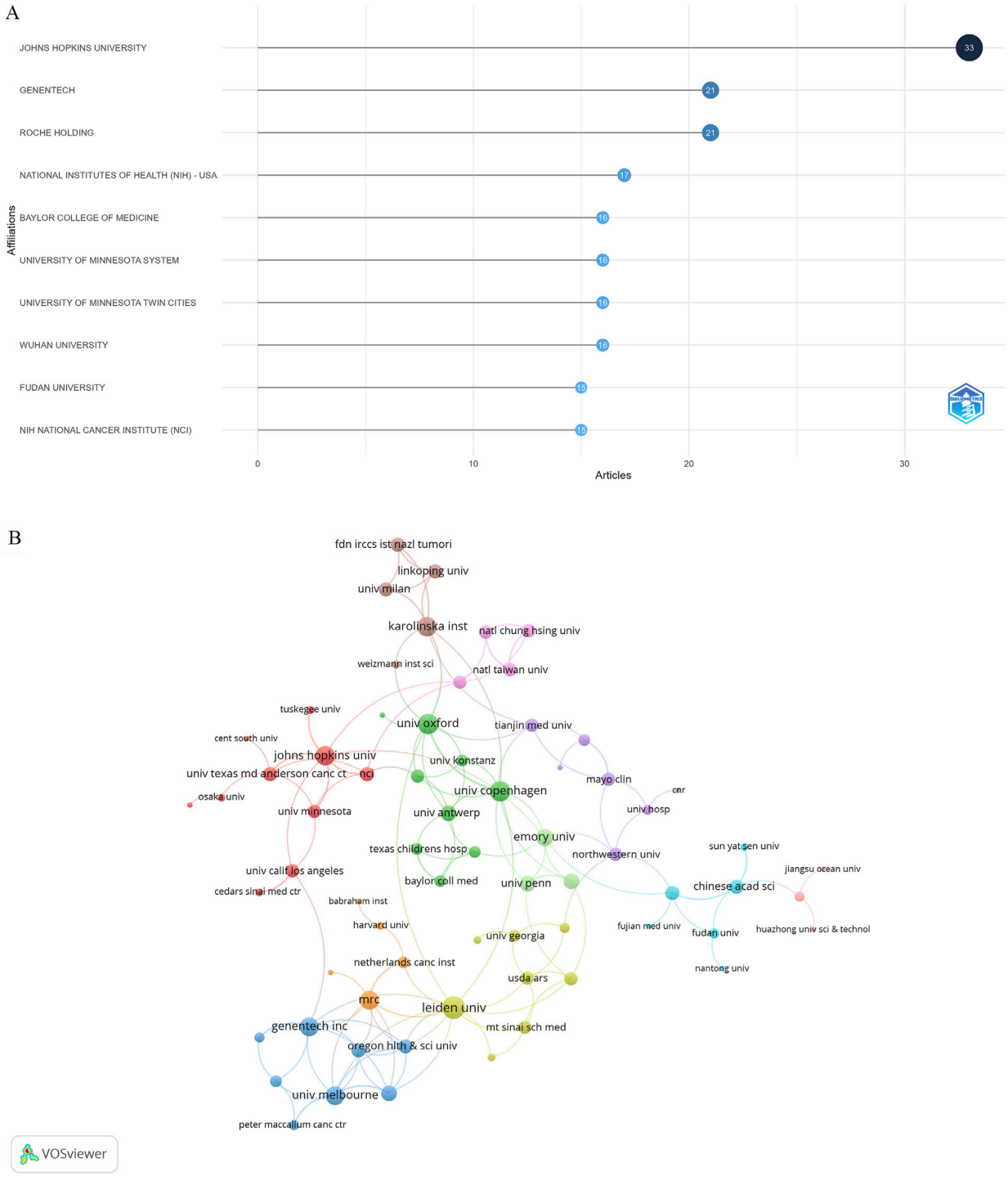

FIGURE 5
Visualization of institutions. **(A)** Publications by institution. **(B)** Collaborative networks of institutions.

Analysis of institutions

Publications related to research on DUBs in ovarian cancer involved 926 institutions. The three institutions with the most publications were Johns Hopkins University (United States, 33 publications), Genentech (United States, 21 publications), and Roche Holding (United States, 21 publications) (Figure 5A). Institutions with at least two publications were included in the analysis of collaborative networks, which were visualized using VOSviewer. The clusters were arranged in different colors based on the frequency of collaboration between institutions (Figure 5B). Johns Hopkins University had the largest node, indicating the highest level of collaboration with other institutions.

TABLE 2 Top 20 productive journals related to DUBs in ovarian cancer.

Journal	IF (2023)	JCR_Quartile	H_index	PY_start	TP	TP_rank	TC	TC_rank	g-index	m-index
Biochemical and Biophysical Research Communications	2.5	Q3	6	2005	9	1	59	36	9	0.300
Journal of Biological Chemistry	4	Q2	6	2003	7	2	527	1	7	0.273
Nature Communications	14.7	Q1	6	2013	6	4	153	11	6	0.500
Oncotarget	N/A	N/A	6	2014	6	5	N/A	N/A	6	0.545
Cell Death and Differentiation	13.7	Q1	5	2016	5	6	64	32	5	0.556
Journal of Virology	4	Q2	5	2010	5	8	193	8	5	0.333
PLOS One	2.9	Q1	5	2010	7	3	146	12	7	0.333
Proceedings of the National Academy of Sciences of the United States of America	9.4	Q1	5	2011	5	10	287	5	5	0.357
International Journal of Oncology	4.5	Q1	4	2004	5	7	N/A	N/A	5	0.190
Oncogene	6.9	Q1	4	1998	5	9	178	9	5	0.148
Science Advances	11.7	Q1	4	2018	5	11	N/A	N/A	5	0.571
Biochemical Journal	4.4	Q2	3	2004	4	12	86	22	4	0.143
Cell Death and Disease	8.1	Q1	3	2022	3	13	50	40	3	1.000
EMBO Journal	9.4	Q1	3	2012	3	14	230	6	3	0.231
Genes Chromosomes and Cancer	3.1	Q2	3	2008	3	15	N/A	N/A	3	0.176
Journal of Experimental and Clinical Cancer Research	11.4	Q1	3	2019	3	17	N/A	N/A	3	0.500
Molecular Cell	14.5	Q1	3	2009	3	18	383	3	3	0.188
Nature	50.5	Q1	3	2004	3	19	486	2	3	0.143
Oncology Reports	3.8	Q2	3	2015	3	20	43	50	3	0.300
Biochemistry	2.9	Q3	2	2016	2	22	N/A	N/A	2	0.222

Note(s): H_index, h-index of the journal, which measures both the productivity and citation impact of the publications; IF, impact factor, indicating the average number of citations to recent articles published in the journal; JCR_Quartile, quartile ranking of the journal in the Journal Citation Reports, indicating the journal ranking relative to others in the same field (Q1: top 25%, Q2: 25%–50%, Q3: 50%–75%, and Q4: bottom 25%); TP, total publications; TP_rank, rank of total publications; TC, total citations; TC_rank, rank of total citations; Average citations, average number of citations per publication; PY_start, publication year start, indicating the year the journal started publication; g_index, g-index of the journal, which provides more weight to highly cited articles; m_index, m-index of the journal, which is the h-index divided by the number of years since the first published paper; N/A, not applicable.

Analysis of journals and co-cited journals

Research on DUBs in ovarian cancer prominently features in 152 journals. *Biochemical and Biophysical Research Communications* leads with nine publications, accounting for 3.70% of the total, followed by the *Journal of Biological Chemistry* and *PLOS One*, each with seven papers, accounting for 2.88% each (Table 2). Co-citation analysis revealed that the five key journals with the highest total link strength were the *Journal of Biological Chemistry* (56), *Proceedings of the National Academy of Sciences of the United States of America* (54), *PLOS One* (48), *Cell* (47), and *EMBO Reports* (40) (Figure 6A). Bibliographic coupling analysis indicated that the five key journals with the highest total link strength were *PLOS One* (1,110), *Proceedings of the National Academy of Sciences of the United States of America* (1,053), *Journal of Biological Chemistry* (1,049), *EMBO Journal* (901), and *Nature Communications* (818) (Figure 6B).

Analysis of authors and collaborations

The 243 articles were contributed by 1,895 authors. The distribution of authors was relatively concentrated, and a high degree of collaboration strength was observed. David Komander, Zhihua Liu, and Richard Roden contributed the highest number of publications, with total citations of 939, 198, and 263, respectively (Table 3). Using

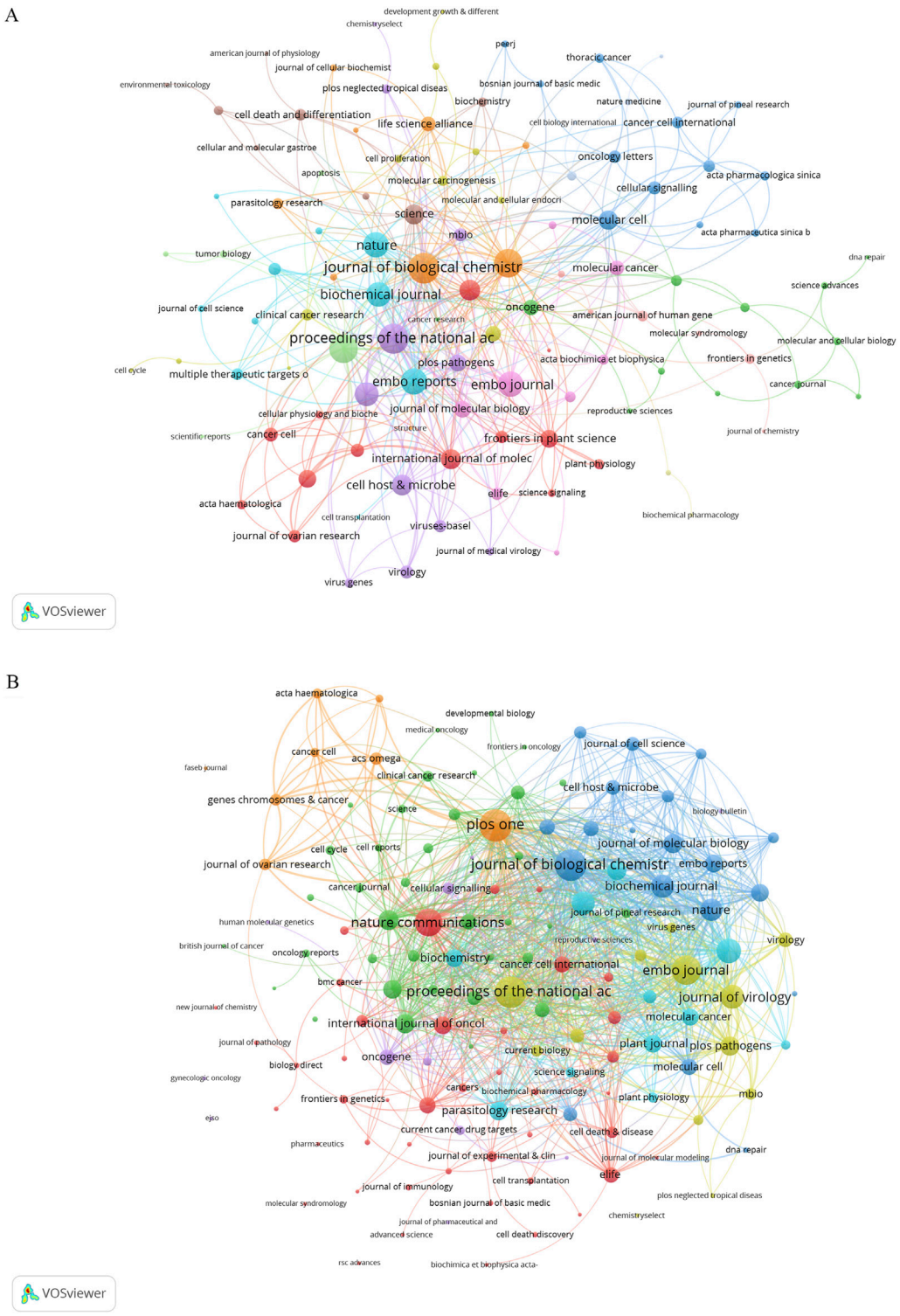

FIGURE 6
Co-citation and bibliographic coupling analysis. **(A)** Co-occurrence networks: journal link strength in co-occurrence networks measures the frequency with which two journals are cited together within the same articles or references. This metric reflects how often the publications from two different journals are associated in the bibliographies of scholarly articles. High link strength implies that the journals are often cited in tandem, indicating a thematic or topical connection between the research they publish. **(B)** Coupling networks: journal link strength in coupling networks assesses the extent to which journals are linked based on the common references cited in their articles. This metric captures the degree to which the research published in two different journals relies on the same body of prior work. Strong link strength in this context signifies that the journals share a substantial number of references, highlighting a shared intellectual foundation or research focus.

TABLE 3 Publication and citation profiles of the top 20 authors.

Authors	H_index	g-index	m-index	PY_start	TP	TP_Frac	TP_rank	TC	TC_rank
Komander David	6	7	0.35	2008	7	1.20	1	939	1
Liu Zhihua	5	5	0.71	2018	5	0.74	4	198	20
Roden Richard B. S	5	7	0.42	2013	7	0.62	2	263	15
Anchoori Ravi K	4	5	0.33	2013	5	0.40	3	164	23
Anderson Lee	4	4	0.24	2008	4	0.49	6	113	33
Fejzo Marlena S	4	4	0.24	2008	4	0.49	8	113	33
Ovaa Huib	4	5	0.33	2013	5	0.41	5	599	7
Pegan Scott D	4	4	0.29	2011	4	0.65	11	116	32
Slamon Dennis J	4	4	0.24	2008	4	0.49	12	113	33
Snijder Eric J	4	4	0.22	2007	4	0.51	13	617	6
Ahel Ivan	3	3	0.75	2021	3	0.42	14	112	37
Akutsu Masato	3	3	0.21	2011	3	0.34	15	653	3
Anchoori Ravi	3	3	0.27	2014	3	0.35	16	118	31
Baek Kwang-Hyun	3	3	0.20	2010	3	0.89	17	35	46
Bazzaro Martina	3	4	0.27	2014	4	0.45	7	135	25
Bergeron Eric	3	3	0.20	2010	3	0.48	18	126	29
Ding Fang	3	3	0.43	2018	3	0.37	19	176	22
Dixit vishva M	3	3	0.17	2007	3	0.18	20	736	2
Frias-Staheli Natalia	3	3	0.17	2007	3	0.34	21	484	12
Fu Hongyong	3	4	0.27	2014	4	0.68	9	69	43

Note(s): H_index, h-index of the journal, which measures both the productivity and citation impact of the publications; g_index, g-index of the journal, which provides more weight to highly-cited articles; m_index, m-index of the journal, which is the h-index divided by the number of years since the first published paper; TP, total publications; TP_rank, rank of total publications; TC, total citations; TC_rank, rank of total citations; Average citations, average number of citations per publication; PY_start, publication year start, indicating the year the journal started publication.

VOSviewer, a collaborative network analysis was conducted on authors with publication volumes of three or more. Among the 170 authors involved in international collaborations, Richard Roden had the highest number of collaborations with other countries (total link strength = 48), followed by Ravik Anchoori (total link strength = 35) and David Komander (total link strength = 27) (Figure 7).

Analysis of research hotspots and frontiers

Keywords succinctly encapsulate the fundamental concepts of a paper, outlining the key areas of research interest. A comprehensive keyword analysis of the selected 243 articles related to DUBs was performed using "Author Keywords" from the Biblioshiny application and "Keywords Plus" provided by the VOSviewer application. In total, 566 keywords were identified. A network visualization map demonstrating the connections among these keyword co-occurrences was generated using VOSviewer. The sizes of the circles correspond to the frequency of occurrence of the keywords. A co-word analysis revealed that "deubiquitinating enzyme," "degradation," "expression," "activation," and "ubiquitin" were the most frequently co-occurring keywords (Figure 8). The top 20 co-occurring keywords are given in Table 4.

Figure 9 presents the top 20 keywords with the highest burst strengths. The most significant citation burst belongs to "deubiquitinating enzyme." Particularly noteworthy is the concentration of keywords such as "cancer," "growth," "specificity," "mechanism," "ubiquitin," "pathway," "ovarian cancer," "resistance," and "enzymes" since 2020, indicating promising developments.

Discussion

Since 1996, studies on DUBs in ovarian cancer have experienced rapid growth, particularly after 2002, driven by their pivotal biological roles in cancer research. It is evident that DUBs have gradually emerged as a hotspot in ovarian cancer, indicated by an average citation of 47.41 per article. Additionally, the number of articles on DUBs in ovarian cancer has steadily increased, with an annual growth rate of 8.57%. Since 2020, keyword concentrations have focused on "cancer," "growth," "specificity," "mechanism," "ubiquitin," "pathway," "ovarian cancer," "resistance," and "enzymes," highlighting future research directions for DUBs in ovarian cancer. Additionally, the most frequently co-occurring keywords are "deubiquitinating

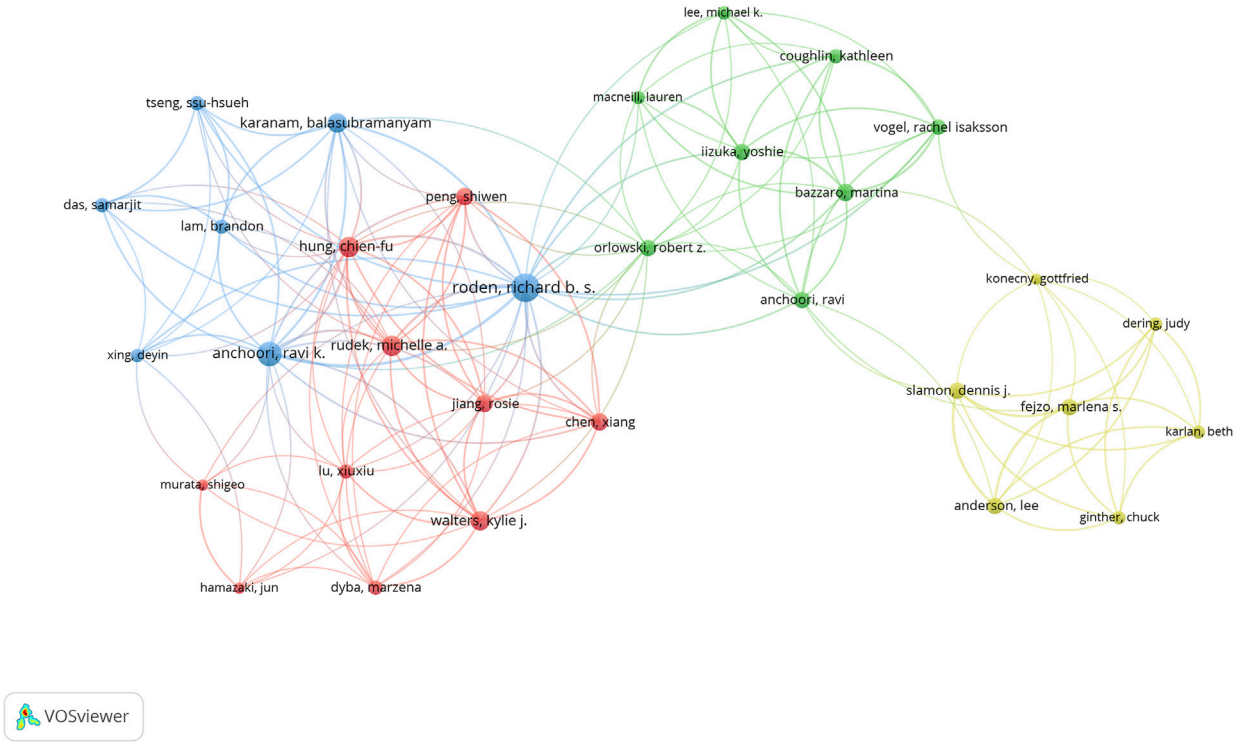

FIGURE 7
Visualization map depicting the collaboration among different authors. Nodes represent authors, with size indicating the publication count. Links represent co-authorships, with thickness showing collaboration strength. Colors indicate different research clusters. The total link strength in collaboration networks measures the frequency of co-authorship between authors, indicating the level of collaborative research.

enzyme," "degradation," "expression," "activation," and "ubiquitin," indicating that a deep understanding of the mechanisms of DUBs in ovarian cancer is a critical medical need. Interestingly, these keywords are centered around the critical regulatory functions of DUBs, suggesting that DUBs are widely entangled with the classic signaling pathways that have been well understood in ovarian cancer development. These findings highlight that DUBs may be of equal importance as the key regulatory proteins in cell division, growth, and proliferation, encouraging research workers to include DUBs as a part of the cellular regulatory network rather than as a simple tool for protein degradation and recycling. Therefore, based on this bibliometric analysis, studies of DUBs on ovarian cancer are likely to continue advancing by understanding their roles in cancer development and their potential as therapeutic targets.

The countries with the highest publication volume are primarily China, the United States, and the United Kingdom. China ranks the first in terms of publication quantity, whereas the United States and the United Kingdom have the highest average citations (all higher than 100) and intermediary centrality, highlighting their active and prominent roles in this field. However, the average citation frequency per paper in China is low, indicating that Chinese authors have lower citation frequencies, highlighting the need of high-quality paper publication. Notably, the top three institutions contributing to the publication volume were all from the United States, indicating a pioneering role in driving DUB-related research in ovarian cancer. Johns Hopkins University, Roche Holding, and Genentech had the highest intermediary centrality, serving as crucial contributors to fundamental DUB research in this disease. The top three cited articles had 1,509, 573, and 429 citations, respectively, and were published in *Nature* (impact factor = 50.5), *Oncogene* (impact factor = 6.9), and *Cell* (impact factor = 45.5) (Wertz et al., 2004; Jensen et al., 1998; Mevissen et al., 2013). All three articles focused on the mechanism of DUBs, highlighting the critical need of the mechanical analysis of this malignant disease.

We summarized the expression profile and biological roles of DUBs in ovarian cancer. Specifically, the following terms were used for the database search without language and regional restrictions: "ovarian cancer" or "ovarian neoplasms" AND "deubiquitinating enzymes" or "deubiquitinases" or "ovarian tumor proteases" or "ubiquitin-specific proteases" or "ubiquitin C-terminal hydrolases" or "Josephin domain-containing proteins" or "motif interacting with Ubcontaining novel DUB family" or "JAB1/MPN/Mov34 metalloenzyme." Other eligible studies were also reviewed from the references of each article. As we retrieved zero results for Josephin domain-containing proteins in ovarian cancer, we mainly focused on the expression and functional role of OTUs, USPs, and UCHs in ovarian cancer (Table 5). Research workers may utilize this information to develop treatments against important molecular targets, such as mutant p53 and PTEN, or explore DUBs as potential therapeutic targets. For instance, USP7 is one of the representative DUBs that have been widely studied in cancer research. It exerts fine-tuned control over diverse protein

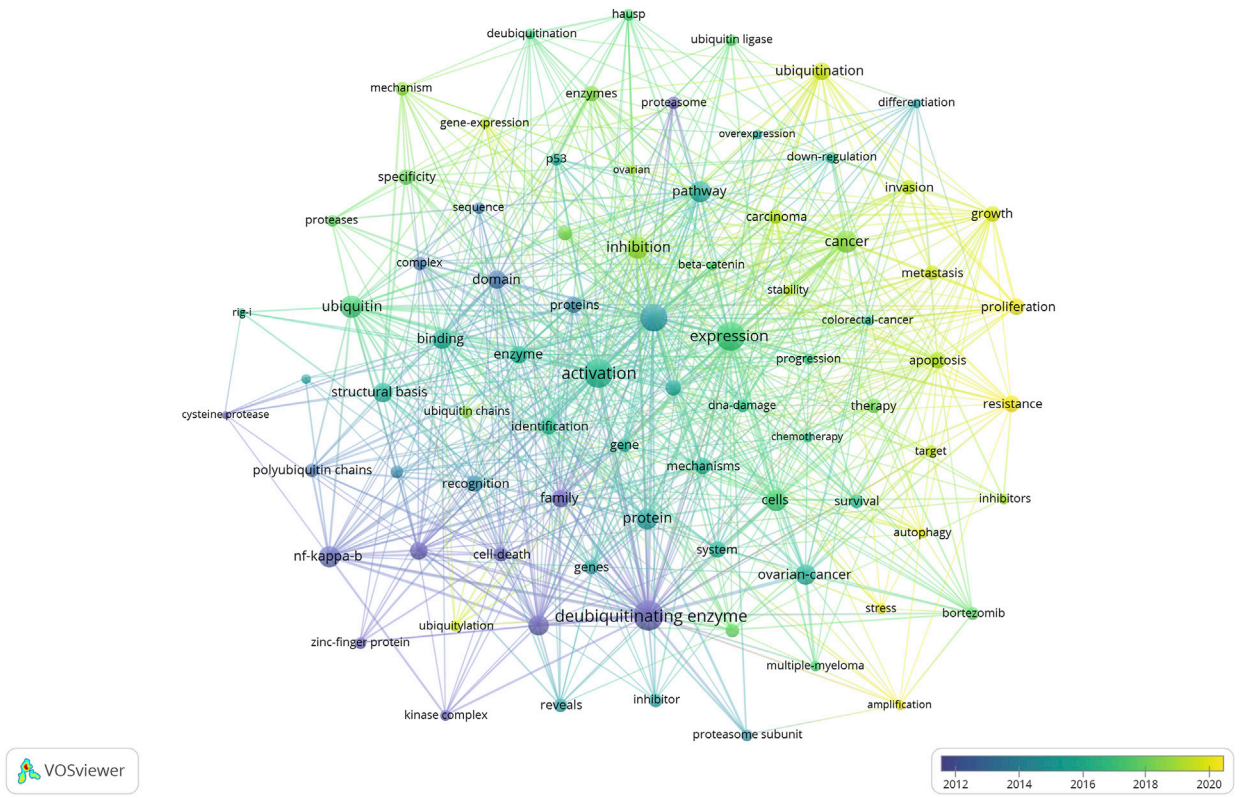

FIGURE 8
Visualization of keyword co-occurrence. This network visualization displays the co-occurrence of keywords in selected literature. Each node represents a keyword, with size indicating its frequency of occurrence. Links between nodes represent co-occurrence in the same documents, with thicker lines showing stronger associations. Colors reflect the average publication year of the articles, as indicated by the color gradient at the bottom right.

Top 20 Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin	End	1996 - 2024
family	1996	2.2	**1996**	2004	
deubiquitinating enzyme	1998	6.51	**2004**	2013	
cell death	2004	1.07	**2004**	2007	
degradation	2003	1.22	**2008**	2009	
cysteine protease	2004	2.76	**2011**	2012	
66k replication protein	2012	1.27	**2012**	2013	
binding	2008	3.22	**2014**	2016	
nf kappa b	2007	1.71	**2014**	2015	
domain	2004	2.26	**2015**	2016	
protein	1996	1.69	**2017**	2018	
activation	2003	1.3	**2018**	2019	
cancer	1996	3.02	**2020**	2024	
growth	2020	2.86	**2020**	2021	
specificity	2020	2.4	**2020**	2022	
mechanism	2016	2.04	**2020**	2021	
ubiquitin	2009	1.11	**2021**	2024	
pathway	2004	1.08	**2021**	2022	
ovarian cancer	1998	1.07	**2021**	2022	
resistance	2022	3.24	**2022**	2024	
enzymes	2016	1.67	**2022**	2024	

FIGURE 9
Top 20 keywords with the strongest citation bursts.

TABLE 4 Top 20 keyword co-occurrence network analysis.

id	Keyword	Occurrences	Total link strength
208	Deubiquitinating enzyme	34	136
15	Activation	35	129
304	Expression	40	127
200	Degradation	29	110
890	Ubiquitin	27	76
425	Inhibition	20	74
100	Cancer	26	74
688	Protein	24	71
129	Cells	21	69
567	nf-kappa-b	17	68
622	Pathway	19	66
595	Ovarian cancer	21	62
181	Cysteine proteases	14	62
71	Binding	14	58
812	Structural basis	17	56
235	Domain	12	54
172	Crystal structure	10	49
314	Family	11	48
910	Ubiquitination	12	48
283	Enzyme	10	47

levels and functions, impacting cell fate decisions and maintaining cellular homeostasis. USP7 is a critical regulator of many cancer-related proteins, including p53, MDM2, PTEN, and FOXO4. Zhang et al. (2016) suggested that USP7 expression is associated with poor prognosis in ovarian cancer, supported by cellular experiments. Ma and Yu (2016) found that USP7 is highly expressed in epithelial ovarian cancer patients, positively correlated with lymphatic invasion, and independently associated with poor overall survival. They concluded that the modulation of USP7 expression could affect ovarian cancer cell viability and invasion (Ma and Yu, 2016). Wang et al. (2017) reported that the inhibition of USP7 could induce cell death in ovarian cancers, regardless of the P53 status. This finding is consistent with that of previous research, showing that USP7 was highly expressed in ovarian cancer and inversely correlated with the differentiation level, and that inhibition of USP7 could lead to cell apoptosis (Qin et al., 2016). Furthermore, Wang et al. (2023b) found that USP7 deubiquitinases TRAF4, and the knockdown of USP7 suppressed ovarian cancer both *in vitro* and *in vivo*. A recent meta-analysis concluded that USP7 promotes ovarian cancer progression and predicts unfavorable clinical outcomes (Kisaï and Koji, 2021). These findings suggest that USP7 may act as an oncoprotein highly expressed in ovarian cancer cells and patients, and may be associated with poor clinical outcomes. In addition, USP14 may be another promising target in ovarian cancer treatment, with the earliest research traced back to 2007 (Yang et al., 2007). Subsequent studies have revealed the critical involvement of USP14 in various pathways, especially in tumor proliferation and chemoresistance (Wang et al., 2015; Wada et al., 2009; Shen et al., 2020; Huang et al., 2017; Luo et al., 2019; Ji et al., 2023). It can thus be hypothesized that targeting USP14 may be an effective strategy for second- and third-line therapies, during which chemoresistance is the major challenge. Moreover, UCHL1 is another interesting target for its broad implications in various ovarian cancer cell lines, as well as animal models and patient samples (Tangri et al., 2021; Okochi-Takada et al., 2006; Jin et al., 2013). Understanding its roles in different cell lines and signaling pathways may reveal common mechanisms in ovarian cancer development. It should be emphasized that although most DUBs are not direct executers in signaling pathways, they may be equally important as they essentially modulate the concentrations of the key regulators. This can be utilized to create novel therapeutic strategies against certain oncoproteins, especially against those with various mutations or thought to be "undruggable" (Lei et al., 2021b). For example, KRAS mutation is known to promote ovarian cancer development (Therachiyil et al., 2022), yet only a few drugs are proven effective against certain mutations of KRAS. Instead of directly inhibiting KRAS, inducing KRAS degradation by activating its DUB(s) may be a promising approach; furthermore, this strategy may be a "one-size-fits-all" solution that is robust against various KRAS mutations (Fraile et al., 2017), which may also be extended to other critical targets in cancer therapy.

Keywords reflect the primary content of publications and encapsulate the main topics covered in the literature. Analyzing keywords can offer insights into current study hotspots and future directions in the research field. By examining the frequency and co-occurrence of keywords, research workers can identify prevailing themes and emerging trends that shape the field trajectory. In this study, "deubiquitinating enzyme," "degradation," "expression," and "activation" were the most frequently co-occurring keywords. These keywords highlight the central themes of current research, emphasizing the role of DUBs in cellular processes. DUBs are known for their ability to remove ubiquitin from target proteins, thereby preventing their degradation. This stabilization affects the activation and localization of various proteins, triggering cascades of biological processes that are crucial for maintaining cellular homeostasis and function. A timeline viewer for keyword analysis reveals the evolution of hotspots in the field over time, showing how the focus within the field has shifted and expanded. This tool helps visualize the progression of key research topics and provides a historical perspective on how the field has developed. For instance, the consistent appearance of terms like "degradation," "expression," and "activation" underscores the ongoing interest in understanding the fundamental mechanisms of DUBs and their broader biological implications. Regarding keywords with the strongest citation bursts, "cancer," "ubiquitin," "resistance," and "enzymes" have been the latest hotspots in ovarian cancer

TABLE 5 Summary of DUB biological function in ovarian cancer.

Family	DUBs	Author	Year	Source	Target	Mechanism
Ovarian tumor protease (OTU)	OTUB1	Wang et al. (2016)	2016	A2780, SKOV3, CAOV3, and ovarian cancer patients	FOXM1	Tumor progression and prognosis
		Wu et al. (2021)	2021	HeLa and SW620	/	Chemoresistance
		Maresca et al. (2015)	2015	Ovarian cancer tissue	/	Tumorigenesis
	OTUD3	Johnson et al. (2020)	2020	Bioinformatics analysis, OVSAHO, PEO1, and OVCAR5	PTEN and RIPK	Necroptosis
	ALG13	Wang (2021)	2021	Bioinformatics analysis	/	Prognosis
	A20	Lin et al. (2016)	2016	SKOV3	CYLD	Chronic inflammation, apoptotic resistance, and invasion
	OTUD7A	Tavares et al. (2021)	2021	Bioinformatics analysis	/	/
Ubiquitin-specific proteases (USPs)	USP1	Sonego et al. (2019)	2019	MDAH-2774, TOV-21G, OV-90, SKOV3, OVCAR3, OVCAR4, OVCAR8, OVSAHO, KURAMOCH, and ovarian cancer tissue	Snail	Platinum resistance and metastasis
		Simoneau et al. (2023)	2023	*BRCA1/2* mutant and wild-type tumor	PCNA	Apoptosis
		Song et al. (2022)	2022	OVCAR8, EFO21, and bioinformatics analysis	S phase	Cell cycle
	USP2	Yang et al. (2007)	2007	Ovarian cancer tissue	/	/
	USP5	Du et al. (2019)	2019	Ovarian serous carcinoma specimen, OVCAR3, A2780, HO-8910, CAOV3, SKOV3, and xenograft model	HDAC2	Apoptosis
	USP7	Zhang et al. (2016)	2016	Primary serous ovarian cancer specimen and SKOV3	March7	Cell proliferation, invasion
		Ma and Yu (2016)	2016	Primary serous ovarian cancer specimen, SKOV3, and OVCAR3	/	Overall survival, lymph node metastasis, cell viability, and invasion
		Wang et al. (2017)	2017	HeyA8 and OVCAR8	/	Cell death and autophagy
		Qin et al. (2016)	2016	Ovarian cancer tissue array, SKOV3, HO-8910 OVCAR3, A2780, A2780/CP70, HeyC2, and xenograft model	Mdm2, Mdmx, and UHRF1	Cell death
		Wang et al. (2023b)	2023	Ovarian cancer tissue, CAOV-3, SKOV3, and xenograft model	TRAF4	Proliferation, migration, and invasion
		Kisaï and Koji (2021)	2021	Meta-analysis	/	Cancer progression and prognosis
	USP8	Corno et al. (2022)	2022	IGROV-1, A2780, PEO1, PEO4, PEO6, IGROV-1/Pt1, A2780/CP, A2780/BBR, and advanced ovarian cancer patients	/	Drug resistance and apoptosis
	USP9X	Hunter et al. (2015)	2015	Low-grade serous ovarian tumor specimen	/	Tumorigenesis
		Habata et al. (2016)	2016	AMOC2, ES2, and primary ovarian cancer specimens	Mcl-1	Chemoresistance
	USP10	Han et al. (2019)	2019	Epithelial ovarian cancer tissue microarray	/	Prognosis
		Gao et al. (2022)	2022	Bioinformatics analysis	Immune infiltration	Prognosis
		Li et al. (2022a)	2022	Ovarian cancer tissue array, OVCAR3, ES2, A2780, SKOV3, and IGROV1	G3BP1	Cancer progression and metastasis
	USP11	Wang et al. (2019a)	2019	Ovarian cancer tissues, OVCAR-3, and SKOV3	Snail	Epithelial-to-mesenchymal transition
		Zhu et al. (2021)	2021	Ovarian cancer specimen, ES2, and 3AO	BIP	Chemoresistance
		Guo et al. (2022) and Stiff et al. (1994)	2022, 1994	Refractory ovarian cancer patients	/	/
	USP13	Han et al. (2016)	2016	Ovarian cancer specimens, CAOV3, OVCAR3, HeyA8, OVCAR8, and SKOV3	PIK3CA	Cancer metabolism
		Zhang et al. (2018)	2018	SW-1573, TOV-21G, xenograft model, and ovarian cancer specimen	MCL1	Proliferation
		Li et al. (2017)	2017	OVCAR3, SKOV3, A2780, FU-OV-1, EFO-27, and xenograft model	RAP80-BRCA1	DNA damage
		Kwon et al. (2022a)	2022	Xenograft model and primary ovarian specimen	/	Cancer development and metastasis
		Kwon et al. (2022b)	2022	HeyA8 and COV318	/	Proliferation
	USP14	Yang et al. (2007)	2007	Ovarian cancer tissue	/	/

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TABLE 5 (*Continued*) Summary of DUB biological function in ovarian cancer.

Family	DUBs	Author	Year	Source	Target	Mechanism
		Wang et al. (2015)	2015	Epithelial ovarian cancer tissue and SKOV3	/	Proliferation, prognosis
		Wada et al. (2009)	2009	SHIN-3	/	Tumorigenesis
		Shen et al. (2020)	2020	A2780, COC1, A2780/CP, and COC1/CP	BCL6	Chemoresistance
		Huang et al. (2017)	2017	A2780, SKOV3, and xenograft model	/	Proliferation and tumor growth
		Luo et al. (2019)	2019	A2780 and A2780/CDDP	Connexin 32	Chemoresistance
		Ji et al. (2023)	2023	A2780, OVCAR8	BACH1	Heme metabolism and invasion
	USP15	Xu et al. (2009)	2009	HeLa	Caspase-3	Apoptosis
		Eichhorn et al. (2012)	2012	/	TβR-I	Tumorigenesis
		Padmanabhan et al. (2018)	2018	SKOV3, SK-BR-3, YK-Nu, OVCAR3, OVCA420, S1GODL, MDAH2774, COV362, and TOV-112D	p53-R175H	Cell death
	USP17	Yildirim et al. (2019)	2019	High-grade, advanced-staged serous ovarian cancer biopsy	/	Epithelial-to-mesenchymal transition
	USP18	Liu et al. (2022)	2022	A2780, SKOV3, and bioinformatics analysis	AKT/mTOR	Proliferation and migration
		Li et al. (2022b)	2022	A2780 and OVCAR8	FBXO6	Tumorigenesis
	USP19	Kang et al. (2021)	2021	Advanced-stage high-grade serous ovarian carcinoma specimen	/	Prognosis
	USP22	Ji et al. (2015)	2015	SKOV3, OVCAR3, epithelial ovarian cancer specimen, and xenograft model	TGFβ1	Proliferation, prognosis and cell cycle
		Gennaro et al. (2018)	2018	/	/	Tumorigenesis, cell cycle
	USP28	Ito et al. (2018)	2018	TU-OC-1, KOC7c, RMG-1, RMG-2, TOV-21G, ES2, and SKOV-3	Claspin	Cell viability
		Shen et al. (2023)	2023	OVCAR3, A2780, and ovarian cancer patients	β-catenin	Proliferation
		Aziz et al. (2018)	2018	High-grade serous ovarian cancer specimens	Cyclin E1	Prognosis
	USP32	Nakae et al. (2021)	2021	SKOV3, OVCAR3, A2780, high-grade serous ovarian cancer specimen, and xenograft model	FDFT1	Progression and prognosis
	USP34	Zhao et al. (2023)	2023	Bioinformatics analysis	/	Prognosis and immune microenvironment
	USP35	Zhang et al. (2021)	2021	Ovarian cancer tissue, VCAR3, SKOV3, VCAR-5, ID8, and xenograft model	STING	Prognosis, immune infiltration, and chemoresistance
	USP36	Li et al. (2008)	2008	A2780, Caov-3, and ovarian cancer tissue	/	/
		Yan et al. (2020)	2020	OVCAR8, SKOV3, OV-90, OVCAR10, IGROV1, OVKATE, OV-56, PEO1, and ovarian cancer specimen	PrimPol	DNA replication and chemoresistance
	USP39	Wang et al. (2021)	2021	Primary ovarian cancer patients, A2780, SKOV3, OVCAR3, OVCAR8, CAOV3, ID8, and xenograft model	HMGA2	Malignancy
		Wang et al. (2019b)	2019	SKOV3, ES2, and xenograft model	/	Malignancy and chemoresistance
		Yan et al. (2019)	2019	HO8910, SKOV3, and xenograft model	p53/p21	Proliferation and epithelial-to-mesenchymal transition
	USP44	Lu et al. (2014)	2014	T80 and SKOV3ip1	/	Cell cycle progression and proliferation
		Tserpeli et al. (2021)	2021	Advanced high-grade serous ovarian cancer	/	/
	USP45	Liu et al. (2023b)	2023	SKOV3, OVCAR3, serous ovarian cancer specimen, and xenograft model	Snail	Tumorigenesis, progression, and chemoresistance
	USP46	Xu et al. (2021)	2021	Ovarian cancer specimen, SKOV3, and SKOV3/DDP	Bcl-2/caspase-3 and ATK	Proliferation, apoptosis, and chemoresistance
	USP47	Hu et al. (2019)	2019	SKOV3, TOV-112D, and ovarian cancer specimen	/	Proliferation
	USP48	Lei et al. (2020)	2020	ES2, 3AO, A2780, ovarian cancer specimen, and xenograft model	/	Chemoresistance and metastasis
	USP51	Zou et al. (2015)	2015	Bioinformatics analysis, SKOV3, SKOV3/DDP, A2780, and A2780/DDP	/	/

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TABLE 5 (*Continued*) Summary of DUB biological function in ovarian cancer.

Family	DUBs	Author	Year	Source	Target	Mechanism
Ubiquitin C-terminal hydrolases (UCHs)	UCHL1	Tangri et al. (2021)	2021	Bioinformatics analysis, high-grade serous ovarian cancer patient specimens, xenograft model, OVCAR4, COV362, OVCAR8, OVCAR3, SKOV3, A2780, and HeyA8	PSMA7-APEH-proteasome	Proliferation, invasion, survival, and tumor growth
		Okochi-Takada et al. (2006)	2006	OV90, MCAS, RMUG-L, RMG-I, RTSG, TYK-nu, TOV112D, ES2, HTOA, KURAMOCHI, JHOS-2, and TOV-21G	/	/
		Jin et al. (2013)	2013	A2780, A2780CP, SKOV3, IGROV1, ES2, OVCAR3, and CAOV3	BCL2, BCL11A, AEN, and XIAP	Proliferation, cell cycle, and chemoresistance
		Gutkin et al. (2019)	2019	High-grade serous ovarian cancer patient specimen	/	Tumorigenesis and immunogenicity
		Alur et al. (2019)	2019	Bioinformatics analysis	/	Progression
	UCHL3	Li and Wang (2019)	2019	SKOV3 and IGROV1	/	Progression
		Zhang et al. (2020)	2020	Bioinformatics analysis, xenograft model, SKOV3, ES2, HO8910, A2780, and COC1	TRAF2	Proliferation, migration, and inflammatory response
	UCHL5	Wang et al. (2014b)	2014	Epithelial ovarian cancer specimen	/	Tumor progression and prognosis
		Fukui et al. (2019)	2019	Tissue microarray, MESOV, SKOV3, OVISE, RMG-1, ES2, and xenograft model	Smad2	Progression-free survival and apoptosis
		Huang et al. (2017)	2017	A2780, SKOV3, and xenograft model	/	Proliferation and tumor growth
	BAP1	Devins et al. (2023)	2023	Ovarian low-grade serous carcinoma specimen	/	/
		Chapel et al. (2017)	2017	Ovarian serous tumor specimen	/	/
		Wang et al. (2022c)	2022	Bioinformatics analysis	/	/
		Chui and Grisham (2023)	2023	Ovarian serous borderline tumor and recurrent low-grade serous carcinoma specimen	/	/
		Davidson et al. (2018)	2018	Ovarian serous tumor specimen	/	/

research since 2020, and the focus on "ubiquitin" and "resistance" as future directions highlights the need for more research into how ubiquitin signaling pathways contribute to cancer progression and treatment outcomes. Understanding these pathways could lead to the development of novel interventions that target specific DUBs or their substrates, potentially overcoming resistance to current therapies and improving patient outcomes.

This bibliometric analysis provides a comprehensive and visual analysis of DUBs in ovarian cancer; however, several limitations should be acknowledged. This study only included articles indexed in the WoSCC, and the language was restricted to English. Therefore, publications in other databases or languages were not included in the analysis. Nevertheless, the WoSCC is a well-recognized database, and given its prominence, the impact of such omissions on the overall findings is expected to be low. Further studies are needed to include additional databases and languages to provide a more accurate and comprehensive analysis. Based on the narrative review and the bibliometric analysis, future studies may need to focus on the potential of DUBs as drug targets for the treatment and management of this disease.

Conclusion and outlook

In summary, a visual analysis of DUBs is presented in this study in the field of ovarian cancer research, facilitated by the use of CiteSpace, VOSviewer, and R4.3.3. The essential functions of DUBs in ovarian cancer biology include DNA repair, cell cycle regulation, apoptosis, oncogenic signaling, chemotherapy response, and chemoresistance. However, the precise functions and mechanisms of DUBs in ovarian cancer remain largely unexplored. Moreover, the expression levels and functions of some DUBs are still under debate; whether these DUBs serve as oncogenic proteins, tumor suppressors, or double-edged swords in ovarian cancer requires further investigation. Understanding the intricate interplay between DUBs and ovarian cancer biology offers promising prospects for developing innovative and more effective treatment strategies, ultimately improving outcomes for patients with this challenging disease. Future efforts are expected to decipher the specific roles of individual DUBs in ovarian cancer, identify potential therapeutic targets, and explore the feasibility of targeting DUBs as a novel approach to treating ovarian cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Author contributions

FQ: writing–original draft, writing–review and editing, funding acquisition, and data curation. YL: data curation, writing–review

and editing, resources, and investigation. LZ: resources, writing–review and editing, and funding acquisition. YiW: supervision and writing–review and editing. YuW: software and writing–review and editing. ZF: writing–review and editing, resources, and software. YWa: methodology and writing–review and editing. DQ: software, supervision, validation, and writing–review and editing. CL: project administration, supervision, and writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1445037/full#supplementary-material

References

Alur, V. C., Raju, V., Vastrad, B., and Vastrad, C. (2019). Mining featured biomarkers linked with epithelial ovarian CancerBased on bioinformatics. *Diagn. Basel, Switz.* 9, 39. doi:10.3390/diagnostics9020039

Arora, T., Mullangi, S., Vadakekut, E. S., and Lekkala, M. R. (2024). in *StatPearls* (StatPearls Publishing).

Aziz, D., Etemadmoghadam, D., Caldon, C. E., Au-Yeung, G., Deng, N., Hutchinson, R., et al. (2018). 19q12 amplified and non-amplified subsets of high grade serous ovarian cancer with overexpression of cyclin E1 differ in their molecular drivers and clinical outcomes. *Gynecol. Oncol.* 151, 327–336. doi:10.1016/j.ygyno.2018.08.039

Chapel, D. B., Husain, A. N., Krausz, T., and McGregor, S. M. (2017). PAX8 expression in a subset of malignant peritoneal mesotheliomas and benign mesothelium has diagnostic implications in the differential diagnosis of ovarian serous carcinoma. *Am. J. Surg. pathology* 41, 1675–1682. doi:10.1097/pas.0000000000000935

Chauhan, D., Tian, Z., Nicholson, B., Kumar, K. G. S., Zhou, B., Carrasco, R., et al. (2012). A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. *Cancer Cell* 22, 345–358. doi:10.1016/j.ccr.2012.08.007

Chui, M. H., and Grisham, R. N. (2023). Somatic BAP1 loss in ovarian serous borderline tumor and recurrent low-grade serous carcinoma from a germline BAP1 mutation carrier. *Int. J. Gynecol. pathology official J. Int. Soc. Gynecol. Pathologists* 42, 432–433. doi:10.1097/pgp.0000000000000932

Corno, C., D'Arcy, P., Bagnoli, M., Paolini, B., Costantino, M., Carenini, N., et al. (2022). The deubiquitinase USP8 regulates ovarian cancer cell response to cisplatin by suppressing apoptosis. *Front. Cell Dev. Biol.* 10, 1055067. doi:10.3389/fcell.2022.1055067

Damgaard, R. B. (2021). The ubiquitin system: from cell signalling to disease biology and new therapeutic opportunities. *Cell Death and Differ.* 28, 423–426. doi:10.1038/s41418-020-00703-w

Davidson, B., Tötsch, M., Wohlschlaeger, J., Hager, T., and Pinamonti, M. (2018). The diagnostic role of BAP1 in serous effusions. *Hum. Pathol.* 79, 122–126. doi:10.1016/j.humpath.2018.05.012

Devins, K. M., Zukerberg, L., Watkins, J. C., Hung, Y. P., and Oliva, E. (2023). BAP1 and claudin-4, but not MTAP, reliably distinguish borderline and low-grade serous ovarian tumors from peritoneal mesothelioma. *Int. J. Gynecol. pathology official J. Int. Soc. Gynecol. Pathologists* 42, 159–166. doi:10.1097/pgp.0000000000000877

Du, Y., Lin, J., Zhang, R., Yang, W., Quan, H., Zang, L., et al. (2019). Ubiquitin specific peptidase 5 promotes ovarian cancer cell proliferation through deubiquitinating HDAC2. *Aging* 11, 9778–9793. doi:10.18632/aging.102425

Duan, Y., Zhang, P., Zhang, T., Zhou, L., and Yin, R. (2023). Characterization of global research trends and prospects on platinum-resistant ovarian cancer: a bibliometric analysis. *Front. Oncol.* 13, 1151871. doi:10.3389/fonc.2023.1151871

Eichhorn, P. J., Rodón, L., Gonzàlez-Juncà, A., Dirac, A., Gili, M., Martínez-Sáez, E., et al. (2012). USP15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. *Nat. Med.* 18, 429–435. doi:10.1038/nm.2619

Fortner, R. T., Poole, E. M., Wentzensen, N. A., Trabert, B., White, E., Arslan, A. A., et al. (2019). Ovarian cancer risk factors by tumor aggressiveness: an analysis from the Ovarian Cancer Cohort Consortium. *Int. J. cancer* 145, 58–69. doi:10.1002/ijc.32075

Fraile, J. M., Manchado, E., Lujambio, A., Quesada, V., Campos-Iglesias, D., Webb, T. R., et al. (2017). USP39 deubiquitinase is essential for KRAS oncogene-driven cancer. *J. Biol. Chem.* 292, 4164–4175. doi:10.1074/jbc.M116.762757

Fukui, S., Nagasaka, K., Miyagawa, Y., Kikuchi-Koike, R., Kawata, Y., Kanda, R., et al. (2019). The proteasome deubiquitinase inhibitor bAP15 downregulates TGF-β/Smad signaling and induces apoptosis via UCHL5 inhibition in ovarian cancer. *Oncotarget* 10, 5932–5948. doi:10.18632/oncotarget.27219

Gao, D., Zhang, Z., Xu, R., He, Z., Li, F., Hu, Y., et al. (2022). The prognostic value and immune infiltration of USP10 in pan-cancer: a potential therapeutic target. *Front. Oncol.* 12, 829705. doi:10.3389/fonc.2022.829705

Gennaro, V. J., Stanek, T. J., Peck, A. R., Sun, Y., Wang, F., Qie, S., et al. (2018). Control of CCND1 ubiquitylation by the catalytic SAGA subunit USP22 is essential for cell cycle progression through G1 in cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* 115, E9298–e9307. doi:10.1073/pnas.1807704115

Giles, E. D., Purcell, S. A., Olson, J., Vrieling, A., Hirko, K. A., Woodruff, K., et al. (2023). Trends in diet and cancer research: a bibliometric and visualization analysis. *Cancers* 15, 3761. doi:10.3390/cancers15153761

Guo, T., Tang, H., Yuan, Z., Zhang, E., and Wang, X. (2022). The dual role of USP11 in cancer. *J. Oncol.* 2022, 9963905. doi:10.1155/2022/9963905

Gutkin, D. W., Shurin, M. R., El Azher, M. A., Shurin, G. V., Velikokhatnaya, L., Prosser, D., et al. (2019). Novel protein and immune response markers of human serous tubal intraepithelial carcinoma of the ovary. *Cancer biomarkers Sect. A Dis. markers* 26, 471–479. doi:10.3233/cbm-190528

Habata, S., Iwasaki, M., Sugio, A., Suzuki, M., Tamate, M., Satohisa, S., et al. (2016). BAG3-mediated Mcl-1 stabilization contributes to drug resistance via interaction with USP9X in ovarian cancer. *Int. J. Oncol.* 49, 402–410. doi:10.3892/ijo.2016.3494

Han, C., Yang, L., Choi, H. H., Baddour, J., Achreja, A., Liu, Y., et al. (2016). Amplification of USP13 drives ovarian cancer metabolism. *Nat. Commun.* 7, 13525. doi:10.1038/ncomms13525

Han, G. H., Chay, D. B., Yi, J. M., Cho, H., Chung, J. Y., and Kim, J. H. (2019). Loss of both USP10 and p14ARF protein expression is an independent prognostic biomarker for poor prognosis in patients with epithelial ovarian cancer. *Cancer genomics and proteomics* 16, 553–562. doi:10.21873/cgp.20157

Harrigan, J. A., Jacq, X., Martin, N. M., and Jackson, S. P. (2018). Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat. Rev. Drug Discov.* 17, 57–78. doi:10.1038/nrd.2017.152

Hu, L., Kolibaba, H., Zhang, S., Cao, M., Niu, H., Mei, H., et al. (2019). MicroRNA-204-5p inhibits ovarian cancer cell proliferation by down-regulating USP47. *Cell Transplant.* 28, 51S–58s. doi:10.1177/0963689719877372

Huang, H., Liu, N., Liao, Y., Liu, N., Cai, J., Xia, X., et al. (2017). Platinum-containing compound platinum pyrithione suppresses ovarian tumor proliferation through proteasome inhibition. *J. Exp. and Clin. cancer Res. CR* 36, 79. doi:10.1186/s13046-017-0547-8

Hunter, S. M., Anglesio, M. S., Ryland, G. L., Sharma, R., Chiew, Y. E., Rowley, S. M., et al. (2015). Molecular profiling of low grade serous ovarian tumours identifies novel candidate driver genes. *Oncotarget* 6, 37663–37677. doi:10.18632/oncotarget.5438

Ito, F., Yoshimoto, C., Yamada, Y., Sudo, T., and Kobayashi, H. (2018). The HNF-1β-USP28-Claspin pathway upregulates DNA damage-induced Chk1 activation in ovarian clear cell carcinoma. *Oncotarget* 9, 17512–17522. doi:10.18632/oncotarget.24776

Jensen, D. E., Proctor, M., Marquis, S. T., Gardner, H. P., Ha, S. I., Chodosh, L. A., et al. (1998). BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene* 16, 1097–1112. doi:10.1038/sj.onc.1201861

Ji, J., Lv, J., Lv, M., Jing, A., Xu, M., Yuan, Q., et al. (2023). USP14 regulates heme metabolism and ovarian cancer invasion through BACH1 deubiquitination and stabilization. *Biochem. biophysical Res. Commun.* 667, 186–193. doi:10.1016/j.bbrc.2023.04.082

Ji, M., Shi, H., Xie, Y., Zhao, Z., Li, S., Chang, C., et al. (2015). Ubiquitin specific protease 22 promotes cell proliferation and tumor growth of epithelial ovarian cancer through synergy with transforming growth factor β1. *Oncol. Rep.* 33, 133–140. doi:10.3892/or.2014.3580

Jin, C., Yu, W., Lou, X., Zhou, F., Han, X., Zhao, N., et al. (2013). UCHL1 is a putative tumor suppressor in ovarian cancer cells and contributes to cisplatin resistance. *J. Cancer* 4, 662–670. doi:10.7150/jca.6641

Jing, B., Liu, M., Yang, L., Cai, H. Y., Chen, J. B., Li, Z. X., et al. (2018). Characterization of naturally occurring pentacyclic triterpenes as novel inhibitors of deubiquitinating protease USP7 with anticancer activity *in vitro*. *Acta Pharmacol. Sin.* 39, 492–498. doi:10.1038/aps.2017.119

Johnson, J., Bales, E. C., Bitler, B. G., and Watson, Z. L. (2020). Eliciting OTUD3/RIPK-dependent necroptosis to prevent epithelial ovarian cancer. *bioRxiv* 2004.2029, 069021. doi:10.1101/2020.04.29.069021

Kang, H., Choi, M. C., Kim, S., Jeong, J. Y., Kwon, A. Y., Kim, T. H., et al. (2021). USP19 and RPL23 as candidate prognostic markers for advanced-stage high-grade serous ovarian carcinoma. *Cancers* 13, 3976. doi:10.3390/cancers13163976

Kisaï, K., and Koji, S. (2021). Prognostic role of USP7 expression in cancer patients: a systematic review and meta-analysis. *Pathology, Res. Pract.* 227, 153621. doi:10.1016/j.prp.2021.153621

Kwon, J., Choi, H., Ware, A. D., Morillo, B. C., Wang, H., Bouker, K. B., et al. (2022a). USP13 promotes development and metastasis of high-grade serous ovarian carcinoma in a novel mouse model. *Oncogene* 41, 1974–1985. doi:10.1038/s41388-022-02224-x

Kwon, J., Zhang, J., Mok, B., and Han, C. (2022b). CK2-Mediated phosphorylation upregulates the stability of USP13 and promotes ovarian cancer cell proliferation. *Cancers* 15, 200. doi:10.3390/cancers15010200

Lee, J. M., Hammarén, H. M., Savitski, M. M., and Baek, S. H. (2023). Control of protein stability by post-translational modifications. *Nat. Commun.* 14, 201. doi:10.1038/s41467-023-35795-8

Lei, H., Wang, J., Hu, J., Zhu, Q., and Wu, Y. (2021b). Deubiquitinases in hematological malignancies. *Biomark. Res.* 9, 66. doi:10.1186/s40364-021-00320-w

Lei, H., Xu, H. Z., Shan, H. Z., Liu, M., Lu, Y., Fang, Z. X., et al. (2021a). Targeting USP47 overcomes tyrosine kinase inhibitor resistance and eradicates leukemia stem/progenitor cells in chronic myelogenous leukemia. *Nat. Commun.* 12, 51. doi:10.1038/s41467-020-20259-0

Lei, X., Li, X., Chen, H., and Liu, Z. (2020). USP48 sustains chemoresistance and metastasis in ovarian cancer. *Curr. cancer drug targets* 20, 689–699. doi:10.2174/1568009620666200503045400

Leng, Y., Li, S., Zhu, J., Wang, X., Luo, F., Wang, Y., et al. (2023). Application of medical imaging in ovarian cancer: a bibliometric analysis from 2000 to 2022. *Front. Oncol.* 13, 1326297. doi:10.3389/fonc.2023.1326297

Li, G., Shi, W., Xu, Y., Li, K., Chen, Z., Lv, M., et al. (2022b). The USP18-FBXO6 axis maintains the malignancy of ovarian cancer. *Biochem. biophysical Res. Commun.* 593, 101–107. doi:10.1016/j.bbrc.2022.01.020

Li, J., Olson, L. M., Zhang, Z., Li, L., Bidder, M., Nguyen, L., et al. (2008). Differential display identifies overexpression of the USP36 gene, encoding a deubiquitinating enzyme, in ovarian cancer. *Int. J. Med. Sci.* 5, 133–142. doi:10.7150/ijms.5.133

Li, M., Tang, Y., Zuo, X., Meng, S., and Yi, P. (2022a). Loss of Ras GTPase-activating protein SH3 domain-binding protein 1 (G3BP1) inhibits the progression of ovarian cancer in coordination with ubiquitin-specific protease 10 (USP10). *Bioengineered* 13, 721–734. doi:10.1080/21655979.2021.2012624

Li, T. T., and Wang, H. J. (2019). UCH-L3 expression in epithelial ovarian cancer and its clinical significance. *Sichuan Da Xue Xue Bao Yi Xue Ban.* 50, 556–560.

Li, Y., Luo, K., Yin, Y., Wu, C., Deng, M., Li, L., et al. (2017). USP13 regulates the RAP80-BRCA1 complex dependent DNA damage response. *Nat. Commun.* 8, 15752. doi:10.1038/ncomms15752

Li, Y., Zhang, R., and Hei, H. (2023). Advances in post-translational modifications of proteins and cancer immunotherapy. *Front. Immunol.* 14, 1229397. doi:10.3389/fimmu.2023.1229397

Lin, F. T., Lin, V. Y., Lin, V. T., and Lin, W. C. (2016). TRIP6 antagonizes the recruitment of A20 and CYLD to TRAF6 to promote the LPA2 receptor-mediated TRAF6 activation. *Cell Discov.* 2, 15048. doi:10.1038/celldisc.2015.48

Lin, X., Zheng, W., Zhao, X., Zeng, M., Li, S., Peng, S., et al. (2024). Microbiome in gynecologic malignancies: a bibliometric analysis from 2012 to 2022. *Transl. cancer Res.* 13, 1980–1996. doi:10.21037/tcr-23-1769

Liu, J., Ma, J., Zhang, J., Li, C., Yu, B., Choe, H. C., et al. (2023a). Corrigendum: bibliometric and visualized analysis of drug resistance in ovarian cancer from 2013 to 2022. *Front. Oncol.* 13, 1228879. doi:10.3389/fonc.2023.1228879

Liu, L., Chen, C., Liu, P., Li, J., Pang, Z., Zhu, J., et al. (2023b). MYH10 combines with MYH9 to recruit USP45 by deubiquitinating snail and promotes serous ovarian cancer carcinogenesis, progression, and cisplatin resistance. *Adv. Sci. Weinheim, Baden-Wurttemberg, Ger.* 10, e2203423. doi:10.1002/advs.202203423

Liu, M., Gao, S., Xu, X., Zhang, L., Xu, B., and Lu, D. (2022). USP18 contributes to the proliferation and migration of ovarian cancer cells by regulating the AKT/mTOR signaling pathway. *Acta biochim. Pol.* 69, 417–422. doi:10.18388/abp.2020_5871

Lu, H., Wang, X., Urvalek, A. M., Xie, H., Yu, L., et al. (2014). Transformation of human ovarian surface epithelial cells by Krüppel-like factor 8. *Oncogene* 33, 10–18. doi:10.1038/onc.2012.545

Luo, H., Wang, X., Ge, H., Zheng, N., Peng, F., Fu, Y., et al. (2019). Inhibition of ubiquitin-specific protease 14 promotes connexin 32 internalization and counteracts cisplatin cytotoxicity in human ovarian cancer cells. *Oncol. Rep.* 42, 1237–1247. doi:10.3892/or.2019.7232

Ma, M., and Yu, N. (2016). Ubiquitin-specific protease 7 expression is a prognostic factor in epithelial ovarian cancer and correlates with lymph node metastasis. *OncoTargets Ther.* 9, 1559–1569. doi:10.2147/ott.s100050

Maresca, L., Spugnesi, L., Lodovichi, S., Cozzani, C., Naccarato, A. G., Tancredi, M., et al. (2015). MSH2 role in BRCA1-driven tumorigenesis: a preliminary study in yeast and in human tumors from BRCA1-VUS carriers. *Eur. J. Med. Genet.* 58, 531–539. doi:10.1016/j.ejmg.2015.09.005

Matan, L. S., Perri, T., Kogan, L., Brandt, B., Meyer, R., and Levin, G. (2022). Ovarian cancer risk management in BRCA-mutation carriers: a comparison of six international and national guidelines. *Eur. J. obstetrics, Gynecol. reproductive Biol.* 278, 166–171. doi:10.1016/j.ejogrb.2022.09.035

Meng, S., Song, S., and Yin, R. (2024). Bibliometric and visual analysis of immune checkpoint inhibitors for ovarian cancer. *Asian J. Surg.* 47, 3205–3206. doi:10.1016/j.asjsur.2024.03.056

Mevissen, T. E., Hospenthal, M. K., Geurink, P. P., Elliott, P. R., Akutsu, M., Arnaudo, N., et al. (2013). OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell* 154, 169–184. doi:10.1016/j.cell.2013.05.046

Nakae, A., Kodama, M., Okamoto, T., Tokunaga, M., Shimura, H., Hashimoto, K., et al. (2021). Ubiquitin specific peptidase 32 acts as an oncogene in epithelial ovarian cancer by deubiquitylating farnesyl-diphosphate farnesyltransferase 1. *Biochem. biophysical Res. Commun.* 552, 120–127. doi:10.1016/j.bbrc.2021.03.049

Okochi-Takada, E., Nakazawa, K., Wakabayashi, M., Mori, A., Ichimura, S., Yasugi, T., et al. (2006). Silencing of the UCHL1 gene in human colorectal and ovarian cancers. *Int. J. cancer* 119, 1338–1344. doi:10.1002/ijc.22025

Padmanabhan, A., Candelaria, N., Wong, K. K., Nikolai, B. C., Lonard, D. M., O'Malley, B. W., et al. (2018). USP15-dependent lysosomal pathway controls p53-R175H turnover in ovarian cancer cells. *Nat. Commun.* 9, 1270. doi:10.1038/s41467-018-03599-w

Qin, D., Wang, W., Lei, H., Luo, H., Cai, H., Tang, C., et al. (2016). CDDO-Me reveals USP7 as a novel target in ovarian cancer cells. *Oncotarget* 7, 77096–77109. doi:10.18632/oncotarget.12801

Sandvei, M. S., Pinborg, A., Gissler, M., Bergh, C., Romundstad, L. B., van Leeuwen, F. E., et al. (2023). Risk of ovarian cancer in women who give birth after assisted reproductive technology (ART)-a registry-based Nordic cohort study with follow-up from first pregnancy. *Br. J. cancer* 128, 825–832. doi:10.1038/s41416-022-02097-7

Shen, J., Hong, L., and Chen, L. (2020). Ubiquitin-specific protease 14 regulates ovarian cancer cisplatin-resistance by stabilizing BCL6 oncoprotein. *Biochem. biophysical Res. Commun.* 524, 683–688. doi:10.1016/j.bbrc.2020.01.150

Shen, J., Xie, M., Xu, Y., Qian, Q., Qiu, T., Shi, W., et al. (2023). Identification of the deubiquitinase USP28 as a novel molecular therapeutic target of ovarian cancer. *Biochem. biophysical Res. Commun.* 638, 184–191. doi:10.1016/j.bbrc.2022.11.055

Siegel, R. L., Miller, K. D., Wagle, N. S., and Jemal, A. (2023). Cancer statistics. *CA a cancer J. Clin.* 73, 17–48. doi:10.3322/caac.21763

Simoneau, A., Engel, J. L., Bandi, M., Lazarides, K., Liu, S., Meier, S. R., et al. (2023). Ubiquitinated PCNA drives USP1 synthetic lethality in cancer. *Mol. cancer Ther.* 22, 215–226. doi:10.1158/1535-7163.mct-22-0409

Singh, H. R., and Ostwal, Y. B. (2019). Post-translational modification, phase separation, and robust gene transcription. *Trends Genet. TIG* 35, 89–92. doi:10.1016/j.tig.2018.11.002

Snyder, N. A., and Silva, G. M. (2021). Deubiquitinating enzymes (DUBs): regulation, homeostasis, and oxidative stress response. *J. Biol. Chem.* 297, 101077. doi:10.1016/j.jbc.2021.101077

Sonego, M., Pellarin, I., Costa, A., Vinciguerra, G. L. R., Coan, M., Kraut, A., et al. (2019). USP1 links platinum resistance to cancer cell dissemination by regulating Snail stability. *Sci. Adv.* 5, eaav3235. doi:10.1126/sciadv.aav3235

Song, B., Jiang, Y., Jiang, Y., Lin, Y., and Liu, J. (2022). ML323 suppresses the progression of ovarian cancer via regulating USP1-mediated cell cycle. *Front. Genet.* 13, 917481. doi:10.3389/fgene.2022.917481

Song, S., Meng, S., and Yin, R. (2024). Application of anti-angiogenic drugs in ovarian cancer from the perspective of bibliometrics. *Asian J. Surg.* 47, 2228–2229. doi:10.1016/j.asjsur.2024.01.110

Stiff, P. J., McKenzie, R. S., Alberts, D. S., Sosman, J. A., Dolan, J. R., Rad, N., et al. (1994). Phase I clinical and pharmacokinetic study of high-dose mitoxantrone combined with carboplatin, cyclophosphamide, and autologous bone marrow rescue: high response rate for refractory ovarian carcinoma. *J. Clin. Oncol. official J. Am. Soc. Clin. Oncol.* 12, 176–183. doi:10.1200/jco.1994.12.1.176

Sung, S., Hong, Y., Kim, B. G., Choi, J. Y., Kim, J. W., Park, S. Y., et al. (2023). Stratifying the risk of ovarian cancer incidence by histologic subtypes in the Korean Epithelial Ovarian Cancer Study (Ko-EVE). *Cancer Med.* 12, 8742–8753. doi:10.1002/cam4.5612

Tangri, A., Lighty, K., Loganathan, J., Mesmar, F., Podicheti, R., Zhang, C., et al. (2021). Deubiquitinase UCHL1 maintains protein homeostasis through the PSMA7-APEH-proteasome Axis in high-grade serous ovarian carcinoma. *Mol. cancer Res. MCR* 19, 1168–1181. doi:10.1158/1541-7786.mcr-20-0883

Tavares, V., Pinto, R., Assis, J., Coelho, S., Brandão, M., Alves, S., et al. (2021). Implications of venous thromboembolism GWAS reported genetic makeup in the clinical outcome of ovarian cancer patients. *pharmacogenomics J.* 21, 222–232. doi:10.1038/s41397-020-00201-9

Therachiyil, L., Anand, A., Azmi, A., Bhat, A., Korashy, H. M., and Uddin, S. (2022). Role of RAS signaling in ovarian cancer. *F1000Research* 11, 1253. doi:10.12688/f1000research.126337.1

Tserpeli, V., Stergiopoulou, D., Londra, D., Giannopoulou, L., Buderath, P., Balgkouranidou, I., et al. (2021). Prognostic significance of SLFN11 methylation in plasma cell-free DNA in advanced high-grade serous ovarian cancer. *Cancers* 14, 4. doi:10.3390/cancers14010004

van Eck, N. J., and Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 84, 523–538. doi:10.1007/s11192-009-0146-3

Wada, T., Yamashita, Y., Saga, Y., Takahashi, K., Koinuma, K., Choi, Y. L., et al. (2009). Screening for genetic abnormalities involved in ovarian carcinogenesis using retroviral expression libraries. *Int. J. Oncol.* 35, 973–976. doi:10.3892/ijo_00000410

Wang, H., Song, Y., Liu, C., Qian, X., Zhang, D., et al. (2022b). Overexpression of Foxc1 ameliorates sepsis-associated encephalopathy by inhibiting microglial migration and neuroinflammation through the IκBα/NF-κB pathway. *Mol. Med. Rep.* 25, 107. doi:10.3892/mmr.2022.12623

Wang, L. (2021). A novel glycosyltransferase-related gene signature for overall survival prediction in patients with ovarian cancer. *Int. J. general Med.* 14, 10337–10350. doi:10.2147/ijgm.s332945

Wang, L., Chen, T., Li, X., Yan, W., Lou, Y., Liu, Z., et al. (2019b). USP39 promotes ovarian cancer malignant phenotypes and carboplatin chemoresistance. *Int. J. Oncol.* 55, 277–288. doi:10.3892/ijo.2019.4818

Wang, L., Chen, Y. J., Xu, K., Wang, Y. Y., and Shen, X. Z. (2014b). High expression of UCH37 is significantly associated with poor prognosis in human epithelial ovarian cancer. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 35, 11427–11433. doi:10.1007/s13277-014-2446-3

Wang, M., Zhang, Y., Wang, T., Zhang, J., Zhou, Z., Sun, Y., et al. (2017). The USP7 inhibitor P5091 induces cell death in ovarian cancers with different P53 status. *Cell. physiology Biochem. Int. J. Exp. Cell. physiology, Biochem. Pharmacol.* 43, 1755–1766. doi:10.1159/000484062

Wang, S., Osgood, A. O., and Chatterjee, A. (2022a). Uncovering post-translational modification-associated protein-protein interactions. *Curr. Opin. Struct. Biol.* 74, 102352. doi:10.1016/j.sbi.2022.102352

Wang, S., Wang, Z., Li, J., Qin, J., Song, J., Li, Y., et al. (2021). Splicing factor USP39 promotes ovarian cancer malignancy through maintaining efficient splicing of oncogenic HMGA2. *Cell death and Dis.* 12, 294. doi:10.1038/s41419-021-03581-3

Wang, T., Zhu, T., Zhang, Y., Bai, J., Xue, Y., Xu, G., et al. (2022c). Pan-cancer analysis of the prognostic and immunological role of BRCA1-associated protein 1 gene (BAP1): friend or foe? *Gene* 840, 146765. doi:10.1016/j.gene.2022.146765

Wang, W., Wang, J., Yan, H., Zhang, K., and Liu, Y. (2019a). Upregulation of USP11 promotes epithelial-to-mesenchymal transition by deubiquitinating Snail in ovarian cancer. *Oncol. Rep.* 41, 1739–1748. doi:10.3892/or.2018.6924

Wang, W., Wei, J., Feng, D., and Ling, B. (2024). Current trends and emerging patterns in the application of nanomaterials for ovarian cancer research: a bibliometric analysis. *Front. Pharmacol.* 15, 1344855. doi:10.3389/fphar.2024.1344855

Wang, Y., Luo, X., Wu, N., Liao, Q., and Wang, J. (2023b). USP7 mediates TRAF4 deubiquitination to facilitate the malignant phenotype of ovarian cancer via the RSK4/PI3K/AKT axis. *J. cancer Res. Ther.* 19, 97–107. doi:10.4103/jcrt.jcrt_517_22

Wang, Y., Wang, J., Zhong, J., Deng, Y., Xi, Q., He, S., et al. (2015). Ubiquitin-specific protease 14 (USP14) regulates cellular proliferation and apoptosis in epithelial ovarian cancer. *Med. Oncol. N. Lond. Engl.* 32, 379. doi:10.1007/s12032-014-0379-8

Wang, Y., Wang, Z., Zhang, Z., Wang, H., Peng, J., and Hong, L. (2023a). Burden of ovarian cancer in China from 1990 to 2030: a systematic analysis and comparison with the global level. *Front. public health* 11, 1136596. doi:10.3389/fpubh.2023.1136596

Wang, Y., Zhou, X., Xu, M., Weng, W., Zhang, Q., Yang, Y., et al. (2016). OTUB1-catalyzed deubiquitination of FOXM1 facilitates tumor progression and predicts a poor prognosis in ovarian cancer. *Oncotarget* 7, 36681–36697. doi:10.18632/oncotarget.9160

Wang, Y.-C., Peterson, S. E., and Loring, J. F. (2014a). Protein post-translational modifications and regulation of pluripotency in human stem cells. *Cell Res.* 24, 143–160. doi:10.1038/cr.2013.151

Wertz, I. E., O'Rourke, K. M., Zhou, H., Eby, M., Aravind, L., Seshagiri, S., et al. (2004). De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* 430, 694–699. doi:10.1038/nature02794

Wu, Q., Huang, Y., Gu, L., Chang, Z., and Li, G. M. (2021). OTUB1 stabilizes mismatch repair protein MSH2 by blocking ubiquitination. *J. Biol. Chem.* 296, 100466. doi:10.1016/j.jbc.2021.100466

Xu, L., Zhang, B., and Li, W. (2021). Downregulated expression levels of USP46 promote the resistance of ovarian cancer to cisplatin and are regulated by PUM2. *Mol. Med. Rep.* 23, 263. doi:10.3892/mmr.2021.11902

Xu, M., Takanashi, M., Oikawa, K., Tanaka, M., Nishi, H., Isaka, K., et al. (2009). USP15 plays an essential role for caspase-3 activation during Paclitaxel-induced apoptosis. *Biochem. biophysical Res. Commun.* 388, 366–371. doi:10.1016/j.bbrc.2009.08.015

Yan, C., Yuan, J., Xu, J., Zhang, G., Li, X., Zhang, B., et al. (2019). Ubiquitin-specific peptidase 39 regulates the process of proliferation and migration of human ovarian cancer via p53/p21 pathway and EMT. *Med. Oncol. N. Lond. Engl.* 36, 95. doi:10.1007/s12032-019-1308-7

Yan, Y., Xu, Z., Huang, J., Guo, G., Gao, M., Kim, W., et al. (2020). The deubiquitinase USP36 Regulates DNA replication stress and confers therapeutic resistance through PrimPol stabilization. *Nucleic acids Res.* 48, 12711–12726. doi:10.1093/nar/gkaa1090

Yang, Y., Hou, J. q., Qu, L. y., Wang, G. q., Ju, H. w., Zhao, Z. w., et al. (2007). Differential expression of USP2, USP14 and UBE4A between ovarian serous cystadenocarcinoma and adjacent normal tissues. *Xi bao yu fen zi mian yi xue za zhi = Chin. J. Cell. Mol. Immunol.* 23, 504–506.

Yildirim, N., Kocal, G. C., Isik, Z., Saatli, B., Saygili, U., Uysal, T., et al. (2019). Ubiquitin-Proteasome Axis, especially ubiquitin-specific protease-17 (USP17) gene family, is a potential target for epithelial-mesenchymal transition in high-grade serous ovarian cancer. *Reprod. Sci. (Thousand Oaks, Calif.)* 26, 794–805. doi:10.1177/1933719118799189

Zhang, J., Chen, Y., Chen, X., Zhang, W., Zhao, L., Weng, L., et al. (2021). Deubiquitinase USP35 restrains STING-mediated interferon signaling in ovarian cancer. *Cell death Differ.* 28, 139–155. doi:10.1038/s41418-020-0588-y

Zhang, L., Wang, H., Tian, L., and Li, H. (2016). Expression of USP7 and MARCH7 is correlated with poor prognosis in epithelial ovarian cancer. *Tohoku J. Exp. Med.* 239, 165–175. doi:10.1620/tjem.239.165

Zhang, M. H., Zhang, H. H., Du, X. H., Gao, J., Li, C., Shi, H. R., et al. (2020). UCHL3 promotes ovarian cancer progression by stabilizing TRAF2 to activate the NF-κB pathway. *Oncogene* 39, 322–333. doi:10.1038/s41388-019-0987-z

Zhang, S., Zhang, M., Jing, Y., Yin, X., Ma, P., Zhang, Z., et al. (2018). Deubiquitinase USP13 dictates MCL1 stability and sensitivity to BH3 mimetic inhibitors. *Nat. Commun.* 9, 215. doi:10.1038/s41467-017-02693-9

Zhao, L., Tang, Y., Yang, J., Lin, F., Liu, X., Zhang, Y., et al. (2023). Integrative analysis of circadian clock with prognostic and immunological biomarker identification in ovarian cancer. *Front. Mol. Biosci.* 10, 1208132. doi:10.3389/fmolb.2023.1208132

Zhu, X., Zhang, Y., Luo, Q., Wu, X., Huang, F., Shu, T., et al. (2021). The deubiquitinase USP11 promotes ovarian cancer chemoresistance by stabilizing BIP. *Signal Transduct. Target. Ther.* 6, 264. doi:10.1038/s41392-021-00580-w

Zou, J., Yin, F., Wang, Q., Zhang, W., and Li, L. (2015). Analysis of microarray-identified genes and microRNAs associated with drug resistance in ovarian cancer. *Int. J. Clin. Exp. pathology* 8, 6847–6858.

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Efficacy and safety of PARP inhibitor maintenance therapy for ovarian cancer: a meta-analysis and trial sequential analysis of randomized controlled trials

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Background: The landscape of poly (ADP-ribose) polymerase (PARP) inhibitor treatment for ovarian cancer (OC) is continually evolving. This research aimed to evaluate the efficacy and safety of PARP inhibitors compared to placebo as a maintenance therapy for OC patients.

Methods: We conducted a search of PubMed, Embase, Web of Science, and the Cochrane Library databases for randomized controlled trials (RCTs) involving the use of PARP inhibitors as maintenance therapy in OC patients, up to 16 June 2024. Data regarding progression-free survival (PFS), overall survival (OS), chemotherapy-free interval (CFI), time to first subsequent therapy or death (TFST), time to second subsequent therapy or death (TSST), and treatment-emergent adverse events (TEAEs) were aggregated. Pooled hazard ratio (HR) and their corresponding 95% confidence intervals (CI) were calculated for PFS, OS, CFI, TFST, and TSST. Additionally, the relative risk (RR) and 95% CI for TEAEs were determined.

Results: This meta-analysis encompassed 20 RCTs involving 7,832 participants. The overall analysis demonstrated that maintenance therapy with PARP inhibitors led to significant improvements in PFS (HR: 0.398, 95% CI = 0.339–0.467, 95% PI = 0.219–0.724), OS (HR: 0.677, 95% CI = 0.582–0.788, 95% PI = 0.546–0.839), CFI (HR: 0.417, 95% CI = 0.368–0.472, 95% PI = 0.265–0.627), TFST (HR: 0.441, 95% CI = 0.391–0.498, 95% PI = 0.308–0.632), and TSST (HR: 0.574, 95% CI = 0.507–0.649, 95% PI = 0.488–0.674) compared with placebo. Subgroup analyses further indicated that PARP inhibitor maintenance treatment significantly improved PFS, regardless of homologous recombination status (all $p < 0.05$). However, the risks of any grade (RR = 1.046, 95% CI = 1.032–1.059, 95% PI = 1.028–1.055) and grade ≥3 TEAEs (RR = 2.931, 95% CI = 2.641–3.253, 95% PI = 2.128–3.792) were increased by PARP inhibitor maintenance therapy compared to placebo.

Conclusion: Our research elucidated the benefits of maintenance therapy with PARP inhibitors in patients with OC, showing improvements in PFS, OS, CFI, TFST, and TSST. Vigilance regarding TEAEs is paramount for clinicians implementing PARP inhibitor maintenance therapy in clinical practice.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42024560286.

KEYWORDS

PARP inhibitors, olaparib, niraparib, rucaparib, placebo, ovarian cancer, meta-analysis

1 Introduction

Ovarian cancer (OC) stands as the primary cause of mortality among gynecological malignancies (Torre et al., 2018). At the time of diagnosis, roughly 75% of OC patients exhibit advanced stages of the disease (Lheureux et al., 2019; Salani et al., 2011). While early-stage OC can be effectively managed with initial platinum-based chemotherapy (CT) and standard cytoreductive surgery, the majority of patients with advanced OC (70%–80%) eventually develop resistance to platinum, leading to poor survival outcomes (Ledermann et al., 2013). Attempts to improve treatment efficacy, including intraperitoneal CT, weekly paclitaxel administration, the incorporation of bevacizumab, and BRAF (v-raf murine sarcoma viral oncogene homolog B1)/MEK (mitogen-activated protein kinase) inhibitors, have had limited success (Burger et al., 2011; Katsumata et al., 2013; Marchetti et al., 2019; Perren et al., 2011; Perrone et al., 2024). Pathogenic or likely pathogenic germline mutations in BRCA1 or BRCA2 genes are present in approximately 10%–20% of OC patients (Cancer Genome Atlas Research Network, 2011), while around 50% exhibit somatic defects in the homologous recombination repair pathway, referred to as homologous recombination deficiency (HRD) (Gupta et al., 2021; Cancer Genome Atlas Research Network, 2011). Mutations in BRCA1/2 heighten the likelihood of OC development in women. Furthermore, OC in women with germline mutations tends to be more aggressive and have a worse prognosis than those with somatic mutations, as BRCA-mutated tumors typically present with higher clinical grades and stages, and a greater potential for metastasis (Musolino et al., 2007). Research in cancer biology has underscored the significance of BRCA1/2 mutations and HRD, paving the way for targeted treatments such as poly (ADP-ribose) polymerase (PARP) inhibitors.

The suppression of PARP results in the persistence of single-strand DNA breaks, which subsequently lead to double-strand breaks necessitating repair via homologous recombination repair (HRR) (Creeden et al., 2021). In the context of pathogenic BRCA1/2 mutations or other HRD, cancer cells exhibit heightened sensitivity to PARP inhibitors due to synthetic lethality. This concurrent deficiency in both repair pathways culminates in cell death (Farmer et al., 2005). Consequently, this therapeutic approach has led to the development of a class of drugs known as PARP inhibitors. The introduction of these inhibitors has broadened the therapeutic options for OC, particularly for patients with BRCA mutations or HRD patients who are characterized by platinum sensitivity and non-BRCA mutation (Purwar et al., 2023). Presently, three PARP inhibitors have received FDA approval for OC treatment: olaparib and niraparib as monotherapies are sanctioned for maintenance therapy following primary and recurrent CT, while rucaparib is approved for maintenance in recurrent OC (Armstrong et al., 2022). Evidence suggested that olaparib, niraparib, and rucaparib are efficacious in the treatment of OC, particularly in extending progression-free survival (PFS) in patients with recurrent OC when compared to placebo (Cancanelli et al., 2022; Mengato et al., 2022; Wang et al., 2021). Additionally, evidence from previous randomized controlled trials (RCTs) indicated that PARP inhibitors markedly enhance PFS when employed as maintenance therapy in recurrent OC patients, irrespective of biomarker status such as BRCA mutation or HRD (Coleman et al., 2017; Ledermann et al., 2012; Mirza et al., 2016; Pujade-Lauraine et al., 2017). More recent RCTs have demonstrated significant improvements in PFS with PARP inhibitor maintenance therapy in newly diagnosed OC patients, regardless of the presence or absence of BRCA mutations or HRD (Banerjee et al., 2021; Coleman et al., 2019; González-Martín et al., 2019; Ray-Coquard et al., 2019).

Moreover, in a recent meta-analysis, Wang et al. demonstrated an improved prognosis for patients with newly diagnosed advanced OC undergoing PARP inhibitor maintenance therapy (Wang et al., 2020). Previous network meta-analyses have established the efficacy of olaparib, niraparib, and rucaparib in prolonging PFS in recurrent OC cases (Wang et al., 2021; Xu et al., 2020). Nonetheless, in recent years, multiple RCTs have provided updated data on PFS, overall survival (OS), chemotherapy-free interval (CFI), time to first subsequent therapy or death (TFST), and time to second subsequent therapy or death (TSST) following PARP inhibitor maintenance therapy for OC (DiSilvestro et al., 2023; González-Martín et al., 2023; Li et al., 2023; Pujade-Lauraine et al., 2023; Wu et al., 2024a; Wu et al., 2024b). Additionally, there remains debate over whether different PARP inhibitor maintenance treatments elevate the risk of any grade treatment-emergent adverse events (TEAEs) compared to placebo (Coleman et al., 2017; Friedlander et al., 2018; Monk et al., 2022). Therefore, we conducted a meta-analysis to evaluate the efficacy and safety of PARP inhibitor maintenance therapy versus placebo in the treatment of OC and its various subtypes.

2 Methods

2.1 Study protocol

This research adhered rigorously to the guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021). The study protocol was prospectively recorded in the PROSPERO database (CRD42024560286).

2.2 Search strategy

A comprehensive literature search was performed across several databases, including PubMed, Web of Science, the Cochrane

Library, and Embase, to locate relevant RCTs published up to 16 June 2024. The search terms utilized included: ("poly (ADP-ribose) polymerase inhibitor," "PARP inhibitor," "PARPi," "PARP inhibitors") OR ("olaparib," "niraparib," "rucaparib," "veliparib," "AZD221," "AG014699," "MK 4827") AND ("ovarian neoplasm," "ovarian cancer," "cancer of ovary," "ovary cancer"). A detailed search strategy is available in Supplementary Files S1. Additionally, references within selected review articles were examined to capture further relevant studies.

2.3 Inclusion and exclusion criteria

The inclusion criteria for the selected articles were as follows: (1) RCTs; (2) participants were adult women (18 years and older) with a histologically or cytologically confirmed diagnosis of OC at any stage; (3) intervention involved maintenance treatment with PARP inhibitors; (4) comparison: treatment with placebo; (5) outcomes included PFS, OS, CFI, TFST, TSST, TEAEs of any grade, or grade ≥3 TEAEs. Articles were excluded if they were: (1) single-arm trials, retrospective or prospective cohort studies; (2) studies involving combination therapy of PARP inhibitors with anti-angiogenic agents or CT in the intervention group; (3) trials lacking relevant outcomes or with duplicated data; (4) conference abstracts, study protocols, case reports, and literature reviews.

2.4 Data extraction

Two independent reviewers undertook the screening, selection, exclusion, and data extraction phases of the study. Extracted data from each eligible study included details such as first author, publication year, trial name, study phase, disease status, sample size, median participant age, specifics of intervention and control regimens, follow-up duration, and outcomes analyzed in the meta-analysis. Primary outcomes focused on PFS and OS, while secondary outcomes encompassed CFI, TFST, TSST, and TEAEs. The CFI was defined as the interval from the final dose of prior CT to the initiation of the next CT (Ledermann et al., 2020). TFST referred to the period from randomization to the first subsequent anti-cancer treatment or death (Wu et al., 2024b), while TSST denoted the time from random assignment to the second subsequent therapy or death (DiSilvestro et al., 2023). In instances where hazard ratio (HR) data extraction was not direct, the Engauge Digitizer Version 10.8 tool and the methodology proposed by Tierney et al. were employed to derive data from Kaplan-Meier curves (Tierney et al., 2007).

2.5 Assessment of risk of bias

The assessment of RCTs for quality and risk of bias employed the modified Jadad scale (Jadad et al., 1996). Two independent reviewers evaluated each study based on criteria encompassing the randomization process, randomization concealment, double-blinding implementation, and the documentation of withdrawals and dropouts. Studies scoring between 0 and 3 points were deemed to be of low quality, whereas those scoring between 4 and 7 points were considered high quality.

2.6 Statistical analysis

The efficacy and safety outcomes are synthesized using HR and relative risk (RR), each accompanied by a 95% confidence interval (CI) and prediction interval (PI). The HR less than 1 indicated a benefit for the intervention group, while HR greater than 1 suggested an advantage for the control group. Cochran's Q test and I^2 statistics were used to statistically probe heterogeneity (Bowden et al., 2011; IntHout et al., 2016). When I^2 exceeded 50% or *p*-values were below 0.10, significant heterogeneity was inferred, prompting the use of a random-effects model; otherwise, a fixed-effects model was employed (Higgins and Thompson, 2002). Subgroup analyses based on homologous recombination (HR) status, OC subtypes, or specific PARP inhibitors were performed only for groups with ≥2 studies included. Sensitivity analysis was performed to validate the stability of the current analysis. Publication bias was ascertained through the visual examination of funnel plots and application of Begg's and Egger's tests (Begg and Mazumdar, 1994; Egger et al., 1997), with any detected bias adjusted using the trim-and-fill method (Duval and Tweedie, 2000). All statistical analyses were conducted using R Version 4.3.1 and STATA Version 12.0, with a two-sided *p*-value of less than 0.05 considered to indicate statistical significance.

2.7 Trial sequential analysis

A trial sequential analysis (TSA) was executed to evaluate the robustness of the evidence and correct potential inaccuracies (Wetterslev et al., 2017). For TEAE outcomes, the TSA was conducted using TSA v0.9.5.10 Beta software to determine the required information size (RIS) and establish trial sequential monitoring boundaries. The RIS estimation and construction of O'Brien-Fleming α-spending boundaries were performed using the TSA software, maintaining a type I error at 5% and a type II error at 20%. The efficacy outcomes of PFS, OS, CFI, TFST, and TSST were analyzed using the "rsource" and "metacumbounds" functions of STATA 12.0, in conjunction with the "ldbounds" and "foreign" packages of R software 4.3.1 (Xie et al., 2022). The RIS was evaluated using an *a priori* information size (APIS) method. If the cumulative Z-curve intersected the trial sequential monitoring or RIS boundary, additional studies were deemed unnecessary, and solid evidence was gathered to either confirm or deny the effect of the intervention.

3 Results

3.1 Study selection procedure

The initial search yielded 3,454 articles, from which 1,357 duplicates were removed. Subsequently, title and abstract screening was performed on the remaining 2,097 articles, resulting in the exclusion of 2,035 due to irrelevance. Of the 62 full-text articles assessed, 42 were excluded for the following reasons: 3 were non-comparative clinical studies; 8 involved repeated trials; 14 lacked essential outcome data; and 17 had intervention and control designs that did not meet the inclusion criteria. Ultimately, 20 studies satisfied the inclusion criteria and

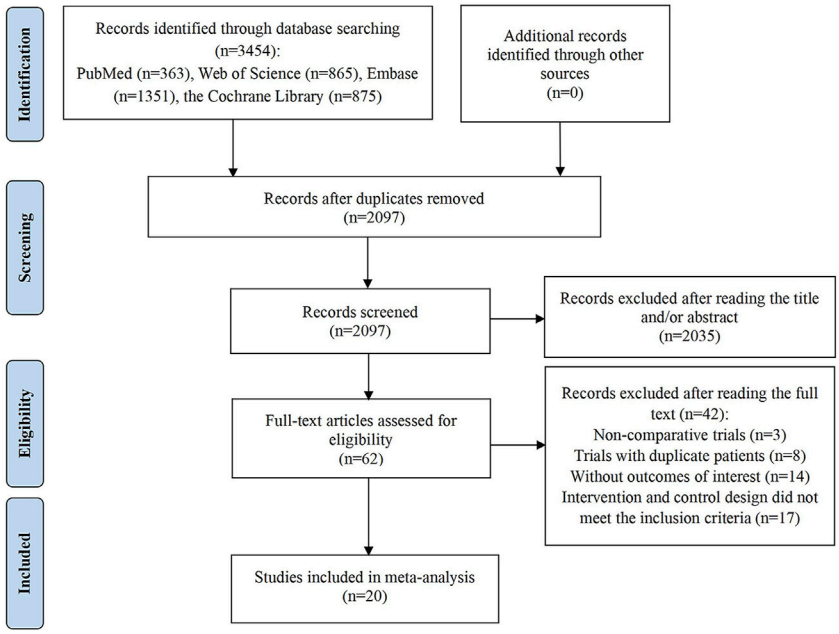

FIGURE 1
Flow diagram of the process of study selection.

were incorporated into the meta-analysis (Banerjee et al., 2021; Coleman et al., 2017; DiSilvestro et al., 2023; Friedlander et al., 2018; González-Martín et al., 2019; González-Martín et al., 2023; Ledermann et al., 2014; Ledermann et al., 2020; Li et al., 2022a; Li et al., 2023; Mirza et al., 2016; Monk et al., 2022; Moore et al., 2018; Poveda et al., 2021; Pujade-Lauraine et al., 2017; Pujade-Lauraine et al., 2023; Wu L. et al., 2021; Wu et al., 2024a; Wu et al., 2024b; Wu X. H. et al., 2021). The study identification and selection process are illustrated in Figure 1.

3.2 Study characteristics and quality assessment

The details of the included studies and their participants are presented in Table 1. This analysis encompassed 20 studies, comprising 2 phase II and 18 phase III trials, all published in English between 2014 and 2024. The subjects were patients with newly diagnosed, recurrent, or advanced OC. Specifically, 8 studies focused on newly diagnosed OC, 11 on recurrent OC, and 1 on advanced OC. A total of 5,204 OC patients were randomly assigned to receive maintenance therapy with PARP inhibitors, while 2,628 patients were allocated to placebo. PARP inhibitors used in the intervention group included olaparib, niraparib, rucaparib, fuzuloparib, and senaparib. Notably, only one study each reported on the efficacy and safety of fuzuloparib and senaparib as maintenance therapies for OC. All included trials were published in high-impact journals, characterized by rigorous designs and comprehensive descriptions. Consequently, all studies were considered to be of high quality. Further information on the quality assessment (Supplementary Table S1) and Risk of Bias graph (Supplementary Figure S1) are available in Supplementary Files S2.

3.3 Pooled effect of primary outcomes

Fifteen studies investigated the PFS benefit of PARP inhibitors in OC patients. A pooled analysis using random-effects model (I^2 = 75.0%, Tau^2 = 0.0701) indicated a 60.2% reduction in the risk of disease progression or mortality with PARP inhibitor maintenance therapy compared to placebo (HR: 0.398, 95% CI = 0.339–0.467, 95% PI = 0.219–0.724) (Table 2; Figure 2A). Subgroup analyses based on HR status demonstrated significant PFS improvements across various HR categories, including HRD (HR: 0.427, 95% CI = 0.368–0.496, 95% PI = 0.232–0.782), BRCA mutation (HR: 0.341, 95% CI = 0.269–0.432, 95% PI = 0.166–0.699), germline BRCA mutation (HR: 0.256, 95% CI = 0.203–0.323, 95% PI = 0.120–0.530), non-germline BRCA mutation (HR: 0.450, 95% CI = 0.376–0.540, 95% PI = 0.303–0.670), BRCA wild-type (HR: 0.523, 95% CI = 0.442–0.620, 95% PI = 0.412–0.665), or HR proficiency (HRP) (HR: 0.615, 95% CI = 0.497–0.761, 95% PI = 0.154–2.452). Notably, PARP inhibitors conferred PFS benefits in both newly diagnosed (HR: 0.479, 95% CI = 0.362–0.633, 95% PI = 0.180–1.273) and recurrent OC cases (HR: 0.354, 95% CI = 0.318–0.395, 95% PI = 0.238–0.524). Analysis by specific PARP inhibitors showed that olaparib (HR: 0.363, 95% CI = 0.312–0.422, 95% PI = 0.240–0.576), niraparib (HR: 0.422, 95% CI = 0.306–0.582, 95% PI = 0.130–1.370), or rucaparib (HR: 0.428, 95% CI = 0.299–0.614) maintenance therapy significantly improved PFS compared with placebo (Table 3; Supplementary Figures S2–S4).

Six studies evaluated OS benefits. These trials exhibited no significant heterogeneity, thus adopting a fixed-effects model for analysis (I^2 = 0%, Tau^2 = 0). Overall, PARP inhibitor maintenance therapy significantly improved OS in OC patients relative to placebo (HR = 0.677, 95% CI = 0.582–0.788; 95% PI = 0.546–0.839) (Table 2; Figure 2B). Stratified analysis by HR status revealed improved OS in

TABLE 1 Characteristics of RCTs included in this meta-analysis.

First author (Year)	Trial name	Study phase	Disease state	Population (I/C)	Median age (range) (y)	Intervention arm	Control arm	Median duration of follow-up (I/C, mo)	Reported outcomes
Monk et al. (2022)	ATHENA-MONO	Phase III	Newly diagnosed, histologically confirmed, advanced, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer; FIGO stage III-IV	427/111	I: 61 (30–83); C: 61 (31–80)	Rucaparib 600 mg twice a day	Placebo	26.1/26.2	1, 6, 7
Banerjee et al. (2021)	SOLO1/GOG 3004	Phase III	Newly diagnosed, histologically confirmed advanced, FIGO stage III or IV, high-grade serous or high-grade endometrioid ovarian cancer; ECOG-PS of 0–1	260/131	18 ears or older	Olaparib 300 mg twice daily	Placebo	57.6/60	1
Wu et al. (2021a)	NORA	Phase III	Histologically confirmed epithelial ovarian, fallopian tube or primary peritoneal carcinoma of high-grade serous histology or no histological restrictions for patients with ovarian cancer carrying a germline BRCA mutation	177/88	I: 53 (35–78); C: 55 (38–72)	Niraparib 300 mg/day	Placebo	15.8	1, 6, 7
González-Martín et al. (2019)	PRIMA/ENGOT-OV26/GOG-3012	Phase III	Newly diagnosed, histologically confirmed advanced cancer of the ovary, peritoneum, or fallopian tube; FIGO stage III or IV	487/246	I: 62 (32–85); C: 62 (33–88)	Niraparib 300 mg once daily	Placebo	13.8	2, 4
Li et al. (2022a)	FZOCUS-2	Phase III	Pathologically confirmed, high-grade (or poorly to moderately differentiated) serous ovarian cancer, primary peritoneal or fallopian tube cancer, or grade ≥2 endometrioid ovarian cancer	167/85	I: 54 (34–75); C: 54 (29–73)	Fuzuloparib 150 mg twice daily	Placebo	8.5	1, 3, 6, 7
Poveda et al. (2021)	SOLO2/ENGOT-Ov21	Phase III	Histologically confirmed, relapsed, high-grade serous or high-grade endometrioid ovarian cancer, including primary peritoneal or fallopian tube cancer; ECOG-PS of 0–1	196/99	I: 56 (IQR 51–63); C: 56 (IQR 49–63)	Olaparib 300 mg twice daily	Placebo	65.7/64.5	2, 4, 5, 6, 7
Coleman et al. (2017)	ARIEL3	Phase III	Platinum-sensitive, high-grade serous or endometrioid ovarian, primary peritoneal, or fallopian tube carcinoma	375/189	I: 61 (IQR 53–67); C: 62 (IQR 53–68)	Rucaparib 600 mg twice daily	Placebo	NR	1, 6, 7
Wu et al. (2021b)	SOLO1 (China cohort)	Phase III	Newly diagnosed, histologically confirmed advanced high-grade	44/20	18 years or older	Olaparib 300 mg twice daily	Placebo	30.5/30.4	1, 4, 5, 6, 7

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TABLE 1 (*Continued*) Characteristics of RCTs included in this meta-analysis.

First author (Year)	Trial name	Study phase	Disease state	Population (I/C)	Median age (range) (y)	Intervention arm	Control arm	Median duration of follow-up (I/C, mo)	Reported outcomes
			serous ovarian cancer or high-grade endometrioid cancer						
Friedlander et al. (2018)	Study 19	Phase II	Recurrent, platinum-sensitive, ovarian, fallopian tube or primary peritoneal cancer with high-grade serous histology	136/129	I: 58 (21–89); C: 59 (33–84)	Olaparib 400 mg twice daily	Placebo	78	2, 4, 5, 6, 7
Ledermann et al. (2014)	Study 19	Phase II	Recurrent, platinum-sensitive, ovarian or fallopian tube cancer, or primary peritoneal cancer, with high-grade (grade 2 or 3) serous features or a serous component	136/129	I: 58 (21–89); C: 59 (33–84)	Olaparib 400 mg twice daily	Placebo	5.6	1
Mirza et al. (2016)	ENGOT-OV16/NOVA	Phase III	Histologically diagnosed ovarian cancer, fallopian tube cancer, or primary peritoneal cancer with predominantly high-grade serous histologic features	372/181	I: NR (33–84); C: NR (34–82)	Niraparib 300 mg once daily	Placebo	16.9	1, 3, 4, 6, 7
Moore et al. (2018)	SOLO1	Phase III	Newly diagnosed, histologically confirmed advanced (FIGO stage III or IV) high-grade serous or endometrioid ovarian cancer, primary peritoneal cancer, or fallopian-tube cancer	260/131	18 years or older	Olaparib 300 mg twice daily	Placebo	40.7/41.2	1, 6
Pujade-Lauraine et al. (2017)	SOLO2/ENGOT-Ov21	Phase III	Histologically confirmed, relapsed, high-grade serous ovarian cancer or high-grade endometrioid cancer; ECOG-PS of 0–1	196/99	I: 56 (IQR 51–63); C: 56 (IQR 49–63)	Olaparib 300 mg twice daily	Placebo	22.1/22.2	1
Wu et al. (2024a)	FLAMES	Phase III	Histologically confirmed advanced (FIGO stage III-IV), high-grade serous or endometrioid cancer or other histological types of epithelial ovarian cancer, fallopian tube cancer or primary peritoneal cancer; ECOG-PS of 0–1	271/133	I: 55 (IQR 50–62); C: 54 (IQR 49–60)	Senaparib 100 mg once daily	Placebo	22.3	1, 3, 4, 6, 7
Li et al. (2023)	PRIME	Phase III	New diagnosis of histologically confirmed, high-grade serous or endometrioid epithelial ovarian cancer, fallopian tube carcinoma, or primary peritoneal carcinoma; FIGO stage III or IV	255/129	I: 53 (32–77); C: 54 (33–77)	Niraparib 200 mg or 300 mg once daily	Placebo	27.5	1, 2, 4, 6, 7

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TABLE 1 (*Continued*) Characteristics of RCTs included in this meta-analysis.

First author (Year)	Trial name	Study phase	Disease state	Population (I/C)	Median age (range) (y)	Intervention arm	Control arm	Median duration of follow-up (I/C, mo)	Reported outcomes
González-Martín et al. (2023)	PRIMA/ENGOT-OV26/GOG-3012	Phase III	Newly diagnosed, advanced (FIGO stage III/IV), high-grade serous or endometrioid ovarian, primary peritoneal, or fallopian tube cancer	487/246	I: 62 (32–85); C: 62 (33–88)	Niraparib 300 mg once daily	Placebo	41.6/41.9	1, 6, 7
DiSilvestro et al. (2023)	SOLO1/GOG 3004	Phase III	Newly diagnosed, histologically confirmed advanced (FIGO stage III or IV) high-grade serous or endometrioid ovarian, primary peritoneal, and/or fallopian tube cancer	260/131	18 years or older	Olaparib 300 mg twice daily	Placebo	88.9/87.4	2, 4, 5, 7
Wu et al. (2024b)	NORA	Phase III	Histologically confirmed, recurrent, (predominantly) high-grade serous epithelial ovarian cancer, fallopian tube carcinoma, or primary peritoneal carcinoma; ECOG-PS of 0 or 1	177/88	I: 53 (35–78); C: 55 (38–72)	Niraparib 300 mg/day	Placebo	58.4/57.0	2, 3, 4
Pujade-Lauraine et al. (2023)	OReO/ENGOT-ov38	Phase III	Relapsed histologically diagnosed non-mucinous epithelial ovarian cancer, primary peritoneal cancer, and/or fallopian tube cancer	146/74	I: NR (29–81); C: NR (43–87)	Olaparib 300 mg twice daily	Placebo	Cohort 1: 4.1/2.8; Cohort 2: 2.9/2.8	1, 2, 4, 5, 6, 7
Ledermann et al. (2020)	ARIEL3	Phase III	Platinum-sensitive, high-grade serous or endometrioid ovarian, primary peritoneal, or fallopian tube carcinoma; ECOG-PS of 0 or 1	375/189	I: 61 (IQR 53–67); C: 62 (IQR 53–68)	Rucaparib 600 mg twice daily	Placebo	28.1	3, 4, 5

I, intervention; C, control; y, year; mo, month; FIGO, international federation of gynecology and obstetrics; ECOG-PS, eastern cooperative oncology group performance status; IQR, interquartile range; NR, not reported; 1, progression-free survival; 2, overall survival; 3, chemotherapy-free interval; 4, time to first subsequent therapy or death; 5, time to second subsequent therapy or death; 6, any grade treatment-emergent adverse events (TEAEs); 7, grade ≥3 TEAEs.

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TABLE 2 Pooled effect of the efficacy and safety of PARP inhibitor maintenance treatment for ovarian cancer.

Outcomes	Number of studies	Meta-analysis				Heterogeneity	
		HR/RR	95% CI	*p*-value	95% PI	I^2, Tau^2	*p*-value
PFS	15	0.398	0.339–0.467	<0.001	0.219–0.724	75.0%, 0.0701	<0.001
OS	6	0.677	0.582–0.788	<0.001	0.546–0.839	0%, 0	0.515
CFI	6	0.417	0.368–0.472	<0.001	0.265–0.627	39.3%, 0.0167	0.144
TFST	13	0.441	0.391–0.498	<0.001	0.308–0.632	50.4%, 0.0229	0.019
TSST	7	0.574	0.507–0.649	<0.001	0.488–0.674	0%, 0	0.579
TEAEs of any grade	13	1.046	1.032–1.059	<0.001	1.028–1.055	0%, 0	0.957
Grade ≥3 TEAEs	13	2.931	2.641–3.253	<0.001	2.128–3.792	25.7%, 0.0131	0.185

PFS, progression-free survival; OS, overall survival; CFI, chemotherapy-free interval; TFST, time to first subsequent therapy or death; TSST, time to second subsequent therapy or death; TEAEs, treatment-emergent adverse events.

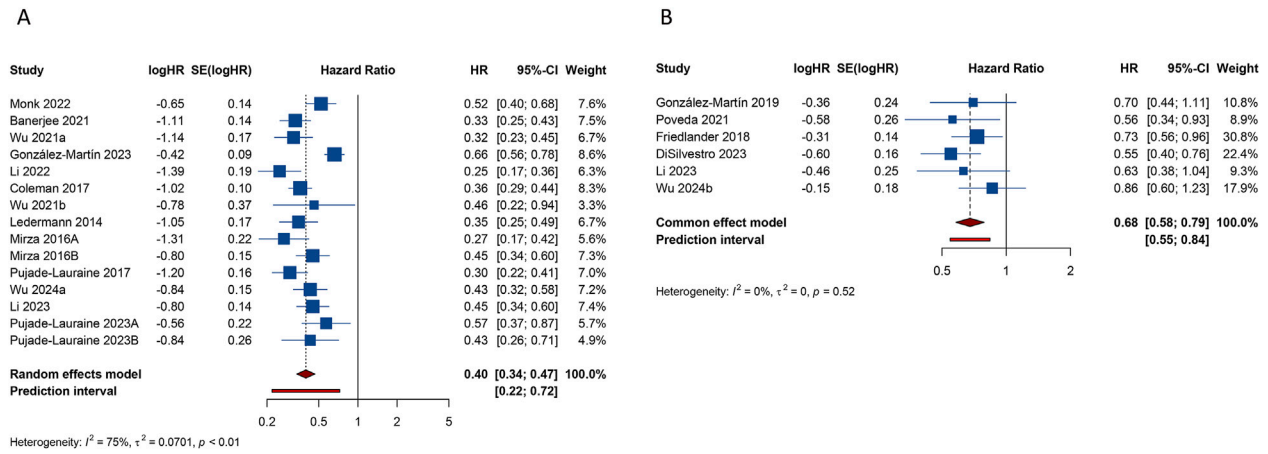

FIGURE 2
Forest plot of primary outcomes after PARP inhibitor maintenance therapy for ovarian cancer. **(A)** progression-free survival; **(B)** overall survival.

OC patients with BRCA mutation (HR = 0.701, 95% CI = 0.509–0.966) or germline BRCA mutation (HR = 0.738, 95% CI = 0.559–0.975). Furthermore, subgroup analyses by OC subtypes revealed an improved OS in patients with newly diagnosed OC (HR: 0.602, 95% CI = 0.477–0.761, 95% PI = 0.133–2.730) or recurrent OC (HR: 0.737, 95% CI = 0.604–0.901, 95% PI = 0.202–2.696). Analysis by specific PARP inhibitors suggested that olaparib (HR: 0.635, 95% CI = 0.524–0.770, 95% PI = 0.181–2.225) or niraparib (HR: 0.752, 95% CI = 0.588–0.962, 95% PI = 0.152–3.716) maintenance therapy significantly improved OS for OC patients (Table 3; Supplementary Figures S5–S7).

3.4 Pooled effect of secondary outcomes

3.4.1 CFI, TFST, and TSST

Six studies reported on the clinical benefit of CFI. The aggregated data indicated that PARP inhibitor maintenance therapy significantly prolonged CFI compared to placebo (HR: 0.417, 95% CI = 0.368–0.472, 95% PI = 0.265–0.627) (Table 2; Figure 3A). Subgroup analyses, stratified by OC subtypes or specific PARP inhibitors, demonstrated that this maintenance therapy notably prolonged CFI in recurrent OC patients (HR: 0.402, 95% CI = 0.326–0.497, 95% PI = 0.213–0.760), with niraparib showing a longer CFI than placebo (HR: 0.407, 95% CI = 0.286–0.581, 95% PI = 0.007–22.336) (Table 3; Supplementary Figure S8).

Thirteen studies examined the TFST outcome. The pooled results revealed that maintenance therapy with PARP inhibitors significantly lengthened TFST relative to placebo (HR: 0.441, 95% CI = 0.391–0.498, 95% PI = 0.308–0.632) (Table 2; Figure 3B), with consistent findings across OC patients with HRD (HR: 0.416, 95% CI = 0.338–0.512), BRCA mutation (HR: 0.366, 95% CI = 0.247–0.543, 95% PI = 0.005–29.785), and in both newly diagnosed (HR: 0.492, 95% CI = 0.364–0.664, 95% PI = 0.139–1.742) and recurrent OC (HR: 0.419, 95% CI = 0.378–0.465, 95% PI = 0.329–0.531) patients. Subsequent analysis grouped by specific PARP inhibitors suggested that olaparib (HR: 0.399, 95% CI = 0.347–0.458, 95% PI = 0.327–0.486) or niraparib (HR: 0.468, 95% CI = 0.367–0.598, 95% PI = 0.201–1.092) maintenance therapy significantly prolonged TFST compared with placebo (Table 3; Supplementary Figures S9–S11).

TABLE 3 Subgroup analysis of the efficacy and safety of PARP inhibitor maintenance treatment for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis				Heterogeneity	
		HR/RR	95% CI	*p*-value	95% PI	I^2, Tau^2	*p*-value
PFS							
Homologous recombination status							
HRD	5	0.427	0.368–0.496	<0.001	0.232–0.782	45.0%, 0.0250	0.122
BRCA mutation	9	0.341	0.269–0.432	<0.001	0.166–0.699	62.3%, 0.0775	0.007
Germline BRCA mutation	5	0.256	0.203–0.323	<0.001	0.120–0.530	31.5%, 0.0331	0.212
Non-germline BRCA mutation	4	0.450	0.376–0.540	<0.001	0.303–0.670	0%, 0	0.932
BRCA wild-type	6	0.523	0.442–0.620	<0.001	0.412–0.665	0%, 0	0.620
HRP	3	0.615	0.497–0.761	<0.001	0.154–2.452	0%, 0	0.386
OC subtypes							
Newly diagnosed OC	5	0.479	0.362–0.633	<0.001	0.180–1.273	79.3%, 0.0741	0.001
Recurrent OC	9	0.354	0.318–0.395	<0.001	0.238–0.524	43.5%, 0.0220	0.078
Types of PARP inhibitors							
Olaparib vs. Placebo	6	0.363	0.312–0.422	<0.001	0.240–0.576	29.5%, 0.0157	0.214
Niraparib vs. Placebo	5	0.422	0.306–0.582	<0.001	0.130–1.370	84.2%, 0.1099	<0.001
Rucaparib vs. Placebo	2	0.428	0.299–0.614	<0.001	—	78.5%, 0.0531	0.031
OS							
Homologous recombination status							
HRD	2	0.752	0.440–1.286	0.298	—	0%, 0	0.508
BRCA mutation	2	0.701	0.509–0.966	0.030	—	4.7%, 0.0029	0.306
Germline BRCA mutation	2	0.738	0.559–0.975	0.033	—	0%, 0	0.587
OC subtypes							
Newly diagnosed OC	3	0.602	0.477–0.761	<0.001	0.133–2.730	0%, 0	0.689
Recurrent OC	3	0.737	0.604–0.901	0.003	0.202–2.696	0%, 0	0.400
Types of PARP inhibitors							
Olaparib vs. Placebo	3	0.635	0.524–0.770	<0.001	0.181–2.225	0.2%, <0.0001	0.367
Niraparib vs. Placebo	3	0.752	0.588–0.962	0.023	0.152–3.716	0%, 0	0.573
CFI							
OC subtypes							
Recurrent OC	5	0.402	0.326–0.497	<0.001	0.213–0.760	51.4%, 0.0283	0.084
Types of PARP inhibitors							
Niraparib vs. Placebo	3	0.407	0.286–0.581	<0.001	0.007–22.336	68.3%, 0.0666	0.043
TFST							
Homologous recombination status							
HRD	2	0.416	0.338–0.512	<0.001	—	0%, 0	0.446
BRCA mutation	3	0.366	0.247–0.543	<0.001	0.005–29.785	65.5%, 0.0794	0.055
OC subtypes							
Newly diagnosed OC	4	0.492	0.364–0.664	<0.001	0.139–1.742	73.2%, 0.0630	0.011
Recurrent OC	8	0.419	0.378–0.465	<0.001	0.329–0.531	19.5%, 0.0058	0.275
Types of PARP inhibitors							
Olaparib vs. Placebo	6	0.399	0.347–0.458	<0.001	0.327–0.486	0%, 0	0.567
Niraparib vs. Placebo	5	0.468	0.367–0.598	<0.001	0.201–1.092	72.6%, 0.0553	0.006
TSST							

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TABLE 3 (*Continued*) Subgroup analysis of the efficacy and safety of PARP inhibitor maintenance treatment for ovarian cancer.

Outcomes and subgroups	Number of studies	Meta-analysis				Heterogeneity	
		HR/RR	95% CI	*p*-value	95% PI	I^2, Tau^2	*p*-value
Homologous recombination status							
BRCA mutation	3	0.529	0.416–0.673	<0.001	0.060–4.694	19.2%, 0.0108	0.290
OC subtypes							
Newly diagnosed OC	2	0.506	0.383–0.668	<0.001	—	0%, 0	0.828
Recurrent OC	5	0.591	0.515–0.679	<0.001	0.473–0.740	0%, 0	0.447
Types of PARP inhibitors							
Olaparib vs. Placebo	6	0.534	0.461–0.619	<0.001	0.433–0.658	0%, 0	0.895
TEAEs of any grade							
OC subtypes							
Newly diagnosed OC	5	1.054	1.032–1.078	<0.001	1.018–1.092	0%, 0	0.968
Recurrent OC	7	1.043	1.025–1.062	<0.001	1.021–1.065	0%, 0	0.999
Types of PARP inhibitors							
Olaparib vs. Placebo	5	1.049	1.018–1.081	0.002	1.004–1.098	0%, 0	0.955
Niraparib vs. Placebo	4	1.053	1.033–1.073	<0.001	1.009–1.095	0%, 0	0.973
Rucaparib vs. Placebo	2	1.041	1.012–1.071	0.005	-	0%, 0	0.850
Grade ≥3 TEAEs							
OC subtypes							
Newly diagnosed OC	5	2.771	2.374–3.235	<0.001	1.614–4.437	30.4%, 0.0152	0.219
Recurrent OC	7	3.026	2.592–3.533	<0.001	1.757–4.802	37.1%, 0.0272	0.145
Types of PARP inhibitors							
Olaparib vs. Placebo	5	2.120	1.715–2.620	<0.001	1.491–2.954	0%, 0	0.927
Niraparib vs. Placebo	4	3.107	2.666–3.621	<0.001	2.221–4.349	0%, 0	0.886
Rucaparib vs. Placebo	2	3.208	2.500–4.115	<0.001	-	48.4%, 0.0305	0.164

PFS, progression-free survival; HRD, homologous recombination deficiency; HRP, homologous recombination proficiency; OC, ovarian cancer; OS, overall survival; CFI, chemotherapy-free interval; TFST, time to first subsequent therapy or death; TSST, time to second subsequent therapy or death; TEAEs, treatment-emergent adverse events.

The TSST was evaluated in 7 studies, with combined estimates showing that PARP inhibitor maintenance therapy significantly extended TSST over placebo (HR: 0.574, 95% CI = 0.507–0.649, 95% PI = 0.488–0.674) (Table 2; Figure 3C). Subgroup analyses further indicated that this therapeutic approach substantially prolonged TSST in patients with BRCA mutation (HR: 0.529, 95% CI = 0.416 to 0.673, 95% PI = 0.060–4.694), and in both newly diagnosed (HR: 0.506, 95% CI = 0.383–0.668) and recurrent OC (HR: 0.591, 95% CI = 0.515–0.679, 95% PI = 0.473–0.740) patients. When stratified by specific PARP inhibitors, olaparib maintenance therapy was associated with a notably longer TSST compared to placebo (HR: 0.534, 95% CI = 0.461 to 0.619, 95% PI = 0.433–0.658) (Table 3; Supplementary Figure S12).

3.4.2 TEAEs

Thirteen studies provided data on any grade TEAEs. The overall analysis revealed that PARP inhibitor maintenance therapy was associated with a higher risk of any grade TEAEs compared to placebo (RR = 1.046, 95% CI = 1.032–1.059, 95% PI = 1.028–1.055) (Table 2; Figure 3D). When categorized by OC subtypes, it was observed that PARP inhibitor maintenance treatment significantly increased the risk of any grade TEAEs in patients with newly diagnosed (RR = 1.054, 95% CI = 1.032–1.078, 95% PI = 1.018–1.092) or recurrent OC (RR = 1.043, 95% CI = 1.025–1.062, 95% PI = 1.021–1.065). Subgroup analyses based on specific PARP inhibitors suggested that olaparib (RR = 1.049, 95% CI = 1.018–1.081, 95% PI = 1.004–1.098), niraparib (RR = 1.053, 95% CI = 1.033–1.073, 95% PI = 1.009–1.095), or rucaparib (RR = 1.041, 95% CI = 1.012–1.071) maintenance treatment significantly increased the incidence of any grade TEAEs compared with placebo (Table 3; Supplementary Figures S13, S14).

Thirteen studies reported on grade ≥3 TEAEs. The overall findings suggested that PARP inhibitor maintenance therapy significantly elevated the risk of grade ≥3 TEAEs compared to placebo (RR = 2.931, 95% CI = 2.641–3.253, 95% PI = 2.128–3.792) (Table 2; Figure 3E). Similar results were also obtained in newly diagnosed (RR = 2.771, 95% CI = 2.374–3.235, 95% PI = 1.614–4.437) or recurrent OC (RR = 3.026, 95% CI = 2.592–3.533, 95% PI = 1.757–4.802) cases. Subgroup analysis according to the types of PARP inhibitors showed that maintenance treatment with olaparib (RR = 2.120, 95% CI = 1.715–2.620, 95% PI = 1.491–2.954), niraparib (RR = 3.107, 95% CI = 2.666–3.621, 95% PI = 2.221–4.349), or rucaparib (RR = 3.208, 95% CI = 2.500–4.115) significantly increased the incidence of

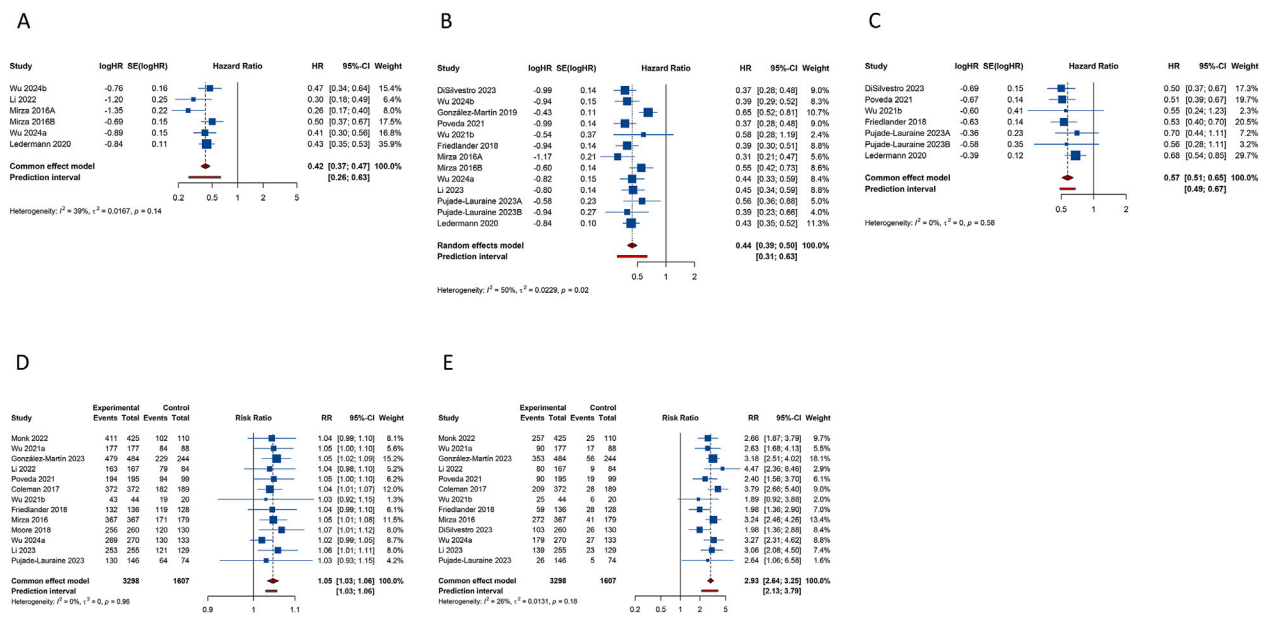

FIGURE 3
Forest plot of secondary outcomes after PARP inhibitor maintenance therapy for ovarian cancer. **(A)** Chemotherapy-free interval; **(B)** Time to first subsequent therapy or death; **(C)** Time to second subsequent therapy or death; **(D)** Any grade treatment-emergent adverse events (TEAEs); **(E)** Grade ≥3 TEAEs.

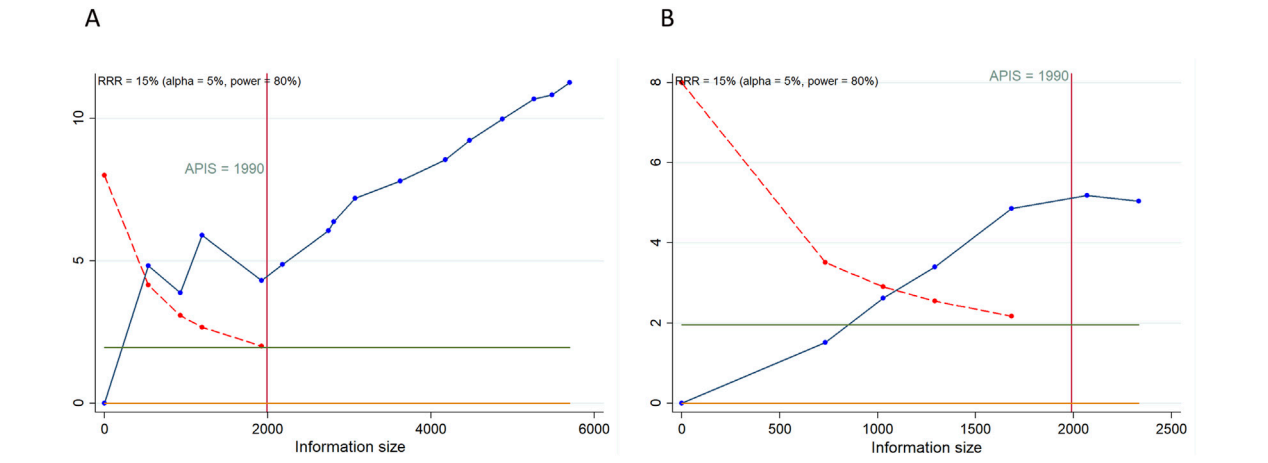

FIGURE 4
Trial sequential analysis of primary outcomes after PARP inhibitor maintenance therapy for ovarian cancer. **(A)** progression-free survival; **(B)** overall survival. Red inward-sloping line to the left represents trial sequential monitoring boundary. Blue line represents evolution of cumulative Z-score. Horizontal green lines represent the conventional boundaries for statistical significance. Heterogeneity-adjusted required information size to demonstrate or reject 15% relative risk (*a priori* estimate) of mortality risk (with alpha of 5% and beta of 20%) is 1990 patients for PFS and OS (vertical red line). Cumulative Z-curve crossing the trial sequential monitoring boundary or the APIS boundary provides firm evidence of effect.

grade ≥3 TEAEs compared to placebo (Table 3; Supplementary Figures S15, S16).

3.5 TSA results

As depicted in Figures 4, 5, a RIS of 1,990 was determined for PFS, OS, CFI, TFST, and TSST. The analysis revealed that all cumulative Z-curves surpassed both the RIS and trial sequential monitoring boundaries, indicating the attainment of a relatively definitive conclusion. For TEAEs, we determined a RIS of 1,680 for any grade TEAEs and 1,554 for grade ≥3 TEAEs. Notably, each cumulative Z-curve crossed either the RIS or trial sequential monitoring boundary, implying that additional research may not be necessary to achieve a conclusive result.

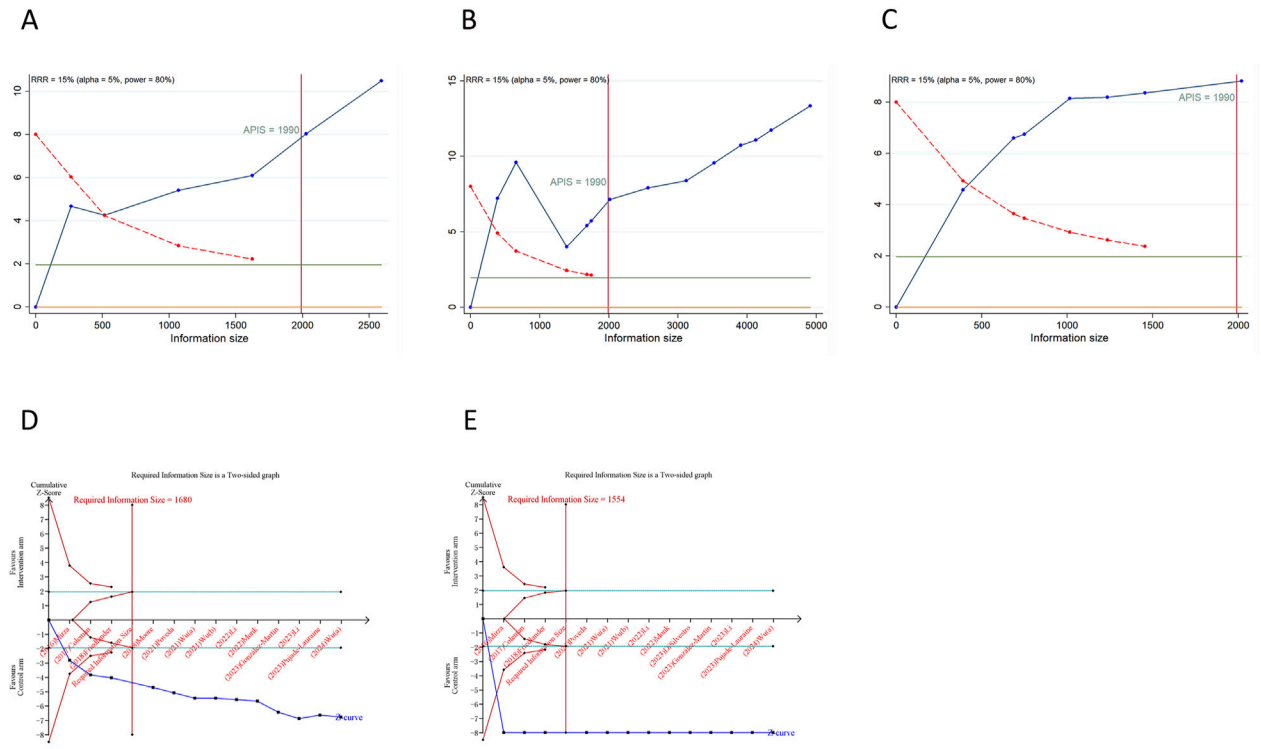

FIGURE 5
Trial sequential analysis of secondary outcomes after PARP inhibitor maintenance therapy for ovarian cancer. **(A)** Chemotherapy-free interval; **(B)** Time to first subsequent therapy or death; **(C)** Time to second subsequent therapy or death; **(D)** Any grade treatment-emergent adverse events (TEAEs); **(E)** Grade ≥3 TEAEs. Uppermost and lowermost red curves represent trial sequential monitoring boundary lines for benefit and harm, respectively. Inner red lines represent the futility boundary. Blue line represents evolution of cumulative Z-score. Horizontal green lines represent the conventional boundaries for statistical significance. Cumulative Z-curve crossing the trial sequential monitoring boundary or the RIS boundary provides firm evidence of effect.

3.6 Sensitivity analysis and publication bias

During the sensitivity analysis, pooled HR or RR along with their 95% CI were calculated, omitting individual studies one by one to assess the influence of each study on the overall outcomes. This analysis indicated that excluding any single study did not notably alter the quantitative results, suggesting that the combined findings are robust and reliable (Supplementary Figures S17, S18). To assess publication bias, Begg's and Egger's tests were utilized, revealing no significant publication bias across all efficacy and safety outcomes (all $p > 0.05$). Detailed funnel plots can be found in Supplementary Figures S19, S20.

4 Discussion

Our meta-analysis comprehensively assessed the efficacy and safety of PARP inhibitor maintenance monotherapy compared with placebo in the treatment of OC by incorporating the outcomes of the latest RCTs. The findings indicated that PARP inhibitor maintenance therapy significantly improved PFS and OS, as well as prolonged CFI, TFST, and TSST in OC patients. Recent systematic reviews and meta-analyses mainly focused on elucidating the effects and toxicity of PARP inhibitor therapy for patients with various subtypes of OC, such as newly diagnosed, recurrent, or advanced cases (Gulia et al., 2022; Maiorano et al., 2022; Wang et al., 2021). Baradács et al.'s summary analysis demonstrated significant PFS benefits with PARP inhibitor maintenance therapy versus placebo in recurrent OC across the entire cohort, BRCA mutation carriers, germline BRCA mutation carriers, and those with wild-type BRCA status. In newly diagnosed OC, PFS was also improved in both the overall population and the BRCA mutation subgroup (Baradács et al., 2024). However, due to immature OS data in the original trials, Baradács et al.'s study has not yet confirmed the OS benefit of PARP inhibitor maintenance therapy. Additionally, Lee et al.'s research confirmed superior PFS in patients with newly diagnosed advanced epithelial OC treated with PARP inhibitors compared to placebo. Moreover, patients with HRD, BRCA wild type, BRCA1/2 mutation, or HRD without BRCA mutation, but not HRP, exhibited significantly better PFS in the PARP inhibitor group than in the placebo group. Patients with BRCA mutation in the PARP inhibitor group also had significantly better OS compared to those in the placebo group (Lee et al., 2023). Our subgroup analysis demonstrated that compared with placebo, PARP inhibitor maintenance therapy significantly improved PFS in patients with HRD, BRCA mutation, germline BRCA mutation, non-germline BRCA mutation, BRCA wild-type, or HRP. The combined analysis of mature OS data further indicated a notable improvement in OS for patients with BRCA mutation or those with germline BRCA mutation, under PARP inhibitor maintenance

therapy. Furthermore, in cases of either newly diagnosed or recurrent OC, the utilization of PARP inhibitors as maintenance therapy has demonstrated significant improvements in both PFS and OS.

The mechanism by which PARP inhibitors operate in treating OC has been extensively researched. As previously noted, PARP plays a pivotal role in DNA single-strand break repair (SSBR). Inhibition of PARP can result in deficiencies in both SSBR and HRD in patients with BRCA1/2 mutations, ultimately causing cell death (Farmer et al., 2005). Homologous recombination represents a vital error-free mechanism for repairing double-strand breaks (DSBs) during cell division, necessitating functional BRCA1/2 proteins. Mutations in BRCA1/2 genes impede the homologous recombination process. Moreover, PARP inhibitors can partially impede the PARP-associated homologous recombination pathway (Lau et al., 2022). While the absence of either an operational base excision repair pathway or homologous recombination alone does not affect cell viability, the concurrent deficiency of both can result in synthetic lethality (Walsh, 2015). PARP inhibitors effectively inhibit the repair of DNA single-strand breaks. In OC cases linked with BRCA mutations or HRD, PARP inhibitors exhibit superior efficacy due to compromised DNA repair mechanisms that culminate in cell demise. Our subgroup analysis based on HR status indicated that the PFS benefit of PARP inhibitors varies, with the advantages diminishing in the following order: germline BRCA mutation (HR = 0.256), BRCA mutation (HR = 0.341), HRD (HR = 0.427), non-germline BRCA mutation (HR = 0.450), BRCA wild-type (HR = 0.523), and HRP (HR = 0.615). This gradient suggests that wider availability and accessibility of tumor HRD testing could be pivotal in guiding therapeutic decisions regarding PARP inhibitor maintenance in OC. Additionally, our subgroup analysis indicated that the OS benefit of PARP maintenance therapy is similar in patients with BRCA mutations (HR = 0.701) and those with germline BRCA mutations (HR = 0.738). Further investigation is necessary to understand the OS benefits of PARP maintenance therapy across different HR statuses, as more comprehensive OS data from future trials become available.

To date, the FDA has approved three PARP inhibitors-olaparib, niraparib, and rucaparib-for clinical use in OC patients. Olaparib, the first PARP inhibitor introduced into clinical practice, has been utilized for both maintenance and treatment of OC, supported by several highly successful clinical trials (Giannini et al., 2023). Study 19 assessed olaparib's efficacy in the maintenance setting for relapsed, platinum-sensitive OC across all patients, demonstrating significantly longer PFS with olaparib compared to placebo (Ledermann et al., 2012). SOLO-2 specifically targeted high-grade serous OC with BRCA1/2 mutations, revealing that olaparib significantly prolonged PFS relative to placebo (Pujade-Lauraine et al., 2017). Rucaparib, the second approved PARP inhibitor, received accelerated FDA approval as a monotherapy, and subsequently for maintenance treatment (Hirschl et al., 2024). The ARIEL 3 trial, which randomized eligible patients to receive either rucaparib or placebo as maintenance therapy, showed that rucaparib significantly enhanced PFS in patients with platinum-sensitive OC who had responded to platinum-based CT. Notably, rucaparib markedly improved PFS in patients with known genomic or somatic BRCA mutations. For the HRD subgroup, PFS was 13.6 months compared to 5.4 months (HR: 0.32, 95% CI: 0.24–0.42), and in the intention-to-treat population, it was 10.8 months versus 5.4 months (HR: 0.36, 95% CI: 0.30–0.45) (Coleman et al., 2017). A recent meta-analysis confirmed rucaparib's significant efficacy in enhancing PFS and objective response rate in OC patients, particularly those with BRCA mutation (Mustafa et al., 2024). Additionally, niraparib is the latest PARP inhibitor approved for maintenance treatment in OC. Similar to the SOLO-2 findings for olaparib, the PRIMA trial included patients without deleterious BRCA1/2 mutations and showed a significant PFS benefit with niraparib monotherapy across the overall population, regardless of HRD status (González-Martín et al., 2019). Our meta-analysis, which synthesized data from existing RCTs, confirmed that maintenance therapy with olaparib, niraparib, or rucaparib significantly improves PFS compared to placebo. Additionally, maintenance therapy with olaparib or niraparib was associated with a significant extension in OS in OC patients. Nevertheless, determining the most effective PARP inhibitor among olaparib, niraparib, and rucaparib for OC remains challenging due to the absence of RCTs that directly compare their efficacies. Moreover, a feasibility study comparing PARP inhibitor maintenance therapies for OC indicated that indirect treatment comparisons, such as network meta-analyses and population-adjusted indirect comparisons, should be performed with caution due to confounding factors that can preclude objective systematic comparison across RCTs (Lorusso et al., 2022). Despite this, our subgroup analysis suggests that olaparib may offer superior efficacy in enhancing PFS and OS when indirectly comparing HR values. This conclusion, however, necessitates further validation through rigorously designed future research.

TFST and TSST serve as valuable endpoints in evaluating disease recurrence and the initiation of subsequent treatments, reflecting a prolonged PFS benefit and indicating a potential OS advantage (Matulonis et al., 2015). Furthermore, an extended in CFI suggests that patients on PARP inhibitors can delay additional cancer therapies, giving them more time to recover from the adverse effects of prior CT and defer the side effects of further anticancer treatments (Ledermann et al., 2020). In this meta-analysis, patients receiving PARP inhibitor maintenance therapy demonstrated a significant improvement in CFI, TFST, and TSST compared to those on placebo. Subgroup analyses further revealed that the benefit of PARP inhibitor maintenance therapy on these endpoints was consistent, irrespective of HR status, OC subtypes, or the specific PARP inhibitor used. Similar enhancements in post-progression outcomes have been documented in clinical trials evaluating PARP inhibitors for second-line maintenance in OC. For instance, the NOVA trial revealed that maintenance therapy with niraparib significantly improved median CFI and TFST compared to placebo, both in patients with germline BRCA mutations and those without (Mirza et al., 2016). Likewise, the SOLO-2 trial showed that maintenance olaparib significantly extended median TFST and TSST in patients harboring BRCA mutations relative to placebo (Pujade-Lauraine et al., 2017).

Beyond demonstrating the substantial efficacy of PARP inhibitor maintenance therapy in OC, our study also verified an increased risk of any grade and grade ≥3 TEAEs. This elevated risk

was consistently observed in all subgroup analyses. Previous investigations have identified fatigue, nausea, anemia, neutropenia, and thrombocytopenia as prevalent grade ≥3 AEs associated with PARP inhibitor therapy (Banerjee et al., 2021; Coleman et al., 2019; DiSilvestro et al., 2023; González-Martín et al., 2019; Li et al., 2022b; Ray-Coquard et al., 2019). Furthermore, a recent meta-analysis has corroborated that PARP inhibitors are linked with a distinct toxicity profile, predominantly involving hematological abnormalities, with a higher incidence of anemia, thrombocytopenia, and neutropenia compared to placebo (Zhou et al., 2024). Another meta-analysis on safety profiles also reported that the most frequent AEs included fatigue, nausea, vomiting, anemia, and neutropenia, a finding supported by the majority of reviewed studies (Baradács et al., 2024). Thus, it is needed for clinicians to continuously monitor OC patients undergoing PARP inhibitor maintenance treatment, ensuring timely identification and management of TEAEs to mitigate potential health risks.

Nonetheless, this research is not without its limitations. First, this analysis was conducted using aggregate study-level data rather than individual patient data. We did not present separate data for the use of PARP inhibitors in initial and recurrent treatments; however, this form of analysis has already been conducted in previously published meta-analysis (Ruscito et al., 2020). Second, the observed heterogeneity in PFS across studies may stem from various factors, including the stage of OC, types of PARP inhibitors, follow-up duration, and the diverse ethnic backgrounds of participants. Third, while the efficacy of PARP inhibitors is well established in population with HRD and BRCA mutations (Shao et al., 2021), further research is needed to explore their role in HRP population. Fourth, OC is predominantly diagnosed in older adults, who constitute the majority of cases observed in clinical settings (Masvidal Hernandez et al., 2024). The insufficient number of included RCTs that provide HRs and 95% CIs for efficacy and safety outcomes across various age groups restricts our ability to perform further age-based subgroup analyses. Furthermore, future research should focus on assessing the effects of PARP inhibitors on quality of life, as the influence of these maintenance therapies on the quality of life of OC patients remains unreported (Masvidal Hernandez et al., 2024). Fifth, prior research has highlighted that the selection of maintenance therapy should be informed by several key considerations: (1) molecular biomarkers, including BRCA1/2 mutations and HRD status; (2) disease-specific factors, such as chemotherapy response score, the stage at diagnosis, and residual disease post-surgery; and (3) patient characteristics, encompassing comorbidities and concurrent medications (Perez-Fidalgo et al., 2024). While our study has considered BRCA1/2 and HRD status, additional subgroup analyses should be conducted based on these other variables. Finally, although olaparib and niraparib have been extensively studied, fuzuloparib and senaparib have only been investigated in a single trial. Additional studies are needed to confirm the efficacy and safety of fuzuloparib and senaparib in women with OC.

5 Conclusion

In conclusion, the findings from this meta-analysis demonstrated that PARP inhibitors play a significant role in maintenance therapy for OC, showing improvements in PFS, OS, CFI, TFST, and TSST. Subgroup analysis further revealed that this maintenance therapy markedly improved PFS compared to placebo, irrespective of HR status. Nevertheless, the use of PARP inhibitors for maintenance was associated with a heightened risk of any grade and grade ≥3 TEAEs. It is crucial for clinicians to monitor and manage TEAEs when utilizing PARP inhibitors for maintenance therapy in OC within clinical practice.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

GS: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing–original draft. YL: Conceptualization, Methodology, Supervision, Validation, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1460285/full#supplementary-material

References

Armstrong, D. K., Alvarez, R. D., Backes, F. J., Bakkum-Gamez, J. N., Barroilhet, L., Behbakht, K., et al. (2022). NCCN Guidelines® insights: ovarian cancer, version 3.2022. *J. Natl. Compr. Canc Netw.* 20 (9), 972–980. doi:10.6004/jnccn.2022.0047

Banerjee, S., Moore, K. N., Colombo, N., Scambia, G., Kim, B. G., Oaknin, A., et al. (2021). Maintenance olaparib for patients with newly diagnosed advanced ovarian cancer and a BRCA mutation (SOLO1/GOG 3004): 5-year follow-up of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 22 (12), 1721–1731. doi:10.1016/s1470-2045(21)00531-3

Baradács, I., Teutsch, B., Váradi, A., Bilá, A., Vincze, Á., Hegyi, P., et al. (2024). PARP inhibitor era in ovarian cancer treatment: a systematic review and meta-analysis of randomized controlled trials. *J. Ovarian Res.* 17 (1), 53. doi:10.1186/s13048-024-01362-y

Begg, C. B., and Mazumdar, M. (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics* 50 (4), 1088–1101. doi:10.2307/2533446

Bowden, J., Tierney, J. F., Copas, A. J., and Burdett, S. (2011). Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Med. Res. Methodol.* 11, 41. doi:10.1186/1471-2288-11-41

Burger, R. A., Brady, M. F., Bookman, M. A., Fleming, G. F., Monk, B. J., Huang, H., et al. (2011). Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N. Engl. J. Med.* 365 (26), 2473–2483. doi:10.1056/NEJMoa1104390

Cancanelli, L., Mengato, D., Di Spazio, L., Rivano, M., Chiumente, M., and Messori, A. (2022). Evaluation of maintenance treatment with PARP inhibitors in ovarian carcinoma patients responding to platinum therapy: use of restricted mean survival time as an index of efficacy. *Int. J. Clin. Pharmacol. Ther.* 60 (1), 32–35. doi:10.5414/cp204113

Cancer Genome Atlas Research Network (2011). Integrated genomic analyses of ovarian carcinoma. *Nature* 474 (7353), 609–615. doi:10.1038/nature10166

Coleman, R. L., Fleming, G. F., Brady, M. F., Swisher, E. M., Steffensen, K. D., Friedlander, M., et al. (2019). Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. *N. Engl. J. Med.* 381 (25), 2403–2415. doi:10.1056/NEJMoa1909707

Coleman, R. L., Oza, A. M., Lorusso, D., Aghajanian, C., Oaknin, A., Dean, A., et al. (2017). Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 390 (10106), 1949–1961. doi:10.1016/s0140-6736(17)32440-6

Creeden, J. F., Nanavaty, N. S., Einloth, K. R., Gillman, C. E., Stanbery, L., Hamouda, D. M., et al. (2021). Homologous recombination proficiency in ovarian and breast cancer patients. *BMC Cancer* 21 (1), 1154. doi:10.1186/s12885-021-08863-9

DiSilvestro, P., Banerjee, S., Colombo, N., Scambia, G., Kim, B. G., Oaknin, A., et al. (2023). Overall survival with maintenance olaparib at a 7-year follow-up in patients with newly diagnosed advanced ovarian cancer and a BRCA mutation: the SOLO1/GOG 3004 trial. *J. Clin. Oncol.* 41 (3), 609–617. doi:10.1200/jco.22.01549

Duval, S., and Tweedie, R. (2000). Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56 (2), 455–463. doi:10.1111/j.0006-341x.2000.00455.x

Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *Bmj* 315 (7109), 629–634. doi:10.1136/bmj.315.7109.629

Farmer, H., McCabe, N., Lord, C. J., Tutt, A. N., Johnson, D. A., Richardson, T. B., et al. (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434 (7035), 917–921. doi:10.1038/nature03445

Friedlander, M., Matulonis, U., Gourley, C., du Bois, A., Vergote, I., Rustin, G., et al. (2018). Long-term efficacy, tolerability and overall survival in patients with platinum-sensitive, recurrent high-grade serous ovarian cancer treated with maintenance olaparib capsules following response to chemotherapy. *Br. J. Cancer* 119 (9), 1075–1085. doi:10.1038/s41416-018-0271-y

Giannini, A., Di Dio, C., Di Donato, V., D'Oria, O., Salerno, M. G., Capalbo, G., et al. (2023). PARP inhibitors in newly diagnosed and recurrent ovarian cancer. *Am. J. Clin. Oncol.* 46 (9), 414–419. doi:10.1097/coc.0000000000001024

González-Martín, A., Pothuri, B., Vergote, I., DePont Christensen, R., Graybill, W., Mirza, M. R., et al. (2019). Niraparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 381 (25), 2391–2402. doi:10.1056/NEJMoa1910962

González-Martín, A., Pothuri, B., Vergote, I., Graybill, W., Lorusso, D., McCormick, C. C., et al. (2023). Progression-free survival and safety at 3.5years of follow-up: results from the randomised phase 3 PRIMA/ENGOT-OV26/GOG-3012 trial of niraparib maintenance treatment in patients with newly diagnosed ovarian cancer. *Eur. J. Cancer* 189, 112908. doi:10.1016/j.ejca.2023.04.024

Gulia, S., Kannan, S., and Gupta, S. (2022). Maintenance therapy with a poly (ADP-ribose) polymerase inhibitor in patients with newly diagnosed advanced epithelial ovarian cancer: updated individual patient data and trial-level meta-analysis. *ESMO Open* 7 (6), 100632. doi:10.1016/j.esmoop.2022.100632

Gupta, S., Rajappa, S., Advani, S., Agarwal, A., Aggarwal, S., Goswami, C., et al. (2021). Prevalence of BRCA1 and BRCA2 mutations among patients with ovarian, primary peritoneal, and fallopian tube cancer in India: a multicenter cross-sectional study. *JCO Glob. Oncol.* 7, 849–861. doi:10.1200/go.21.00051

Higgins, J. P., and Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21 (11), 1539–1558. doi:10.1002/sim.1186

Hirschl, N., Leveque, W., Granitto, J., Sammarco, V., Fontillas, M., and Penson, R. T. (2024). PARP inhibitors: strategic use and optimal management in ovarian cancer. *Cancers (Basel)* 16 (5), 932. doi:10.3390/cancers16050932

IntHout, J., Ioannidis, J. P., Rovers, M. M., and Goeman, J. J. (2016). Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open* 6 (7), e010247. doi:10.1136/bmjopen-2015-010247

Jadad, A. R., Moore, R. A., Carroll, D., Jenkinson, C., Reynolds, D. J., Gavaghan, D. J., et al. (1996). Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin. Trials* 17 (1), 1–12. doi:10.1016/0197-2456(95)00134-4

Katsumata, N., Yasuda, M., Isonishi, S., Takahashi, F., Michimae, H., Kimura, E., et al. (2013). Long-term results of dose-dense paclitaxel and carboplatin versus conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (JGOG 3016): a randomised, controlled, open-label trial. *Lancet Oncol.* 14 (10), 1020–1026. doi:10.1016/s1470-2045(13)70363-2

Lau, C. H., Seow, K. M., and Chen, K. H. (2022). The molecular mechanisms of actions, effects, and clinical implications of PARP inhibitors in epithelial ovarian cancers: a systematic review. *Int. J. Mol. Sci.* 23 (15), 8125. doi:10.3390/ijms23158125

Ledermann, J., Harter, P., Gourley, C., Friedlander, M., Vergote, I., Rustin, G., et al. (2012). Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N. Engl. J. Med.* 366 (15), 1382–1392. doi:10.1056/NEJMoa1105535

Ledermann, J., Harter, P., Gourley, C., Friedlander, M., Vergote, I., Rustin, G., et al. (2014). Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol.* 15 (8), 852–861. doi:10.1016/s1470-2045(14)70228-1

Ledermann, J. A., Oza, A. M., Lorusso, D., Aghajanian, C., Oaknin, A., Dean, A., et al. (2020). Rucaparib for patients with platinum-sensitive, recurrent ovarian carcinoma (ARIEL3): post-progression outcomes and updated safety results from a randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 21 (5), 710–722. doi:10.1016/s1470-2045(20)30061-9

Ledermann, J. A., Raja, F. A., Fotopoulou, C., Gonzalez-Martin, A., Colombo, N., Sessa, C., et al. (2013). Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 24 (Suppl. 6), vi24–32. doi:10.1093/annonc/mdt333

Lee, B., Chang, S. J., Kwon, B. S., Son, J. H., Lim, M. C., Kim, Y. H., et al. (2023). Impact of PARP inhibitor maintenance therapy in newly diagnosed advanced epithelial ovarian cancer: a meta-analysis. *PLoS One* 18 (11), e0294647. doi:10.1371/journal.pone.0294647

Lheureux, S., Gourley, C., Vergote, I., and Oza, A. M. (2019). Epithelial ovarian cancer. *Lancet* 393 (10177), 1240–1253. doi:10.1016/s0140-6736(18)32552-2

Li, N., Zhang, Y., Wang, J., Zhu, J., Wang, L., Wu, X., et al. (2022a). Fuzuloparib maintenance therapy in patients with platinum-sensitive, recurrent ovarian carcinoma (FZOCUS-2): a multicenter, randomized, double-blind, placebo-controlled, phase III trial. *J. Clin. Oncol.* 40 (22), 2436–2446. doi:10.1200/jco.21.01511

Li, N., Zhu, J., Yin, R., Wang, J., Pan, L., Kong, B., et al. (2022b). Efficacy and safety of niraparib as maintenance treatment in patients with newly diagnosed advanced ovarian cancer using an individualized starting dose (PRIME Study): a randomized, double-blind, placebo-controlled, phase 3 trial (LBA 5). *Gynecol. Oncol.* 166, S50–S51. doi:10.1016/S0090-8258(22)01298-7

Li, N., Zhu, J., Yin, R., Wang, J., Pan, L., Kong, B., et al. (2023). Treatment with niraparib maintenance therapy in patients with newly diagnosed advanced ovarian cancer: a phase 3 randomized clinical trial. *JAMA Oncol.* 9 (9), 1230–1237. doi:10.1001/jamaoncol.2023.2283

Lorusso, D., Guy, H., Samyshkin, Y., Hawkes, C., Estenson, K., and Coleman, R. L. (2022). Feasibility study of a network meta-analysis and unanchored population-adjusted indirect treatment comparison of niraparib, olaparib, and bevacizumab as maintenance therapies in patients with newly diagnosed advanced ovarian cancer. *Cancers (Basel)* 14 (5), 1285. doi:10.3390/cancers14051285

Maiorano, B. A., Maiorano, M. F. P., Lorusso, D., Di Maio, M., and Maiello, E. (2022). Efficacy and safety of PARP inhibitors in elderly patients with advanced ovarian cancer: a systematic review and meta-analysis. *Int. J. Gynecol. Cancer* 32 (11), 1410–1418. doi:10.1136/ijgc-2022-003614

Marchetti, C., De Felice, F., Perniola, G., Palaia, I., Musella, A., Di Donato, V., et al. (2019). Role of intraperitoneal chemotherapy in ovarian cancer in the platinum-taxane-based era: a meta-analysis. *Crit. Rev. Oncol. Hematol.* 136, 64–69. doi:10.1016/j.critrevonc.2019.01.002

Masvidal Hernandez, M., Cros Costa, S., Salvador Coloma, C., Quilez Cutillas, A., Barretina-Ginesta, M. P., and Cotes Sanchís, A. (2024). First-line PARP inhibitor maintenance treatment in ovarian carcinoma for older adult women: a review of the current literature. *Clin. Transl. Oncol.* doi:10.1007/s12094-024-03609-y

Matulonis, U. A., Oza, A. M., Ho, T. W., and Ledermann, J. A. (2015). Intermediate clinical endpoints: a bridge between progression-free survival and overall survival in ovarian cancer trials. *Cancer* 121 (11), 1737–1746. doi:10.1002/cncr.29082

Mengato, D., Cancanelli, L., Di Spazio, L., Rivano, M., Chiumente, M., and Messori, A. (2022). Indirect comparison of three PARP inhibitors (olaparib, niraparib, and rucaparib) as maintenance treatment in ovarian carcinoma patients responding to platinum therapy. *Int. J. Clin. Pharmacol. Ther.* 60 (8), 370–372. doi:10.5414/cp204243

Mirza, M. R., Monk, B. J., Herrstedt, J., Oza, A. M., Mahner, S., Redondo, A., et al. (2016). Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N. Engl. J. Med.* 375 (22), 2154–2164. doi:10.1056/NEJMoa1611310

Monk, B. J., Parkinson, C., Lim, M. C., O'Malley, D. M., Oaknin, A., Wilson, M. K., et al. (2022). A randomized, phase III trial to evaluate rucaparib monotherapy as maintenance treatment in patients with newly diagnosed ovarian cancer (ATHENA-MONO/GOG-3020/ENGOT-ov45). *J. Clin. Oncol.* 40 (34), 3952–3964. doi:10.1200/jco.22.01003

Moore, K., Colombo, N., Scambia, G., Kim, B. G., Oaknin, A., Friedlander, M., et al. (2018). Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 379 (26), 2495–2505. doi:10.1056/NEJMoa1810858

Musolino, A., Bella, M. A., Bortesi, B., Michiara, M., Naldi, N., Zanelli, P., et al. (2007). BRCA mutations, molecular markers, and clinical variables in early-onset breast cancer: a population-based study. *Breast* 16 (3), 280–292. doi:10.1016/j.breast.2006.12.003

Mustafa, M. T., Abushanab, A. K., Mousa, M. T., Qawaqzeh, R. A., Alakhras, H. M., Othman, A. S., et al. (2024). Safety and efficacy of Rucaparib in the treatment of ovarian cancer and patients with BRCA mutation: a systematic review and meta-analysis of phase III randomized clinical trials. *Expert Rev. Anticancer Ther.* 24 (1-2), 71–79. doi:10.1080/14737140.2024.2309177

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj* 372, n71. doi:10.1136/bmj.n71

Perez-Fidalgo, J. A., Gálvez-Montosa, F., Guerra, E. M., Madariaga, A., Manzano, A., Martin-Lorente, C., et al. (2024). SEOM-GEICO clinical guideline on epithelial ovarian cancer (2023). *Clin. Transl. Oncol.* doi:10.1007/s12094-024-03531-3

Perren, T. J., Swart, A. M., Pfisterer, J., Ledermann, J. A., Pujade-Lauraine, E., Kristensen, G., et al. (2011). A phase 3 trial of bevacizumab in ovarian cancer. *N. Engl. J. Med.* 365 (26), 2484–2496. doi:10.1056/NEJMoa1103799

Perrone, C., Angioli, R., Luvero, D., Giannini, A., Di Donato, V., Cuccu, I., et al. (2024). Targeting BRAF pathway in low-grade serous ovarian cancer. *J. Gynecol. Oncol.* 35 (4), e104. doi:10.3802/jgo.2024.35.e104

Poveda, A., Floquet, A., Ledermann, J. A., Asher, R., Penson, R. T., Oza, A. M., et al. (2021). Olaparib tablets as maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a final analysis of a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 22 (5), 620–631. doi:10.1016/s1470-2045(21)00073-5

Pujade-Lauraine, E., Ledermann, J. A., Selle, F., Gebski, V., Penson, R. T., Oza, A. M., et al. (2017). Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 18 (9), 1274–1284. doi:10.1016/s1470-2045(17)30469-2

Pujade-Lauraine, E., Selle, F., Scambia, G., Asselain, B., Marmé, F., Lindemann, K., et al. (2023). Maintenance olaparib rechallenge in patients with platinum-sensitive relapsed ovarian cancer previously treated with a PARP inhibitor (OReO/ENGOT-ov38): a phase IIIb trial. *Ann. Oncol.* 34 (12), 1152–1164. doi:10.1016/j.annonc.2023.09.3110

Purwar, R., Ranjan, R., Pal, M., Upadhyay, S. K., Kumar, T., and Pandey, M. (2023). Role of PARP inhibitors beyond BRCA mutation and platinum sensitivity in epithelial ovarian cancer: a meta-analysis of hazard ratios from randomized clinical trials. *World J. Surg. Oncol.* 21 (1), 157. doi:10.1186/s12957-023-03027-4

Ray-Coquard, I., Pautier, P., Pignata, S., Pérol, D., González-Martín, A., Berger, R., et al. (2019). Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N. Engl. J. Med.* 381 (25), 2416–2428. doi:10.1056/NEJMoa1911361

Ruscito, I., Bellati, F., Ray-Coquard, I., Mirza, M. R., du Bois, A., Gasparri, M. L., et al. (2020). Incorporating parp-inhibitors in primary and recurrent ovarian cancer: a meta-analysis of 12 phase II/III randomized controlled trials. *Cancer Treat. Rev.* 87, 102040. doi:10.1016/j.ctrv.2020.102040

Salani, R., Backes, F. J., Fung, M. F., Holschneider, C. H., Parker, L. P., Bristow, R. E., et al. (2011). Posttreatment surveillance and diagnosis of recurrence in women with gynecologic malignancies: society of Gynecologic Oncologists recommendations. *Am. J. Obstet. Gynecol.* 204 (6), 466–478. doi:10.1016/j.ajog.2011.03.008

Shao, F., Duan, Y., Zhao, Y., Li, Y., Liu, J., Zhang, C., et al. (2021). PARP inhibitors in breast and ovarian cancer with BRCA mutations: a meta-analysis of survival. *Aging (Albany NY)* 13 (6), 8975–8988. doi:10.18632/aging.202724

Tierney, J. F., Stewart, L. A., Ghersi, D., Burdett, S., and Sydes, M. R. (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 8, 16. doi:10.1186/1745-6215-8-16

Torre, L. A., Trabert, B., DeSantis, C. E., Miller, K. D., Samimi, G., Runowicz, C. D., et al. (2018). Ovarian cancer statistics, 2018. *CA Cancer J. Clin.* 68 (4), 284–296. doi:10.3322/caac.21456

Walsh, C. S. (2015). Two decades beyond BRCA1/2: homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. *Gynecol. Oncol.* 137 (2), 343–350. doi:10.1016/j.ygyno.2015.02.017

Wang, H., Wu, M., Liu, H., Zhou, H., Zhao, Y., Geng, Y., et al. (2021). Comparison of the efficacy and safety of PARP inhibitors as a monotherapy for platinum-sensitive recurrent ovarian cancer: a network meta-analysis. *Front. Oncol.* 11, 785102. doi:10.3389/fonc.2021.785102

Wang, Y., Ren, F., Song, Z., Wang, X., Zhang, C., and Ouyang, L. (2020). PARP inhibitors in patients with newly diagnosed advanced ovarian cancer: a meta-analysis of randomized clinical trials. *Front. Oncol.* 10, 1204. doi:10.3389/fonc.2020.01204

Wetterslev, J., Jakobsen, J. C., and Gluud, C. (2017). Trial Sequential Analysis in systematic reviews with meta-analysis. *BMC Med. Res. Methodol.* 17 (1), 39. doi:10.1186/s12874-017-0315-7

Wu, L., Zhu, J., Yin, R., Wu, X., Lou, G., Wang, J., et al. (2021a). Olaparib maintenance therapy in patients with newly diagnosed advanced ovarian cancer and a BRCA1 and/or BRCA2 mutation: SOLO1 China cohort. *Gynecol. Oncol.* 160 (1), 175–181. doi:10.1016/j.ygyno.2020.10.005

Wu, X., Liu, J., Wang, J., Wang, L., Lin, Z., Wang, X., et al. (2024a). Senaparib as first-line maintenance therapy in advanced ovarian cancer: a randomized phase 3 trial. *Nat. Med.* 30 (6), 1612–1621. doi:10.1038/s41591-024-03003-9

Wu, X., Zhu, J., Yin, R., Yang, J., Liu, J., Wang, J., et al. (2024b). Niraparib maintenance therapy using an individualised starting dose in patients with platinum-sensitive recurrent ovarian cancer (NORA): final overall survival analysis of a phase 3 randomised, placebo-controlled trial. *EClinicalMedicine* 72, 102629. doi:10.1016/j.eclinm.2024.102629

Wu, X. H., Zhu, J. Q., Yin, R. T., Yang, J. X., Liu, J. H., Wang, J., et al. (2021b). Niraparib maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer using an individualized starting dose (NORA): a randomized, double-blind, placebo-controlled phase III trial(☆). *Ann. Oncol.* 32 (4), 512–521. doi:10.1016/j.annonc.2020.12.018

Xie, M., Zhong, Y., Yang, Y., Shen, F., and Nie, Y. (2022). Extended adjuvant endocrine therapy for women with hormone receptor-positive early breast cancer: a meta-analysis with trial sequential analysis of randomized controlled trials. *Front. Oncol.* 12, 1039320. doi:10.3389/fonc.2022.1039320

Xu, Y., Ding, L., Tian, Y., Bi, M., Han, N., and Wang, L. (2020). Comparative efficacy and safety of PARP inhibitors as maintenance therapy in platinum sensitive recurrent ovarian cancer: a network meta-analysis. *Front. Oncol.* 10, 573801. doi:10.3389/fonc.2020.573801

Zhou, S., Jiang, Y., Luo, C., and Yuan, L. (2024). Comparison of poly (ADP-ribose) polymerase inhibitors (PARPis) as maintenance therapy for newly-diagnosed and platinum-sensitive recurrent ovarian cancer with BRCA mutational status: a systematic review and network meta-analysis. *Expert Rev. Anticancer Ther.* 24 (1-2), 59–69. doi:10.1080/14737140.2023.2298832

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Emerging strategies to overcome ovarian cancer: advances in immunotherapy

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Ovarian cancer is the second most common malignant neoplasm of gynecological origin and the leading cause of death from cancer in the female reproductive system worldwide. This scenario is largely due to late diagnoses, often in advanced stages, and the development of chemoresistance by cancer cells. These challenges highlight the need for alternative treatments, with immunotherapy being a promising option. Cancer immunotherapy involves triggering an anti-tumor immune response and developing immunological memory to eliminate malignant cells, prevent recurrence, and inhibit metastasis. Some ongoing research investigate potentially immunological advancements in the field of cancer vaccines, immune checkpoint blockade, CAR-T cell, and other strategies.

KEYWORDS

ovarian cancer, immunotherapy, cancer vaccines, CAR-T cell therapy, antibody therapy

1 Introduction to ovarian cancer immunotherapy

Ovarian cancer (OC) ranks first among deaths caused by gynecological malignant neoplasms around the world (American Cancer Society, 2024). OC's dramatic epidemiological scenario is related to diagnoses in advanced stages of the disease, due to the absence of pathognomonic signs and symptoms for early diagnosis (Doubeni et al., 2016), coupled with the first-choice therapeutic regimens chemoresistance acquisition by OC cells (Ghoneum et al., 2021). These conditions require other ways to treat these patients, other than surgeries and non-specific conventional chemotherapy. In consequence, different immunotherapy approaches have arisen as relevant alternatives to overcome this treatment obstacle (Bund et al., 2022).

OC immunotherapy involves the induction of an anti-tumor immune response and the development of immunological memory. This process not only can eradicate malignant cells within the primary tumor site, thereby averting recurrence, but also hampers the metastatic spread to distant anatomical locations (Cha et al., 2020). Presently, the Food and Drugs Administration (FDA) has sanctioned some distinct immunotherapeutic modalities for OC or is actively investigating them in clinical trials (Cha et al., 2020). These approaches can be categorized into active and passive immunotherapies.

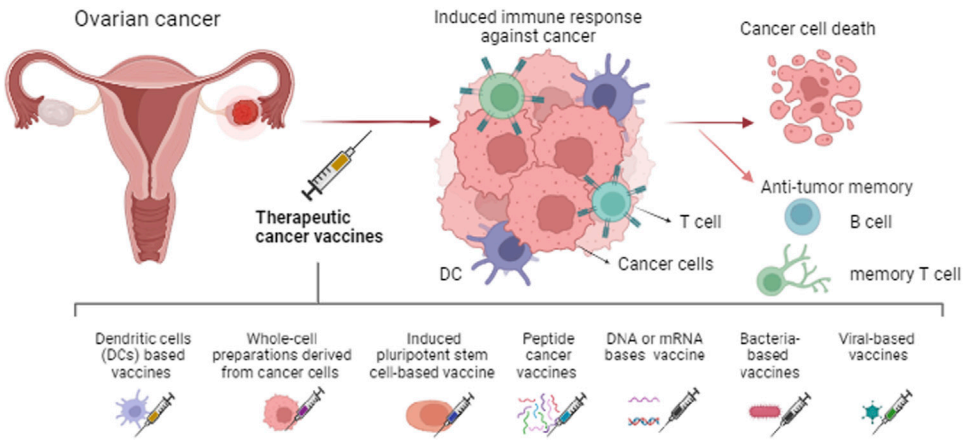

FIGURE 1
Types of therapeutic cancer vaccines and the main mechanism induced. The immune system response is generated against the tumor leading to cancer cell death and immunological memory.

Active immunotherapy harnesses the immune system to identify and target specific cancer antigens. It includes vaccines that stimulate the patient's immune response, or chimeric antigen receptor (CAR) T-cell therapy, which involves the reintroduction of genetically engineered T-cells in the patient (Rui et al., 2023). On the other hand, passive immunotherapy modulates the activity of a patient's immune system response, as observed with immune checkpoint inhibitors (ICIs) molecules (Rui et al., 2023). In this review, we compile the latest findings concerning OC immunotherapy strategies.

2 Therapeutic OC vaccines

To handle the adverse effects of common therapies for cancer, immunotherapy strategies emerged as a cancer-specific alternative capable of targeting the tumor and causing minimal impact on normal tissues (Aly, 2012; Zhu and Yu, 2022). They are significant considering the usual therapeutic approaches such as surgery, chemotherapy, and radiotherapy which besides the adverse effects show a lack of specificity for tumors (Kaczmarek et al., 2023). Therapeutic cancer vaccination is a strategy of immunotherapy developed to elicit or boost antitumor adaptive immune responses to detect and eliminate them (Luo, et al., 2024; Chambers, 2011). This response is specifically direct against malignant cells leading to the inhibition of tumor growth and/or recurrence (Siminiak et al., 2022). Cancer vaccines use diverse mechanisms to provoke the immune system and develop a specific anti-tumor response (Shafabakhsh et al., 2019; American Cancer Society, 2020) and immunological memory that may prevent recurrences (Janes et al., 2024).

OC, which is a challenging disease to diagnose and treat, usually shows resistance to available chemotherapies and frequently relapses with more aggressiveness (Acharya et al., 2024). The clinical characteristics demonstrate the importance of developing novel therapeutic strategies to treat and overcome chemoresistance in OC. In this scenario, different cancer vaccines have been studied in OC. The main mechanisms of cancer vaccines involve the induction of dendritic cells (DCs) potent antigen-presenting cells (APCs), these cells identify and present the antigen for other cells using major histocompatibility complex (MHC) molecules (Lin M. et al., 2022). Also secrete IL-10, IL-12, IL-23, and TNF-β to stimulate the differentiation of immune system cells (Zhang X. et al., 2021). $CD8^+$ cytotoxic T lymphocytes (CTLs) recognize the antigens presented on MHC class I molecules, leading to their activation and proliferation, consequently, attacking and destroying the tumor (Kaczmarek et al., 2023). $CD4^+$ helper T cells recognize peptides presented on MHC class II molecules and provide support to other immune cells. B cells can also be activated resulting in the production of antibodies specific to the tumor-associated antigens (TAAs) (Janes et al., 2024). These antibodies can directly bind to tumor cells, aiding in their destruction. The vaccine also aims to induce a memory response, which enhances immune protection and provides a more robust response upon future encounters with tumor cells expressing the same TAAs (Fan et al., 2023), see Figure 1.

DCs based vaccines depend on *ex vivo* modification of DCs from the patient or cells created in the laboratory. Immune-stimulating agents or tumor-specific antigens (TSAs) obtained from tumor cells or genetic material are applied to mature and activate these cells followed by reinfusion into the patient. Once reinfused, these cells interact with T cells, B cells, and natural killer (NK) cells (Lv et al., 2020; Fan et al., 2023). The activation of immune system cells, as mentioned above, enhances the immune response and destroys cancer cells (Laureano et al., 2022). The use of this kind of vaccine has shown relevant results, for example, a study using an autologous dendritic cell-based vaccine with tumor lysate after systemic chemotherapy resulted in a decrease in progression rate, as well as improved overall survival in OC (Zhang X. et al., 2021). A Th17-inducing folate receptor alpha (FRα)-loaded DCs vaccine, resulted in the development of Th1, Th17, and antibody responses to FRα in most patients. These processes are associated with prolonged recurrence-free survival and induce antigen-specific immunity (Block et al., 2020). Another approach combined a whole tumor lysate-pulsed dendritic cell vaccine with bevacizumab, cyclophosphamide, aspirin, and interleukin-2, this

vaccine produced T-cell responses and was associated with increased overall survival of patients (Tanyi et al., 2021).

A similar mechanism is induced by the whole-cell preparations or lysates derived from cancer cells reintroduced into the patient (Chiang et al., 2011). Cells are sourced from the patient's tumor or established cancer cell lines, aiming to prevent their growth and pathogenicity the cells are inactivated or genetically modified (Kaczmarek et al., 2023; Pérez-Baños et al., 2023). Another approach utilizes induced pluripotent stem cell (iPSC)-based cancer vaccines. iPSCs are created from somatic cells and then differentiated into tumor microenvironment (TME)-specific cells, such as tumor-associated fibroblasts, endothelial cells, or immune cells (Chehelgerdi et al., 2023). These iPSC-derived cells express antigens characteristic of the TME, including TSAs or molecules associated with immunosuppression. When administered to the patient, these cells are recognized by immune cells, triggering a robust immune response (Ouyang et al., 2019). Zhang Z. et al. (2012) used human embryonic stem cells as a OC prevention vaccine in rats, this vaccine caused anti-tumor responses and enhanced tumor rejection in the animal models.

Peptide cancer vaccines are also an emerging treatment for OC, using specific epitope peptides derived from TAAs or TSAs (Abd-Aziz and Poh, 2022). This vaccine can stimulate the immune system after being administered and taked up by APCs (Wada et al., 2016; Liu et al., 2024a). Recent studies in phase I or II use mutated p53 peptides (The cancer-testis antigen, named New York esophageal squamous cell carcinoma-1, NY-ESO-1) and also apply diverse technologies to treat OC in association with co-therapies (Odunsi, 2017; Siminiak et al., 2022). Vaccines made from a peptide or antigen may help the body build an effective immune response to kill tumor cells, functioning as a booster for the patient's anti-tumor immune response and the combination with chemotherapy may induce the death of more tumor cells (Bund et al., 2022; Odunsi, 2017).

In a phase I/IIa trial (Brown et al., 2019) used E39 in patients HLA-A2+, this is an immunogenic peptide derived from the folate-binding protein, frequently found overexpressed in multiple malignancies. When associated with granulocyte macrophage-colony stimulating factor (GM-CSF) was able to improve disease-free survival (DFS) of endometrial cancer and OC patients (90.0% vs. Control Group: 42.9%). Targeting folate receptor (FR) a vaccine was tested in patients with OC or breast cancer. The vaccine stimulated or increased immunity in more than 90% of patients and the FR T cell responses were detectable for at least 12 months. The results demonstrate the benefits of boosting immunity to tumors expressing FR antigen (Kalli et al., 2018). O'Cearbhaill et al. (2019) combined a polyvalent vaccine conjugate responsible for inducing antibody responses (Globo-H, GM2, MUC1-TN, TF) with adjuvant OPT-821 in patients with OC in remission after chemotherapy. Vaccine + OPT-821 compared to OPT-821 alone was modestly more immunogenic.

Cancer vaccines can also involve genetic material (DNA and RNA) encoding TAAs. This DNA or RNA is taken up by cells, such as DCs, and the TAAs are presented on the surface of APCs after being processed. In this process, the activation and proliferation of $CD8^+$ CTLs are induced and $CD4^+$ helper T cells provide support to other immune cells (Pandya et al., 2023). Additionally, B cells can be activated by presented TAAs and induce the production of antibodies. These antibodies can bind directly to tumor cells, aiding in their destruction (Barbier et al., 2022). The vaccine also aims to induce a memory response, which enhances immune protection and ensures a more effective response upon future encounters with tumor cells expressing the same TAAs (Wang B. et al., 2023a). Lu et al. (2023) using immuno-bioinformatics developed a model of a multi-epitope mRNA self-adjuvant vaccine targeting CA-125 neoantigen in breast and ovarian cancers. This *in silico* analysis provided evidence of using this neoantigen in a mRNA-based vaccine. Posity results were observed using the SynCon FSHR DNA vaccine. In this study synthetic consensus (SynCon) approach was capable of breaking immune tolerance to follicle-stimulating hormone receptor (FSHR). The treatment induced robust $CD8^+$ and $CD4^+$ cellular immune responses and FSHR-redirected antibodies in mice, as well, delayed the progression of aggressive OC model with peritoneal carcinomatosis (Perales-Puchalt et al., 2019).

Neoantigen DNA vaccines were used by Bhojnagarwala et al. (2021) to target ~40 neoantigens. These plasmid-based vaccines were able to provoke long-term immune responses against lung and ovarian cancer and protected animals from tumor growth for 89 days after the final vaccination. Another DNA vaccine platform targeting tumor neoantigens was applied against lung and ovarian cancers affecting the tumor progression and survival in mouse models. In this pre-clinical study, the vaccine was able to generate potent $CD8^+$ T-cell antitumor–specific responses *in vivo*. Interestingly, when neoantigen-specific T cells were expanded from immunized mice they were also able to kill tumor cells *ex vivo* (Duperret et al., 2019).

Bacteria-based cancer vaccines use engineered bacteria to stimulate the immune system (Zhou et al., 2023). These modified bacteria interact with immune cells, initiating an inflammatory response and triggering the production of pro-inflammatory cytokines, chemokines, and other signaling molecules (Zalatan et al., 2024). Viral-based cancer vaccines use engineered viruses to stimulate the immune response directly. These modified viruses interact with immune cells such as DCs, macrophages, and NK cells, triggering an inflammatory response along with the release of pro-inflammatory cytokines and chemokines (Xu et al., 2024). Immune cells then phagocytose the virus particles, and TAAs expressed by the virus or introduced into infected cells are processed and presented to T cells (Muthukutty and Yoo, 2023). Cowpea mosaic virus co-delivered with irradiated OC cells comprises an prophylactic vaccine against a model of OC in mice. After two vaccinations most of the mice (72%) reject the tumor challenges, and survived subsequent rechallenges, indicating immunologic memory (Stump et al., 2021).

These approaches highlight the diverse strategies being employed to develop effective vaccines for OC, with ongoing research focused on optimizing these therapies and evaluating their clinical efficacy. The actual scenario for cancer vaccines is due to years of research and discoveries. Nevertheless, the heterogeneity of the immune system and the capacity of cancer cells to evade immune system attacks, even when naturally endogenous or when induced by vaccine makes this process a challenge. This is why more in-depth studies must be completed to enable the large use of these therapies.

TABLE 1 Ongoing studies evaluating CAR technology in OC and other tumors treatments. Clinical trials that have recently started using CAR cell technology in OC are currently in "recruiting" status. Some CAR cells have undergone modifications to become more specific or to avoid some side effects, such as CRS.

CAR technology	Modification	Target	Clinical study phase	References
CAR T cell - iC9-CAR.B7-H3	Presence of an inducible suicide gene, caspase 9 (iC9). CAR T cells are eliminated in a severe CRS event	B7-H3 Immune checkpoint most expressed in tumors, associated with poor prognosis	I	NCT06305299 Miyamoto et al. (2022)
CAR T cell - 27T51	Presence of an anti-MUC16 site	MUC-16 Antigen commonly expressed in OC. Increased efficacy *in vivo*	Ia/Ib	NCT06469281 Chekmasova et al. (2010)
CAR T cell	CAR T cells specific for Cluster of differentiation 70 (CD70)	CD70 Glycoprotein related to chemoresistance in OC.	I	NCT06215950 NCT06383507 NCT06010875 Aggarwal et al. (2009)
CAR T cell Tmod™	Activation in presence of MSLN. Addition of HLA-A*02 inhibitor	Tumors that express second-generation MSLN and have lost HLA-A*02 expression. Associated to poor prognosis	I and II	NCT06051695 Andersson et al. (2012) Tokatlian et al. (2022)
CAR-iNK cell (FT536)	Affinity for MICA and MICB. IL-15 expression (improves the complex performance)	MICA and MICB (overexpressed in OC)	I	NCT06342986 Li K. et al. (2009) Lee D. et al. (2023)
CAR-iNK cell	Umbilical cord blood-derived NK cells transduced with IL-15 and engineered with CAR TROP2	TROP2 Overexpressed protein and associated with proliferation and invasion in OC.	I and II	NCT05922930 Wu et al. (2017)
CAR-iNK cell - SynKIR-110	Presence of a killer cell immunoglobulin-like receptor (KIR)	MSLN. Glycoprotein commonly overexpressed in OC and associated with tumor progression	I	NCT 05568680 NCT06256055 Liang et al. (2021) Hilliard (2018)

3 CAR-T cell therapy in OC

CAR-T cells are genetically engineered to recognize and attack TSAs (June et al., 2018), bypassing the need of MHC molecules presentation, and behaving as active drugs against tumors (Maus and June, 2016). FDA approved CAR-T therapy in 2017 (reviewed by Yi-Ju et al., 2023), with two treatments, Yescarta (axicabtagene ciloleucel) and Kymriah (tisagenlecleucel), specifically for certain lymphomas and leukemia (Food and Drug Administration, 2024). Despite its clinical success in treating blood cancers, CAR-T therapy can lead to serious complications (reviewed by Brudno and Kochenderfer, 2024). These include cytokine release syndrome (CRS), which can cause extreme symptoms like high fevers, organ failure, and even death (Reagan and Neelapu, 2021). Another risk is "on-target, off-tumor toxicity," where CAR-T cells attack healthy tissues, causing severe harm (Flugel et al., 2023). Additionally, the required lymphodepleting chemotherapy before CAR-T infusion is genotoxic, raising the risk of secondary cancers and other diseases (Yeh et al., 2020). Since then, extensive global research has been conducted on various hematologic and solid tumors to evaluate the safety and efficacy of CAR-T therapy and it has shown significant success in treating hematologic cancers, with six other FDA approvals, and holds promise as a new treatment option for OC (reviewed by Cappell and Kochenderfer, 2023).

Solid tumors present significant challenges for CAR-T cell therapy due to their heterogeneity and the scarcity of known tumor-specific epitopes (Labanieh and Mackall, 2023). Unlike hematological malignancies, solid tumors often result in toxicity when targeting overexpressed antigens (reviewed by Baker et al., 2023). Additionally, the TME creates physical and immunological barriers that limit CAR-T cell effectiveness (reviewed by Albelda, 2024). To overcome these obstacles, researchers are exploring intratumoral injections (Tchou et al., 2017), peptide and nanoparticle booster vaccines (MA et al., 2019; Reinhard et al., 2020), engineered cytokine-driven expansion (Sockolosky et al., 2018), and modifying the TME with oncolytic viruses and genome editing techniques like CRISPR-Cas9 (reviewed by Baker et al., 2023).

Emerging clinical data show promise for CAR-T cells targeting solid tumors, including prostate cancer (prostate-specific membrane antigen) (Narayan et al., 2022), gastrointestinal cancer (CLDN18.2) (Qi et al., 2022), glioblastoma (IL13RA2 or EGFRv3) (Sampson et al., 2020), and neuroblastoma (GD2) (Del Bufalo et al., 2023). Despite these advances, challenges persist due to the scarcity of unique, tumor-specific targets (Macpherson et al., 2020). In OC, potential targets identified include mesothelin (MSLN) (Schuster et al., 2017), Muc16 (Coelho et al., 2018), TAG72 (Murad et al., 2018), FR (Rodriguez-Garcia et al., 2017), and FSHR (Perales-Puchalt et al., 2017). Furthermore, recent studies have explored the feasibility, safety, and anti-tumor activity of the first-in-human approach of targeting CLDN6 with CAR-T therapy and combining it with a CAR-amplifying vaccine (Mackensen et al., 2023), given the frequent detection of high-level CLDN6 in epithelial OC, endometrial carcinoma, and other solid tumors (Jaeger et al., 2014). Hence, CAR technology using NK cells is being studied for a range of solid tumors, as well as OC (reviewed by Dagher and

Posey, 2023). Table 1 highlights some studies that evaluate CAR technology use in OC and other cancer types.

4 Exosomes in OC treatment

Exosomes represent a promising tool and target for immunotherapy in OC (Zhou W. et al., 2021). Although they are physiological components, their role in cancer remains somewhat ambiguous. In the context of immunotherapy, these lipophilic vesicles are crucial for facilitating communication among immune system cells, which can either elicit positive immune responses or lead to immunosuppression (Kugeratski and Raghu, 2021). Taylor et al. (2003) demonstrated that membrane fragments, which include exosomes and other lipid vesicles, derived from OC cells can induce T cell apoptosis. The influence of exosomes and similar membrane fragments on orchestrating immune system responses has been explored in various cancer types, including breast (Morrissey et al., 2021), lung (Alipoor et al., 2018), pancreatic (Shen et al., 2020), glioma (Li M. et al., 2022), and colorectal cancer (Zhao S. et al., 2020). Consequently, several key aspects regarding the role of exosomes in immunotherapy will be discussed below.

Exosomes are a category of extracellular vesicles with a lipid bilayer, measuring approximately 30–150 nm, found in various body fluids such as blood, urine, saliva, and cerebrospinal fluid (He et al., 2018; Gong et al., 2023). They are believed to have a dual role in the TME (Li X. and Wang, 2017). Exosomes can both promote and inhibit tumors and carry many potential biomarkers for OC (Gong et al., 2023). In normal cells, these small vesicles can interact with membrane receptors or fuse with cells to release components such as proteins, RNA, DNA, mRNA, miRNA, long non-coding RNA (lncRNA), and lipids, aiding in cellular communication, extracellular matrix maintenance, and immune system modulation (Pegtel and Gould, 2019; Kaushik and Cuervo, 2015; Ramirez and Marcilla, 2021; Zhu et al., 2024; Tian et al., 2022). In cancer cells, exosomes perform similar functions but carry components that promote proliferation, migration, invasion, chemoresistance, and other processes that enhance malignancy, complicating treatment, such as modulation of the TME (Bhattacharya et al., 2024; Yim et al., 2020; Li X. et al., 2021b).

In OC, exosomes play a dual role in the acquisition of chemoresistance, a process caused by the lack of cancer cells response to chemotherapy, often resulting in treatment failure (Tian et al., 2022; Liu H. et al., 2024b; Carmi et al., 2024). In this context, their malignant role in OC was elucidated by Pan et al. (2024). Their study found that exosomes derived from OC stem cells were responsible for increasing chemoresistance and proliferation while inhibiting apoptosis in the cisplatin-resistant SKOV3 cell line. Meanwhile, exosomes derived from ascites were observed to carry a lncRNA that sensitized high-grade serous ovarian cancer (HGSOC) cells to cisplatin chemotherapy, a standard drug for this OC subtype. Additionally, it was demonstrated that exosomes carried a lncRNA that reduced cell proliferation, migration, and invasion in both *in vitro* and *in vivo* experiments (Liu H. et al., 2024b).

Another factor complicating chemotherapy treatment is the low oxygenation within tumors, resulting from reduced blood perfusion. Wang Q. et al. (2024) analyzed this process and observed that tumor-derived exosomes contributed, in part, to the decreased oxygenation through the previously mentioned mechanism, by altering the tumor vascular network and thereby impeding chemotherapy.

In addition to their ambiguous role, exosomes may serve as a potential tool for OC therapy, as demonstrated in the study by Shimizu et al. (2024). In this study, exosomes were extracted from a cell culture of fibroblasts from OC patients and were loaded with siRNAs targeting a proto-oncogene, the MET receptor. This treatment inhibited OC cells proliferation, migration, and invasion. Another study showed that it is possible to create targeted exosomes for OC treatment (Mousaei Ghasroldasht et al., 2024). Mousaei Ghasroldasht et al. (2024) developed what they termed “enhanced exosomes” using a culture of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs), observing that these exosomes contained proteins and miRNAs capable of regulating and sensitizing OC. Another study, by Kim et al. (2023), used a nanotechnology-modified exosome in glioma to evaluate its effectiveness. The results indicate that there was regulation of the TME and decreased tumor progression both *in vitro* and *in vivo*. Furthermore, exosomes can be utilized as biomarkers for an improved and earlier diagnosis, addressing the delays often seen in most cases (Bhavsar et al., 2024; Zhu et al., 2024; Xiao et al., 2022). There is also evidence that these vesicles carry RNAs related to chemoresistance and, therefore, may serve as biomarkers for this process, which precedes clinical interventions (Asare-Werehene et al., 2020; Li T. et al., 2021a). These findings suggest that exosomes have intriguing therapeutic potential warranting further investigation. Therefore, deepening studies in this area is crucial to better understand the contribution of these components in OC immunotherapy and the underlying mechanisms of different kinds of exosomes and how they influence on tumor response to treatment (Figure 2).

5 Antibody-based therapies for OC treatment

Therapeutic monoclonal antibodies have been successfully developed for the treatment of various cancer types (Hafeez et al., 2020).

In this context, with the biotechnology advancement, antibody-drug conjugates (ADCs) have been developed, representing one of the newest classes of cancer medications, with approvals for the treatment of solid tumors as well as hematological malignancies. ADCs exhibit high selectivity for tumors, thereby minimizing their systemic exposure, which potentially leads to an improved therapeutic index, offering greater efficacy and fewer side effects (Dean et al., 2021). To minimize off-target toxicity, the target antigen should be exclusively or preferentially expressed in cancer cells, with minimal expression in healthy tissues (Hafeez et al., 2020). Several monoclonal antibody-based immunotherapies have already been approved by FDA (Zhou et al., 2023). However, numerous clinical trials are still underway with promising prospects for the treatment of OC, including ADCs such as JNJ-78306358, ivonescimab, ipilimumab, durvalumab, oregovomab, catumaxomab, abagovomab, daclizumab and

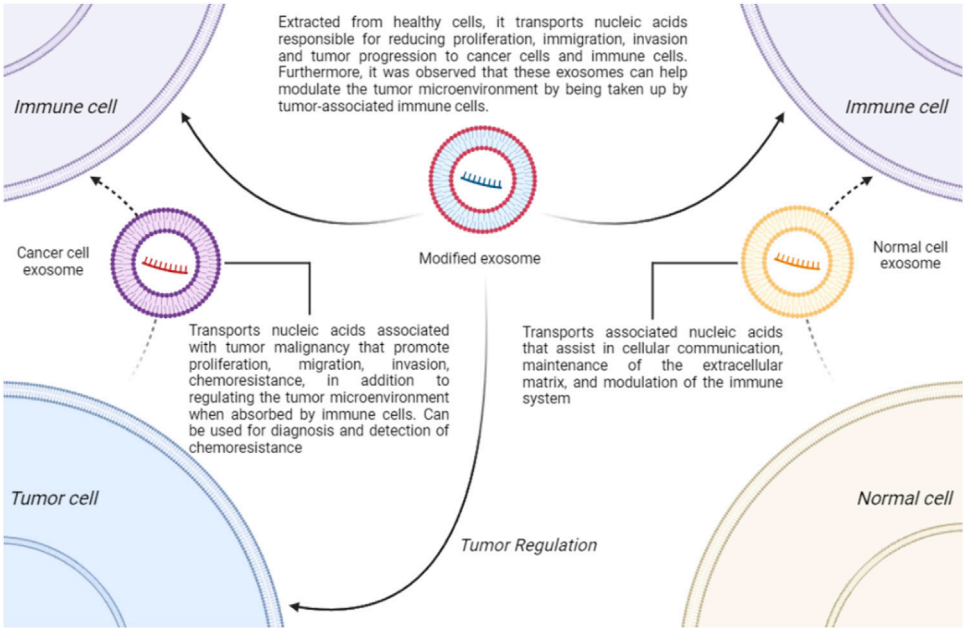

FIGURE 2
The Role of Exosomes. Exosomes play a physiological role in cellular communication, immune system modulation, and maintenance of the extracellular matrix. In OC, they are associated with tumor progression, proliferation, migration, invasion, and regulation of TME. Tumor cell-derived exosomes can serve as biomarkers for diagnosis and early detection of chemoresistance. Additionally, modified exosomes, such as those derived from hUC-MSCs or engineered using nanotechnology, may aid in treatment by reducing tumor progression and potentially modulating the TME.

mirvetuximab, which was approved by the FDA in 2022 but remains under study for application in OC treatment (Dilawari et al., 2023).

5.1 Immune checkpoint inhibitors (ICIs)

Cancer cells develop several complex mechanisms to evade the immune system in the TME, among which the inhibition of T cell activity by the PD-1/PD-L1 (Daud et al., 2016) and CTLA-4/B7 pathways can be highlighted (Tang Q. et al., 2022a). PD-1 is an immune receptor expressed on the surface of various immune cells, and the interaction between PD-1 and PD-L1, which is highly expressed on the surface of tumor cells and tumor-infiltrating cells, results in the inhibition of T cell activity, rendering the anti-tumor immune response ineffective and favoring immune evasion (Daud et al., 2016; Naimi et al., 2022; Tang Q. et al., 2022a). Furthermore, the binding of PD-1 to its ligand can inhibit T cell proliferation, B lymphocyte differentiation, and the production of cytokines such as Interferon-gamma (IFN-Y) (Tang S. et al., 2022b).

On the other hand, another immune checkpoint associated with tumor cell evasion is CTLA-4, an inhibitory receptor belonging to the immunoglobulin superfamily (Van Coillie et al., 2020). CTLA-4 is primarily expressed on activated T cells and, like PD-1, has an immunomodulatory function (Tang S. et al., 2022b). The interaction of CTLA-4 with its ligands, B7-1 (CD80) and B7-2 (CD86), expressed on APCs and tumor cells, transmits a signal that negatively regulates or interrupts T cell activity, thereby decreasing the immune response against cancer cells (Naimi et al., 2022; Tang S. et al., 2022b; Van Coillie et al., 2020).

From this perspective, ICIs represent a promising class of drugs in immunotherapy against OC, targeting PD-1/PD-L1 and CTLA-4. They have already demonstrated broad bioactivity and stable response in the treatment of various types of tumors (Naimi et al., 2022; Tang S. et al., 2022b), including OC (Disis et al., 2019).

5.1.1 PD-1/PD-L1 inhibitors

Recent studies conducted by Friedman et al. (2024) involving 35 patients demonstrated that the use of nivolumab, a PD-1 inhibitory monoclonal antibody, in the treatment of uterine cancer and OC with DNA mismatch repair deficiency (dMMR) showed clinical efficacy with an objective response rate (ORR) of 57%. Additionally, 64.7% of patients experienced progression-free survival (PFS) at 24 weeks, and treatment toxicity was moderate. However, while the results are promising, further studies with a larger cohort representing the population of patients with OC-dMMR are necessary, as well as the identification of additional predictive biomarkers for treatment response and resistance.

On the other hand, another notable ICI is ivonescimab, also known as AK112 and SMT112. It is a humanized bispecific antibody whose single-chain variable fragments (ScFv) bind to the C-terminus of each anti-VEGF antibody heavy chain (Wang L. et al., 2023b), forming a complex with high affinity for PD-1 (Zhao et al., 2023). Ivonescimab is currently being evaluated in clinical studies for its anti-PD-1 and anti-VEGF-A activities, with the goal of preventing tumor progression through the inhibition of angiogenesis (Apte et al., 2019). The anticipated outcomes of this inhibition include reduced immunosuppression and decreased tumor angiogenesis (Dhillon, 2024). However, clinical trials have encountered challenges in achieving satisfactory results.

In the phase Ia study by Frentzas et al. (2024), the activity of ivonescimab was evaluated in 19 patients with platinum-resistant OC. Among these patients, 68.4% had received more than three lines of prior therapy. Of the 19 patients, five achieved a partial response, including 3 with high-grade serous pathology and 2 with clear cell pathology, resulting in an ORR of 26.3%. Additionally, the study observed that the disease remained stable for more than 12 months in four patients who had previously been treated with bevacizumab. However, further clinical studies are needed to determine more appropriate dosages and to conduct additional analyses in combination therapies.

5.1.2 CTLA-4 inhibitors

One of the promising antibody-drugs in this class is ipilimumab, a monoclonal antibody targeting CTLA-4 (Saad and Kasi, 2023). Knisely et al. (2024) conducted a phase Ib study evaluating intraperitoneal ipilimumab and nivolumab in patients with recurrent gynecological neoplasms with peritoneal carcinomatosis. The study included 23 patients: 18 with OC, 2 with uterine cancer, and 3 with cervical cancer. In this study, a partial response was observed in two patients (8.7%), one with OC and one with uterine cancer, with a response duration of 14.8 months. Additionally, the treatment safety was assessed, revealing that two patients (8.7%) experienced adverse effects classified as grade 3 or higher. Despite these adverse effects, the study found that treatment with ipilimumab and nivolumab can produce lasting responses in the treatment of OC.

5.1.3 Combined therapies

Hinchcliff et al. (2024) conducted a phase II randomized clinical trial comparing durvalumab (PD-L1 inhibitory monoclonal antibody) and tremelimumab (anti-CTLA-4 antibody) administered either as a combination therapy or sequentially in patients with platinum-resistant OC. Among the patients, 38 received sequential therapy (tremelimumab followed by durvalumab), while 23 received combination therapy (tremelimumab and durvalumab together, followed by durvalumab alone). There was no significant difference in PFS between the combination therapy group (1.84 months) and the sequential therapy group (1.87 months) (p = 0.402). Partial responses were observed in two patients (8.7%) and stable disease in 1 patient (4.4%), with all responses occurring in the combination therapy group.

Landry et al. (2023) reported promising results from a phase Ib study investigating the combination of durvalumab with eribulin, a microtubule inhibitor with established benefits in metastatic breast cancer (MBC). The study included four patients with recurrent OC and five patients with HER2-negative MBC, all of whom received escalating doses of eribulin along with durvalumab. The results indicated an ORR of 55%, with four patients experiencing stable disease, and a PFS of 6.2 months.

On the other hand, Konstantinopoulos et al. (2019) demonstrated that the combination of niraparib, a PARP inhibitor (PARPi), with pembrolizumab (anti-PD-1 antibody) showed promising activity in the treatment of platinum-resistant recurrent OC patients. This combination resulted in reduced tumor size and observed disease stabilization. Furthermore, the study indicated that the combination enhanced treatment efficacy, achieving an ORR of 19%, compared to monotherapy with each agent. No new signs of toxicity were reported in this study. Hence, those studies suggest that the combination between ICIs with other drug classes may offer a viable alternative for improved treatment outcomes.

5.2 Antibody therapies using ADCs

5.2.1 JNJ-78306358

It is well established that human leukocyte antigen G (HLA-G) is minimally expressed in healthy cells but highly expressed in various types of human cancer cells (Lin A. and Yan, 2018), including OC. HLA-G functions as an immune checkpoint and interacts with inhibitory receptors (Geva et al., 2024).

In this context, the phase I study by Geva et al. (2024) found that JNJ-78306358, an ADC that binds simultaneously to the α3 domain of HLA-G isoforms on tumor cells and the CD3 receptor complex on T cells, facilitated the formation of immune synapses and the killing of tumor cells by CTLs in renal cell carcinoma, OC, and colorectal cancer in 39 patients. Conversely, no interaction of this ADC was found with cells that do not express HLA-G, demonstrating its specificity for certain types of tumor cells. In this study, all 39 patients (100%) discontinued treatment. The most frequent reasons for discontinuation were disease progression (82.1%) and death (5.1%), with none attributed to the ADC JNJ-78306358.

5.2.2 Mirvetuximab

Among the highly important and promising ADCs for OC treatment, mirvetuximab was approved by FDA in 2022, based on the results from the SORAYA study (Matulonis et al., 2023). This ADC consists of an IgG1 monoclonal antibody targeting the folate receptor alpha (FRα) conjugated to the cytotoxic maytansinoid DM4, which has demonstrated significant clinical activity in patients with FRα-positive OC (González-Ochoa et al., 2023).

Richardson et al. (2024) presented results from a phase Ib study combining mirvetuximab soravtansine with carboplatin and bevacizumab in patients with platinum-sensitive OC. In this study, 41 patients were enrolled, of whom 34 exhibited an anti-tumor response, resulting in an ORR of 83%. Most adverse effects were graded as two or lower, indicating an acceptable safety profile.

Another study involving mirvetuximab was conducted by Moore et al. (2023), who reported results from a global, phase III, confirmatory, open-label, randomized, and controlled trial for the treatment of platinum-resistant FRα-positive HGSOC. Among the patients, 227 were assigned to the mirvetuximab group and 226 to the chemotherapy group (paclitaxel, pegylated liposomal doxorubicin, or topotecan). The results showed a median PFS of 5.62 months and an ORR of 42.3% in the mirvetuximab group. During treatment, fewer grade 3 or higher adverse events occurred with mirvetuximab (41.7%) compared to chemotherapy (54.1%), as well as fewer serious adverse events of any grade (23.9% vs. 32.9%) and events leading to discontinuation (9.2% vs. 15.9%), demonstrating greater safety with the ADC treatment.

5.2.3 Oregovomab

The ADC oregovomab is a murine monoclonal antibody that binds to cancer antigen-125 (CA-125) in blood and local tissues

(Battaglia et al., 2020). It is administered to induce targeted therapeutic immunity against cancer. The oregovomab-CA125 complex has enhanced efficacy in antigen capture and cross-presentation, which activates cellular immune response (Brewer et al., 2020).

In this context, Brewer et al. (2020) conducted a phase II, international, randomized, multicenter study to evaluate the results of chemoimmunotherapy in OC using carboplatin-paclitaxel and indirect immunization with oregovomab. The study involved 94 patients who were randomly assigned to receive either carboplatin-paclitaxel alone or carboplatin-paclitaxel with oregovomab addition. Results showed that all patients achieved cytoreduction to less than 1 cm of residual disease or no macroscopic residual disease. Furthermore, the median PFS was 41.8 months in patients receiving additional oregovomab compared to 12.2 months in the control group, demonstrating a significant difference between the two groups ($p = 0.0027$).

Additionally, a multicenter phase II study by Park et al. (2024) examined the efficacy of non-platinum-based chemotherapy with the use of oregovomab in patients with recurrent OC. This study demonstrated promising efficacy, achieving a PFS of 11 weeks and a median overall survival of 70.4 weeks.

5.2.4 Catumaxomab (Removab)

Catumaxomab is a trifunctional bispecific ADC and targets epithelial cell adhesion molecule (EpCAM) and CD3 T-cell antigen (Ruf et al., 2021). Its anti-tumor effect results from a complex immune reaction at the tumor site involving T cell-mediated lysis, which includes T cell-mediated destruction of tumor cells, antibody-dependent cellular cytotoxicity, and phagocytosis (Knödler et al., 2018).

Studies with this ADC have demonstrated its success as an immunotherapy (Fossati et al., 2015), leading to its approval by the European Medicines Agency (EMA) in 2009 for the intraperitoneal treatment of malignant ascites. However, the approval of this ADC was withdrawn in 2017 due to commercial reasons (Ruf et al., 2021).

5.2.5 Abagovomab

The murine anti-idiotypic monoclonal antibody abagovomab was developed to functionally mimic the three-dimensional structure of CA-125 and induce a specific immune response directed against the original antigen (Battaglia et al., 2017). In this context, a phase III placebo-controlled study known as MIMOSA was conducted, but it showed that the survival rate of patients with OC was not increased by abagovomab (Battaglia et al., 2017). However, a study by Battaglia et al. (2017) aimed to demonstrate that a healthy immune system conditions the response to this ADC. In their research, 80 patients received abagovomab, and 31 patients received placebo. Patients treated with abagovomab who had a percentage of $CD8^+$ T cells producing IFN-γ above the cutoff point showed better recurrence-free survival ($p = 0.042$) than those with a percentage of $CD8^+$ T cells producing IFN-γ below the cutoff point. Additionally, this study demonstrated that the recurrence-free survival of patients treated with abagovomab with both a percentage of $CD8^+$ T cells producing IFN-γ and absolute cell counts below the respective cutoff points was identical to that of patients in the placebo group. In this regard, it is concluded that further studies are needed to clarify the effects of abagovomab in OC patients.

5.2.6 Daclizumab (Zenapax)

Daclizumab (Zenapax) is a humanized IgG1 monoclonal antibody specific to IL-2 receptor-α subunit (CD25) (Tse et al., 2014). It irreversibly blocks CD25, thereby preventing signaling through the high-affinity IL-2R while increasing the bioavailability of IL-2 to bind to the low-affinity receptor (Ranganath et al., 2020). As a result, ADC induces various immunological changes, including inhibition of T cell activation, reduction in the frequency and survival of regulatory T cells, and expansion of CD56bright NK cells (Ranganath et al., 2020).

Within this scenario, an interventional phase I clinical trial was conducted with patients with recurrent ovarian, fallopian tube, or primary peritoneal cancer using this ADC. However, the study was terminated in 2018, and the results were not published. Additionally, this drug was suspended by EMA in 2018 due to 12 reported worldwide cases of severe brain inflammation, three of which were fatal (European Medicines Agency, 2018). Table 2 highlights some studies that evaluate ICIs and ADCs technologies in OC treatment.

5.3 T- and NK-cell engaging bispecific antibodies (BsAbs)

Bispecific antibodies (BsAbs) are engineered molecules designed to bind simultaneously to two distinct epitopes or antigens. This dual targeting mechanism allows them to interact with tumor antigens on cancer cells while activating receptors on immune cells, offering a novel approach to immunotherapy (Wang Q. et al., 2019). Recent studies have focused on the roles of T and NK cells in this context, as BsAbs can effectively bring these immune cells into proximity with tumor cells (Wu Z. and Cheung, 2018). By simultaneously binding to tumor antigens on cancer cells and activating receptors such as CD3 on T cells or CD16 on NK cells, BsAbs enhance the capacity of these immune cells to recognize and eliminate malignant cells. This strategy positions engaging BsAbs as a promising approach for cancer immunotherapy (Tapia-Galisteo et al., 2023).

In the context of hematological tumors, numerous clinical trials have demonstrated favorable outcomes with T cell-engaging bispecific antibodies (BsAbs). Notable examples include epcoritamab (Thieblemont et al., 2022), odronextamab (Bannerji et al., 2022), mosunetuzumab (Budde et al., 2022), and glofitamab (Hutchings et al., 2021). These CD3xCD20 T cell-engaging BsAbs bind to T cells via CD3 receptors, effectively directing them to eliminate malignant $CD20^+$ B cells in patients with heavily pretreated B-cell non-Hodgkin lymphoma (van de Donk and Zweegman, 2023). Additionally, Reusing et al. (2021) reported that CD16xCD33 NK cell-engaging BsAbs activated Killer immunoglobulin-like receptor (KIR) signaling, thereby enhancing NK cell-mediated lysis of acute myeloid leukemia (AML) blasts.

Regarding solid tumors, particularly OC, Crawford and colleagues (2019) reported on the BsAb REGN4018, which targets both MUC16, a highly expressed marker in OC cells, and

TABLE 2 Ongoing studies evaluating ICIs and ADCs technologies in OC.

Agents	Targets	Clinical study phase	Results	References
Nivolumab	PD-1	II	64.7% of patients experienced PFS at 24 weeks, and treatment toxicity was moderate	Friedman et al. (2024)
Ivonescimab (AKT112/SMT112)	PD-1/VEGF-A	Ia	Among 19 patients, 5 achieved a partial response, including 3 with high-grade serous pathology, resulting in an ORR of 26.3%. Furthermore, was observed that the disease remained stable for more than 12 months in 4 patients who had previously been treated with bevacizumab	Frentzas et al. (2024)
Ipilimumab + Nivolumab	CTLA-4 and PD-1	Ib and II	A partial response was observed in 2 patients, with a response duration of 14.8 months. In addition, 2 of 23 patients demonstrated adverse effects classified as grade 3 or higher	Knisely et al. (2024)
Durvalumab + Tremelimumab	PD-L1 and CTLA-4	II	There was no significant difference in PFS between the combination therapy group and the sequential therapy group In addition, partial responses were observed in 2 patients and stable disease in 1 patient, with all responses occurring in the combination therapy group	Hinchcliff et al. (2024)
Durvalumab + Eribulin	PD-L1 and microtubules	Ib	ORR of 55%, with 4 patients experiencing stable disease, and a PFS of 6.2 months	Landry et al. (2023)
Niraparib + Pembrolizumab	PARP and PD-1	I and II	ORR of 19%, compared to monotherapy with each agent, with no signs of toxicity	Konstantinopoulos et al. (2019)
JNJ-78306358	α3 domain of HLA-G and CD3	I	The therapy facilitated the formation of immune synapses and the killing of tumor cells by CTLs. Furthermore, no interaction of this ADC was found with cells that do not express HLA-G, demonstrating its specificity for certain types of tumor cells	Geva et al. (2024)
Mirvetuximab	FRα	III	Patients showed median PFS of 5.62 months and ORR of 42.3% During treatment, was demonstrating greater safety in relation to the group with the another treatment	Moore et al. (2023)
Mirvetuximab + Carboplatin + Bevacizumab	FRα and VEGF	Ib	Patients showed an ORR of 83%. Most adverse effects were graded as 2 or lower	Richardson et al. (2024)
Oregovomab	CA-125	II	All patients achieved cytoreduction and the PFS demonstrating a significant difference between the control group and the treated group	Brewer et al. (2020) Junsik et al., 2024
Abagovomab	EpCAM and CD3	III	Patients showed better recurrence-free survival	Battaglia et al., 2017

CD3, a receptor on T cells. Overall, their findings indicated that REGN4018 exhibited robust antitumor activity and favorable tolerability, warranting its clinical evaluation in patients with MUC16-expressing advanced OC (Crawford et al., 2019). Oladapo et al. (2021) similarly investigated T cell-engaging BsAbs targeting MUC16. Their findings indicate that these antibodies demonstrate efficacy against OC, both as a monotherapy and in combination with other agents such as PD-1 and VEGF inhibitors (Oladapo et al., 2021). In the other hand, Lee E. and colleagues (2021) examined a BsAb targeting LYPD1, an antigen associated with high-grade serous OC, and their data suggested its compelling efficacy and safety profiles, supporting its potential use as a treatment for high-grade serous OC (Lee E. et al., 2021).

Furthermore, Avanzino and colleagues (2022) studied TNB-928B, a T-cell engaging BsAb that binds to FRα to selectively target FRα overexpressing tumor cells. It was shown that TNB-928B induced preferential effector T-cell activation, proliferation, and selective cytotoxic activity on high FRα expressing OC cells, and also promoted T-cell infiltration and antitumor activity in OC mouse models (Avanzino et al., 2022). Additionally, Vallera et al. (2020) evaluated cam1615B7H3, a tri-specific killer engager that has a camelid CD16 antibody fragment, a wild-type IL-15 moiety, and an anti-B7-H3 single-chain variable fragment, in various types of solid tumors. Their findings suggest that cam1615B7H3 improves NK cell function, expansion, targeted cytotoxicity against various types of B7-H3-positive human cancer cell lines, and delivers an anti-cancer effect *in vivo* in a solid tumor setting, including in OC (Vallera et al., 2020).

Given the studies conducted, further research is necessary to ensure the safety of these ADCs in OC treatment.

6 Discussion

Overall, immunotherapy for OC faces significant challenges, yet the field holds substantial potential for advancement. Ongoing efforts aim to overcome immune suppression and improve the efficacy of OC immunotherapy. These strategies include combining immunotherapy with other drugs, utilizing targeted and precision-guided particles, developing innovative antigen vaccine delivery systems, and implementing prolonged low-dose immunotherapy regimens. Consequently, recent progress in both active and passive immunotherapy approaches has introduced new perspectives and insights, thereby enhancing the effectiveness of

immune-based treatments for OC. Indeed, to handle the adverse effects of common therapies for cancer, immunotherapy strategies emerged as a cancer-specific alternative capable of targeting the tumor and causing minimal impact on normal tissues (Aly, 2012; Zhu and Yu, 2022). They are significant considering the usual therapeutic approaches such as surgery, chemotherapy, and radiotherapy which besides the adverse effects show a lack of specificity for tumors (Kaczmarek et al., 2023). Therapeutic cancer vaccination is a strategy of immunotherapy developed to elicit or boost antitumor adaptive immune responses to detect and eliminate them (Luo et al., 2024; Chambers, 2011). Moreover, CAR-T cells are genetically engineered to recognize and attack tumor-specific antigens (June et al., 2018), bypassing the need of MHC molecules presentation, and behaving as active drugs against tumors (Maus and June, 2016). In turn, exosomes are a category of extracellular vesicles with a lipid bilayer, measuring approximately 30–150 nm, found in various body fluids such as blood, urine, saliva, and cerebrospinal fluid (He et al., 2018; Gong et al., 2023). In addition to their ambiguous role, exosomes may serve as a potential tool for OC therapy (Shimizu et al., 2024). Also of clinical relevance, therapeutic monoclonal antibodies have been successfully developed for the treatment of various cancer types (Hafeez et al., 2020). Numerous clinical trials are still underway with promising prospects for the treatment of OC, including ADCs such as JNJ-78306358, ivonescimab, ipilimumab, durvalumab, oregovomab, catumaxomab, abagovomab, daclizumab and mirvetuximab, which was approved by the FDA in 2022 but remains under study for application in OC treatment (Dilawari et al., 2023). Yet, ICIs represent a promising class of drugs in immunotherapy against OC, targeting PD-1/PD-L1 and CTLA-4. They have already demonstrated broad bioactivity and stable response in the treatment of various types of tumors (Naimi et al., 2022; Tang S. et al., 2022b), including OC (Disis et al., 2019). Therefore, OC immunotherapy involves the induction of an anti-tumor immune response and the development of immunological memory. This process not only can eradicate malignant cells within the primary tumor site, thereby averting recurrence, but also hampers the metastatic spread to distant anatomical locations (Cha et al., 2020).

Author contributions

TM: Writing–original draft, Writing–review and editing. JC: Writing–original draft, Writing–review and editing. BS: Writing–original draft, Writing–review and editing. SS: Writing–original draft, Writing–review and editing. JS: Writing–original draft, Writing–review and editing. MG: Writing–original draft, Writing–review and editing. GS: Writing–original draft, Writing–review and editing. LR: Project administration, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abd-Aziz, N., and Poh, C. L. (2022). Development of peptide-based vaccines for cancer. *J. Oncol.* 2022, 9749363. doi:10.1155/2022/9749363

Acharya, G., Mani, C., Sah, N., Saamarthy, K., Young, R., Reedy, M. B., et al. (2024). CHK1 inhibitor induced PARylation by targeting PARG causes excessive replication and metabolic stress and overcomes chemoresistance in ovarian cancer. *Cell Death Discov.* 10 (1), 278. doi:10.1038/s41420-024-02040-0

Aggarwal, S., He, T., Fitzhugh, W., Rosenthal, K., Feild, B., Heidbrink, J., et al. (2009). Immune modulator CD70 as a potential cisplatin resistance predictive marker in ovarian cancer. *Gynecol. Oncol.* 115 (3), 430–437. doi:10.1016/j.ygyno.2009.08.031

Albelda, S. M. (2024). CAR T cell therapy for patients with solid tumours: key lessons to learn and unlearn. *Nat. Rev. Clin. Oncol.* 21 (1), 47–66. doi:10.1038/s41571-023-00832-4

Alipoor, S. D., Mortaz, E., Varahram, M., Movassaghi, M., Kraneveld, A. D., Garssen, J., et al. (2018). The potential biomarkers and immunological effects of tumor-derived exosomes in lung cancer. *Front. Immunol.* 9, 819. doi:10.3389/fimmu.2018.00819

Aly, H. A. (2012). Cancer therapy and vaccination. *J. Immunol. methods* 382 (1-2), 1–23. doi:10.1016/j.jim.2012.05.014

American Cancer Society (2020). Cancer vaccines and their side effects. Available at: https://www.cancer.org/cancer/managing-cancer/treatment-types/immunotherapy/cancer-vaccines.html (Accessed July 29, 2024).

American Cancer Society (2024). *Key statistics for ovarian cancer*. Atlanta: ACS Publications. Available at: https://www.cancer.org/cancer/types/ovarian-cancer/about/key-statistics.html (Accessed July 30, 2024).

Andersson, E., Villabona, L., Bergfeldt, K., Carlson, J. W., Ferrone, S., Kiessling, R., et al. (2012). Correlation of HLA-A02* genotype and HLA class I antigen down-regulation with the prognosis of epithelial ovarian cancer. *Cancer Immunol. Immunother. CII.* 61 (8), 1243–1253. doi:10.1007/s00262-012-1201-0

Apte, R. S., Chen, D. S., and Ferrara, N. (2019). VEGF in signaling and disease: beyond discovery and development. *Cell* 176 (6), 1248–1264. doi:10.1016/j.cell.2019.01.021

Asare-Werehene, M., Nakka, K., Reunov, A., Chiu, C. T., Lee, W. T., Abedini, M. R., et al. (2020). The exosome-mediated autocrine and paracrine actions of plasma gelsolin in ovarian cancer chemoresistance. *Oncogene* 39 (7), 1600–1616. doi:10.1038/s41388-019-1087-9

Avanzino, B. C., Prabhakar, K., Dalvi, P., Hartstein, S., Kehm, H., Balasubramani, A., et al. (2022). A T-cell engaging bispecific antibody with a tumor-selective bivalent folate receptor alpha binding arm for the treatment of ovarian cancer. *Oncoimmunology* 11 (1), 2113697. doi:10.1080/2162402X.2022.2113697

Battaglia, A., Buzzonetti, A., Fossati, M., Scambia, G., Fattorossi, A., Madiyalakan, M. R., et al. (2020). ranslational immune correlates of indirect antibody immunization in a

randomized phase II study using scheduled combination therapy with carboplatin/paclitaxel plus oregovomab in ovarian cancer patients. *Cancer immunology, immunotherapy: CII.* 69 (3), 383–397. doi:10.1007/s00262-019-02456-z

Battaglia, A., Fossati, M., Buzzonetti, A., Scambia, G., and Fattorossi, A. (2017). A robust immune system conditions the response to abagovomab (anti-idiotypic monoclonal antibody mimicking the CA125 protein) vaccination in ovarian cancer patients. *Immunology letters.* 191, 35–39. doi:10.1016/j.imlet.2017.09.006

Baker, D. J., Arany, Z., Baur, J. A., Epstein, J. A., and June, C. H. (2023). CAR T therapy beyond cancer: the evolution of a living drug. *Nature* 619 (7971), 707–715. doi:10.1038/s41586-023-06243-w

Bannerji, R., Arnason, J. E., Advani, R. H., Brown, J. R., Allan, J. N., Ansell, S. M., et al. (2022). Odronextamab, a human CD20×CD3 bispecific antibody in patients with CD20-positive B-cell malignancies (ELM-1): results from the relapsed or refractory non-Hodgkin lymphoma cohort in a single-arm, multicentre, phase 1 trial. *Lancet Haematol.* 9 (5), 327–339. doi:10.1016/s2352-3026(22)00072-2

Barbier, A. J., Jiang, A. Y., Zhang, P., Wooster, R., and Anderson, D. G. (2022). The clinical progress of mRNA vaccines and immunotherapies. *Nat. Biotechnol.* 40 (6), 840–854. doi:10.1038/s41587-022-01294-2

Bhattacharya, B., Nag, S., Mukherjee, S., Kulkarni, M., Chandane, P., Mandal, D., et al. (2024). Role of exosomes in epithelial-mesenchymal transition. *ACS Appl. bio Mater.* 7 (1), 44–58. doi:10.1021/acsabm.3c00941

Bhavsar, D., Raguraman, R., Kim, D., Ren, X., Munshi, A., Moore, K., et al. (2024). Exosomes in diagnostic and therapeutic applications of ovarian cancer. *J. ovarian Res.* 17 (1), 113. doi:10.1186/s13048-024-01417-0

Bhojnagarwala, P. S., Perales-Puchalt, A., Cooch, N., Sardesai, N. Y., and Weiner, D. B. (2021). A synDNA vaccine delivering neoAg collections controls heterogenous, multifocal murine lung and ovarian tumors via robust T cell generation. *Mol. Ther. oncolytics* 21, 278–287. doi:10.1016/j.omto.2021.04.005

Block, M. S., Dietz, A. B., Gustafson, M. P., Kalli, K. R., Erskine, C. L., Youssef, B., et al. (2020). Th17-inducing autologous dendritic cell vaccination promotes antigen-specific cellular and humoral immunity in ovarian cancer patients. *Nat. Commun.* 11 (1), 5173. doi:10.1038/s41467-020-18962-z

Brewer, M., Angioli, R., Scambia, G., Lorusso, D., Terranova, C., Panici, P. B., et al. (2020). Front-line chemo-immunotherapy with carboplatin-paclitaxel using oregovomab indirect immunization in advanced ovarian cancer: a randomized phase II study. *Gynecol. Oncol.* 156 (3), 523–529. doi:10.1016/j.ygyno.2019.12.024

Brown, T. A., Byrd, K., Vreeland, T. J., Clifton, G. T., Jackson, D. O., Hale, D. F., et al. (2019). Final analysis of a phase I/IIa trial of the folate-binding protein-derived E39 peptide vaccine to prevent recurrence in ovarian and endometrial cancer patients. *Cancer Med.* 8 (10), 4678–4687. doi:10.1002/cam4.2378

Brudno, J. N., and Kochenderfer, J. N. (2024). Current understanding and management of CAR T cell-associated toxicities. *Nat. Rev. Clin. Oncol.* 21, 501–521. doi:10.1038/s41571-024-00903-0

Budde, L. E., Assouline, S., Sehn, L. H., Schuster, S. J., Yoon, S.-S., Yoon, D. H., et al. (2022). Single-agent mosunetuzumab shows durable complete responses in patients with relapsed or refractory B-cell lymphomas: phase I dose-escalation study. *J. Clin. Oncol.* 40 (5), 481–491. doi:10.1200/JCO.21.00931

Bund, V., Azais, H., Bibi-Triki, S., Lecointre, L., Betrian, S. B., Angeles, M. A., et al. (2022). Basics of immunotherapy for epithelial ovarian cancer. *J. Gynecol. Obstetrics Hum. Reproduction* 51 (2), 102283. doi:10.1016/j.jogoh.2021.102283

Cappell, K. M., and Kochenderfer, J. N. (2023). Long-term outcomes following CAR T cell therapy: what we know so far. *Nat. Rev. Clin. Oncol.* 20 (6), 359–371. doi:10.1038/s41571-023-00754-1

Carmi, Y. K., Agbarya, A., Khamaisi, H., Farah, R., Shechtman, Y., Korobochka, R., et al. (2024). Ovarian cancer ascites confers platinum chemoresistance to ovarian cancer cells. *Transl. Oncol.* 44 (101939), 101939. doi:10.1016/j.tranon.2024.101939

Cha, J. H., Chan, L. C., Song, M. S., and Hung, M. C. (2020). New approaches on cancer immunotherapy. *Cold Spring Harb. Perspect. Med.* 10 (8), a036863. doi:10.1101/cshperspect.a036863

Chambers, C. V. (2011). Cancer vaccines. *Prim. Care Clin. Office Pract.* 38 (4), 703–715. doi:10.1016/j.pop.2011.07.008

Chehelgerdi, M., Behdarvand Dehkordi, F., Chehelgerdi, M., Kabiri, H., Salehian-Dehkordi, H., Abdolvand, M., et al. (2023). Exploring the promising potential of induced pluripotent stem cells in cancer research and therapy. *Mol. Cancer* 22 (1), 189. doi:10.1186/s12943-023-01873-0

Chekmasova, A. A., Rao, T. D., Nikhamin, Y., Park, K. J., Levine, D. A., Spriggs, D. R., et al. (2010). Successful eradication of established peritoneal ovarian tumors in SCID-Beige mice following adoptive transfer of T cells genetically targeted to the MUC16 antigen. *Clin. cancer Res. official J. Am. Assoc. Cancer Res.* 16 (14), 3594–3606. doi:10.1158/1078-0432.ccr-10-0192

Chiang, C. L. L., Kandalaft, L. E., and Coukos, G. (2011). Adjuvants for enhancing the immunogenicity of whole tumor cell vaccines. *Int. Rev. Immunol.* 30 (2-3), 150–182. doi:10.3109/08830185.2011.572210

Coelho, R., Marcos-Silva, L., Ricardo, S., Ponte, F., Costa, A., Lopes, J. M., et al. (2018). Peritoneal dissemination of ovarian cancer: role of MUC16-mesothelin interaction and implications for treatment. *Expert Rev. anticancer Ther.* 18 (2), 177–186. doi:10.1080/14737140.2018.1418326

Crawford, A., Haber, L., Kelly, M. P., Vazzana, K., Canova, L., Ram, P., et al. (2019). A Mucin 16 bispecific T cell-engaging antibody for the treatment of ovarian cancer. *Sci. Transl. Med.* 11 (497), eaau7534. doi:10.1126/scitranslmed.aau7534

Dagher, O. K., and Posey Jr, A. D. (2023). Forks in the road for CAR T and CAR NK cell cancer therapies. *Nat. Immunol.* 24 (12), 1994–2007. doi:10.1038/s41590-023-01659-y

Daud, A. I., Wolchok, J. D., Robert, C., Hwu, W., Weber, J. S., Ribas, A., et al. (2016). Programmed death-ligand 1 expression and response to the anti-programmed death 1 antibody pembrolizumab in melanoma. *J. Clin. Oncol.* 34 (34), 4102–4109. doi:10.1200/JCO.2016.67.2477

Dean, A. Q., Luo, S., Twomey, J. D., and Zhang, B. (2021). Targeting cancer with antibody-drug conjugates: Promises and challenges. *mAbs* 13 (1), 1951427. doi:10.1080/19420862.2021.1951427

Del Bufalo, F., De Angelis, B., Caruana, I., Del Baldo, G., De Ioris, M. A., Serra, A., et al. (2023). GD2-CART01 for relapsed or refractory high-risk neuroblastoma. *N. Engl. J. Med.* 388 (14), 1284–1295. doi:10.1056/NEJMoa2210859

Dhillon, S. (2024). Ivonescimab: first approval. *Drugs* 84, 1135–1142. doi:10.1007/s40265-024-02073-w

Dilawari, A., Shah, M., Ison, G., Gittleman, H., Fiero, M. H., Shah, A., et al. (2023). FDA approval summary: mirvetuximab soravtansine-gynx for FRα-positive, platinum-resistant ovarian cancer. *Clin. Cancer Res.* 29 (19), 3835–3840. doi:10.1158/1078-0432.CCR-23-0991

Disis, M. L., Taylor, M. H., Kelly, K., Beck, J. T., Gordon, M., Moore, K. M., et al. (2019). Efficacy and safety of avelumab for patients with recurrent or refractory ovarian cancer: phase 1b results from the JAVELIN Solid Tumor trial. *JAMA Oncol.* 5 (3), 393–401. doi:10.1001/jamaoncol.2018.6258

Doubeni, C. A., Doubeni, A. R., and Myers, A. E. (2016). Diagnosis and management of ovarian cancer. *Am. Fam. physician.* 93 (11), 937–944.

Duperret, E. K., Perales-Puchalt, A., Stoltz, R., G H, H., Mandloi, N., Barlow, J., et al. (2019). A synthetic DNA, multi-neoantigen vaccine drives predominately MHC class I CD8+ T-cell responses, impacting tumor challenge. *Cancer Immunol. Res.* 7 (2), 174–182. doi:10.1158/2326-6066.CIR-18-0283

European Medicines Agency (2018). Multiple sclerosis medicine Zinbryta suspended in the EU. Available at: https://www.ema.europa.eu/system/files/documents/referral/wc500245166_en.pdf (Accessed July 27, 2024).

Fan, T., Zhang, M., Yang, J., Zhu, Z., Cao, W., and Dong, C. (2023). Therapeutic cancer vaccines: advancements, challenges, and prospects. *Signal Transduct. Target. Ther.* 8 (1), 450. doi:10.1038/s41392-023-01674-3

Flugel, C. L., Majzner, R. G., Krenciute, G., Dotti, G., Riddell, S. R., Wagner, D. L., et al. (2023). Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. *Nat. Rev. Clin. Oncol.* 20 (1), 49–62. doi:10.1038/s41571-022-00704-3

Food and Drug Administration (2024). Package insert and medication guide - Yescarta. Available at: https://www.fda.gov/media/108377/download (Accessed August 1, 2024).

Fossati, M., Buzzonetti, A., Monego, G., Catzola, V., Scambia, G., Fattorossi, A., et al. (2015). Immunological changes in the ascites of cancer patients after intraperitoneal administration of the bispecific antibody catumaxomab (anti-EpCAM×anti-CD3). *Gynecol. Oncol.* 138 (2), 343–351. doi:10.1016/j.ygyno.2015.06.003

Frentzas, S., Mislang, A. R. A., Lemech, C., Nagrial, A., Underhill, C., Wang, W., et al. (2024). Phase 1a dose escalation study of ivonescimab (AK112/SMT112), an anti-PD-1/VEGF-A bispecific antibody, in patients with advanced solid tumors. *J. Immunother. Cancer* 12 (4), e008037. doi:10.1136/jitc-2023-008037

Friedman, C. F., Manning-Geist, B. L., Zhou, Q., Soumerai, T., Holland, A., Paula, A. D. C., et al. (2024). Nivolumab for mismatch-repair-deficient or hypermutated gynecologic cancers: a phase 2 trial with biomarker analyses. *Nat. Med.* 30 (5), 1330–1338. doi:10.1038/s41591-024-02942-7

Geva, R., Vieito, M., Ramon, J., Perets, R., Pedregal, M., Corral, E., et al. (2024). Safety and clinical activity of JNJ-78306358, a human leukocyte antigen-G (HLA-G) x CD3 bispecific antibody, for the treatment of advanced stage solid tumors. *Cancer Immunol. Immunother.* 73 (10), 205. doi:10.1007/s00262-024-03790-7

Ghoneum, A., Almousa, S., Warren, B., Abdulfattah, A. Y., Shu, J., Abouelfadl, H., et al. (2021). Exploring the clinical value of tumor microenvironment in platinum-resistant ovarian cancer. *Seminars cancer Biol.* 77, 83–98. doi:10.1016/j.semcancer.2020.12.024

Gong, X., Chi, H., Strohmer, D. F., Teichmann, A. T., Xia, Z., and Wang, Q. (2023). Exosomes: a potential tool for immunotherapy of ovarian cancer. *Front. Immunol.* 13, 1089410. doi:10.3389/fimmu.2022.1089410

Gonzalez-Ochoa, E., Veneziani, A. C., and Oza, A. M. (2023). Mirvetuximab soravtansine in platinum-resistant ovarian cancer. *Clin. Med. Insights Oncol.* 17, 11795549231187264. doi:10.1177/11795549231187264

Hafeez, U., Parakh, S., Gan, H. K., and Scott, A. M. (2020). Antibody-drug conjugates for cancer therapy. *Molecules* 25 (20), 4764. doi:10.3390/molecules25204764

He, C., Zheng, S., Luo, Y., and Wang, B. (2018). Exosome theranostics: biology and translational medicine. *Theranostics* 8 (1), 237–255. doi:10.7150/thno.21945

Hilliard, T. S. (2018). The impact of mesothelin in the ovarian cancer tumor microenvironment. *Cancers* 10 (9), 277. doi:10.3390/cancers10090277

Hinchcliff, E. M., Knisely, A., Adjei, N., Fellman, B., Yuan, Y., Patel, A., et al. (2024). Randomized phase 2 trial of tremelimumab and durvalumab in combination versus sequentially in recurrent platinum-resistant ovarian cancer. *Cancer.* 130 (7), 1061–1071. doi:10.1002/cncr.35126

Hutchings, M., Morschhauser, F., Iacoboni, G., Carlo-Stella, C., Offner, F. C., Sureda, A., et al. (2021). Glofitamab, a novel, bivalent CD20-targeting T-cell–engaging bispecific antibody, induces durable complete remissions in relapsed or refractory B-cell lymphoma: a phase I trial. *J. Clin. Oncol.* 39 (18), 1959–1970. doi:10.1200/jco.20.03175

Jaeger, D., Sahin, U., and Tureci, O. (2014). A first-in-human dose escalation and dose-finding phase I/II trial of IMAB027 in patients with recurrent advanced ovarian cancer (GM-IMAB-002-01). *J. Clin. Oncol.* 32 (15), TPS5623. doi:10.1200/jco.2014.32.15_suppl.tps5623

Janes, M. E., Gottlieb, A. P., Park, K. S., Zhao, Z., and Mitragotri, S. (2024). Cancer vaccines in the clinic. *Bioeng. & Transl. Med.* 9 (1), e10588. doi:10.1002/btm2.10588

June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., and Milone, M. C. (2018). CAR T cell immunotherapy for human cancer. *Science* 359 (6382), 1361–1365. doi:10.1126/science.aar6711

Kaczmarek, M., Poznańska, J., Fechner, F., Michalska, N., Paszkowska, S., Napierała, A., et al. (2023). Cancer vaccine therapeutics: limitations and effectiveness—a literature review. *Cells* 12 (17), 2159. doi:10.3390/cells12172159

Kalli, K. R., Block, M. S., Kasi, P. M., Erskine, C. L., Hobday, T. J., Dietz, A., et al. (2018). Folate receptor alpha peptide vaccine generates immunity in breast and ovarian cancer patients. *Clin. Cancer Res.* 24 (13), 3014–3025. doi:10.1158/1078-0432.CCR-17-2499

Kaushik, S., and Cuervo, A. M. (2015). Proteostasis and aging. *Nat. Med.* 21 (12), 1406–1415. doi:10.1038/nm.4001

Kim, J., Zhu, Y., Chen, S., Wang, D., Zhang, S., Xia, J., et al. (2023). Anti-glioma effect of ginseng-derived exosomes-like nanoparticles by active blood-brain-barrier penetration and tumor microenvironment modulation. *J. nanobiotechnology* 21 (1), 253. doi:10.1186/s12951-023-02006-x

Knisely, A., Hinchcliff, E., Fellman, B., Mosley, A., Lito, K., Hull, S., et al. (2024). Phase 1b study of intraperitoneal ipilimumab and nivolumab in patients with recurrent gynecologic malignancies with peritoneal carcinomatosis. *Med* 5 (4), 311–320.e3. doi:10.1016/j.medj.2024.02.003

Knödler, M., Körfer, J., Kunzmann, V., Trojan, J., Daum, S., Schenk, M., et al. (2018). Randomised phase II trial to investigate catumaxomab (anti-EpCAM × anti-CD3) for treatment of peritoneal carcinomatosis in patients with gastric cancer. *Br. J. Cancer* 119 (3), 296–302. doi:10.1038/s41416-018-0150-6

Konstantinopoulos, P. A., Waggoner, S., Vidal, G. A., Mita, M., Moroney, J. W., Holloway, R., et al. (2019). Single-Arm phases 1 and 2 trial of niraparib in combination with pembrolizumab in patients with recurrent platinum-resistant ovarian carcinoma. *JAMA Oncol.* 5 (8), 1141–1149. doi:10.1001/jamaoncol.2019.1048

Kugeratski, F. G., and Raghu, K. (2021). Exosomes as mediators of immune regulation and immunotherapy in cancer. *FEBS J.* 288 (1), 10–35. doi:10.1111/febs.15558

Labanieh, L., and Mackall, C. L. (2023). CAR immune cells: design principles, resistance and the next generation. *Nature* 614 (7949), 635–648. doi:10.1038/s41586-023-05707-3

Landry, C. A., Blanter, J., Ru, M., Fasano, J., Klein, P., Shao, T., et al. (2023). Results of a phase Ib study investigating durvalumab in combination with eribulin in patients with HER2-negative metastatic breast cancer and recurrent ovarian cancer. 102, 9, 16. doi:10.1159/000533420

Laureano, R. S., Sprooten, J., Vanmeerbeerk, I., Borras, D. M., Govaerts, J., Naulaerts, S., et al. (2022). Trial watch: dendritic cell (DC)-based immunotherapy for cancer. *Oncoimmunology* 11 (1), 2096363. doi:10.1080/2162402X.2022.2096363

Lee, D., Dunn, Z. S., Guo, W., Rosenthal, C. J., Penn, N. E., Yu, Y., et al. (2023). Unlocking the potential of allogeneic Vδ2 T cells for ovarian cancer therapy through CD16 biomarker selection and CAR/IL-15 engineering. *Nat. Commun.* 14 (1), 6942. doi:10.1038/s41467-023-42619-2

Lee, E., Szvetecz, S., Polli, R., Grauel, A., Chen, J., Judge, J., et al. (2021). PAX8 lineage-driven T cell engaging antibody for the treatment of high-grade serous ovarian cancer. *Sci. Rep.* 11 (1), 14841. doi:10.1038/s41598-021-93992-1

Li, K., Mandai, M., Hamanishi, J., Matsumura, N., Suzuki, A., Yagi, H., et al. (2009). Clinical significance of the NKG2D ligands, MICA/B and ULBP2 in ovarian cancer: high expression of ULBP2 is an indicator of poor prognosis. *Cancer Immunol. Immunother.* 58 (5), 641–652. doi:10.1007/s00262-008-0585-3

Li, M., Xu, H., Qi, Y., Pan, Z., Li, B., Gao, Z., et al. (2022). Tumor-derived exosomes deliver the tumor suppressor miR-3591-3p to induce M2 macrophage polarization and promote glioma progression. *Oncogene* 41 (41), 4618–4632. doi:10.1038/s41388-022-02457-w

Li, T., Lin, L., Liu, Q., Gao, W., Chen, L., Sha, C., et al. (2021a). Exosomal transfer of miR-429 confers chemoresistance in epithelial ovarian cancer. *Am. J. cancer Res.* 11 (5), 2124–2141.

Li, X., Liu, Y., Zheng, S., Zhang, T., Wu, J., Sun, Y., et al. (2021b). Role of exosomes in the immune microenvironment of ovarian cancer. *Oncol. Lett.* 21 (5), 377. doi:10.3892/ol.2021.12638

Li, X., and Wang, X. (2017). The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer. *Mol. cancer* 16 (1), 92. doi:10.1186/s12943-017-0659-y

Liang, Z., Dong, J., Yang, N., Li, S. D., Yang, Z. Y., Huang, R., et al. (2021). Tandem CAR-T cells targeting FOLR1 and MSLN enhance the antitumor effects in ovarian cancer. *Int. J. Biol. Sci.* 17 (15), 4365–4376. doi:10.7150/ijbs.63181

Lin, A., and Yan, W. H. (2018). Heterogeneity of HLA-G expression in cancers: facing the challenges. *Front. Immunol.* 9, 2164. doi:10.3389/fimmu.2018.02164

Lin, M. J., Svensson-Arvelund, J., Lubitz, G. S., Marabelle, A., Melero, I., Brown, B. D., et al. (2022). Cancer vaccines: the next immunotherapy frontier. *Nat. cancer* 3 (8), 911–926. doi:10.1038/s43018-022-00418-6

Liu, D., Liu, L., Li, X., Wang, S., Wu, G., and Che, X. (2024a). Advancements and challenges in peptide-based cancer vaccination: a multidisciplinary perspective. *Vaccines* 12, 950. doi:10.3390/vaccines12080950

Liu, H., Deng, S., Yao, X., Liu, Y., Qian, L., Wang, Y., et al. (2024b). Ascites exosomal lncRNA PLADE enhances platinum sensitivity by inducing R-loops in ovarian cancer. *Oncogene* 43 (10), 714–728. doi:10.1038/s41388-024-02940-6

Lu, L., Ma, W., Johnson, C. H., Khan, S. A., Irwin, M. L., and Pusztai, L. (2023). *In silico* designed mRNA vaccines targeting CA-125 neoantigen in breast and ovarian cancer. *Vaccine* 41 (12), 2073–2083. doi:10.1016/j.vaccine.2023.02.048

Luo, J., Mo, F., Zhang, Z., Hong, W., Lan, T., Cheng, Y., et al. (2024). Engineered mitochondria exert potent antitumor immunity as a cancer vaccine platform. *Cell. & Mol. Immunol.*, 1–15. doi:10.1038/s41423-024-01203-4

Lv, L., Huang, J., Xi, H., and Zhou, X. (2020). Efficacy and safety of dendritic cell vaccines for patients with glioblastoma: a meta-analysis of randomized controlled trials. *Int. Immunopharmacol.* 83, 106336. doi:10.1016/j.intimp.2020.106336

Ma, L., Dichwalkar, T., Chang, J. Y., Cossette, B., Garafola, D., Zhang, A. Q., et al. (2019). Enhanced CAR–T cell activity against solid tumors by vaccine boosting through the chimeric receptor. *Science* 365 (6449), 162–168. doi:10.1126/science.aav8692

Mackensen, A., Haanen, J. B., Koenecke, C., Alsdorf, W., Wagner-Drouet, E., Borchmann, P., et al. (2023). CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. *Nat. Med.* 29 (11), 2844–2853. doi:10.1038/s41591-023-02612-0

Macpherson, A. M., Barry, S. C., Ricciardelli, C., and Oehler, M. K. (2020). Epithelial ovarian cancer and the immune system: biology, interactions, challenges and potential advances for immunotherapy. *J. Clin. Med.* 9 (9), 2967. doi:10.3390/jcm9092967

Matulonis, U. A., Lorusso, D., Oaknin, A., Pignata, S., Dean, A., Denys, H., et al. (2023). Efficacy and safety of mirvetuximab soravtansine in patients with platinum-resistant ovarian cancer with high folate receptor alpha expression: results from the SORAYA study. *J. Clin. Oncol.* 41 (13), 2436–2445. doi:10.1200/JCO.22.01900

Maus, M. V., and June, C. H. (2016). Making better chimeric antigen receptors for adoptive T-cell therapy. *Clin. cancer Res.* 22 (8), 1875–1884. doi:10.1158/1078-0432.CCR-15-1433

Miyamoto, T., Murakami, R., Hamanishi, J., Tanigaki, K., Hosoe, Y., Mise, N., et al. (2022). B7-H3 suppresses antitumor immunity via the CCL2-CCR2-M2 macrophage Axis and contributes to ovarian cancer progression. *Cancer Immunol. Res.* 10 (1), 56–69. doi:10.1158/2326-6066.cir-21-0407

Moore, K. N., Angelergues, A., Konecny, G. E., García, Y., Banerjee, S., Lorusso, D., et al. (2023). Mirvetuximab soravtansine in FRα-positive, platinum-resistant ovarian cancer. *N. Engl. J. Med.* 389 (23), 2162–2174. doi:10.1056/NEJMoa2309169

Morrissey, S. M., Zhang, F., Ding, C., Montoya-Durango, D. E., Hu, X., Yang, C., et al. (2021). Tumor-derived exosomes drive immunosuppressive macrophages in a pre-metastatic niche through glycolytic dominant metabolic reprogramming. *Cell metab.* 33 (10), 2040–2058.e10. doi:10.1016/j.cmet.2021.09.002

Mousaei Ghasroldasht, M., Liakath Ali, F., Park, H. S., Hadizadeh, M., Weng, S. H. S., Huff, A., et al. (2024). A comparative analysis of naïve exosomes and enhanced exosomes with a focus on the treatment potential in ovarian disorders. *J. personalized Med.* 14 (5), 482. doi:10.3390/jpm14050482

Murad, J. P., Kozlowska, A. K., Lee, H. J., Ramamurthy, M., Chang, W. C., Yazaki, P., et al. (2018). Effective targeting of TAG72+ peritoneal ovarian tumors via regional delivery of CAR-engineered T cells. *Front. Immunol.* 9, 2268. doi:10.3389/fimmu.2018.02268

Muthukutty, P., and Yoo, S. Y. (2023). Oncolytic virus engineering and utilizations: cancer immunotherapy perspective. *Viruses* 15 (8), 1645. doi:10.3390/v15081645

Naimi, A., Mohammed, R. N., Raji, A., Chupradit, S., Yumashev, A. V., Suksatan, W., et al. (2022). Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. *Cell Commun. Signal. CCS* 20 (1), 44. doi:10.1186/s12964-022-00854-y

Narayan, V., Barber-Rotenberg, J. S., Jung, I. Y., Lacey, S. F., Rech, A. J., Davis, M. M., et al. (2022). PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nat. Med.* 28 (4), 724–734. doi:10.1038/s41591-022-01726-1

O'Cearbhaill, R. E., Deng, W., Chen, L. M., Lucci III, J. A., Behbakht, K., Spirtos, N. M., et al. (2019). A phase II randomized, double-blind trial of a polyvalent Vaccine-KLH

conjugate (NSC 748933 IND# 14384)+ OPT-821 versus OPT-821 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer who are in second or third complete remission: an NRG Oncology/GOG study. *Gynecol. Oncol.* 155 (3), 393–399. doi:10.1016/j.ygyno.2019.09.015

Odunsi, K. (2017). Immunotherapy in ovarian cancer. *Ann. Oncol.* 28, viii1–viii7. doi:10.1093/annonc/mdx444

Oladapo, O. Y., Rao, T. D., Laster, I., Kononenko, A. P., Purdon, T. J., Wang, P., et al. (2021). Bispecific T-cell engaging antibodies against MUC16 demonstrate efficacy against ovarian cancer in monotherapy and in combination with PD-1 and VEGF inhibition. *Front. Immunol.* 12, 663379. doi:10.3389/fimmu.2021.663379

Ouyang, X., Telli, M. L., and Wu, J. C. (2019). Induced pluripotent stem cell-based cancer vaccines. *Front. Immunol.* 10, 1510. doi:10.3389/fimmu.2019.01510

Park, J., Cho, H. W., Lim, M. C., Choi, C. H., and Lee, J. Y. (2024). OPERA: a phase II trial of oregovomab plus non-platinum chemotherapy in PARP inhibitor/platinum-resistant ovarian cancer. *Future oncology.* 20 (26), 1893–1899. doi:10.1080/14796694.2024.2357533

Pan, X., Shi, X., Zhang, H., Chen, Y., Zhou, J., Shen, F., et al. (2024). Exosomal miR-4516 derived from ovarian cancer stem cells enhanced cisplatin tolerance in ovarian cancer by inhibiting GAS7. *Gene* 927, 148738. doi:10.1016/j.gene.2024.148738

Pandya, A., Shah, Y., Kothari, N., Postwala, H., Shah, A., Parekh, P., et al. (2023). The future of cancer immunotherapy: DNA vaccines leading the way. *Med. Oncol.* 40 (7), 200. doi:10.1007/s12032-023-02060-3

Pegtel, D. M., and Gould, S. J. (2019). Exosomes. *Annu. Rev. Biochem.* 88, 487–514. doi:10.1146/annurev-biochem-013118-111902

Perales-Puchalt, A., Svoronos, N., Rutkowski, M. R., Allegrezza, M. J., Tesone, A. J., Payne, K. K., et al. (2017). Follicle-stimulating hormone receptor is expressed by most ovarian cancer subtypes and is a safe and effective immunotherapeutic target. *Clin. Cancer Res.* 23 (2), 441–453. doi:10.1158/1078-0432.CCR-16-0492

Perales-Puchalt, A., Wojtak, K., Duperret, E. K., Yang, X., Slager, A. M., Yan, J., et al. (2019). Engineered DNA vaccination against follicle-stimulating hormone receptor delays ovarian cancer progression in animal models. *Mol. Ther.* 27 (2), 314–325. doi:10.1016/j.ymthe.2018.11.014

Pérez-Baños, A., Gleisner, M. A., Flores, I., Pereda, C., Navarrete, M., Araya, J. P., et al. (2023). Whole tumour cell-based vaccines: tuning the instruments to orchestrate an optimal antitumour immune response. *Br. J. Cancer* 129 (4), 572–585. doi:10.1038/s41416-023-02327-6

Qi, C., Gong, J., Li, J., Liu, D., Qin, Y., Ge, S., et al. (2022). Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. *Nat. Med.* 28 (6), 1189–1198. doi:10.1038/s41591-022-01800-8

Ramirez, M. I., and Marcilla, A. (2021). Pathogens and extracellular vesicles: new paths and challenges to understanding and treating diseases. Editorial opinion. *Mol. Immunol.* 139, 155–156. doi:10.1016/j.molimm.2021.09.006

Ranganath, T., Simpson, L. J., Ferreira, A. M., Seiler, C., Vendrame, E., Zhao, N., et al. (2020). Characterization of the impact of daclizumab beta on circulating natural killer cells by mass cytometry. *Front. Immunol.* 11, 714. doi:10.3389/fimmu.2020.00714

Reagan, P. M., and Neelapu, S. S. (2021). How I manage: pathophysiology and management of toxicity of chimeric antigen receptor T-cell therapies. *J. Clin. Oncol.* 39 (5), 456–466. doi:10.1200/JCO.20.01616

Reinhard, K., Rengstl, B., Oehm, P., Michel, K., Billmeier, A., Hayduk, N., et al. (2020). An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. *Science* 367 (6476), 446–453. doi:10.1126/science.aay5967

Reusing, S. B., Vallera, D. A., Manser, A. R., Vatrin, T., Bhatia, S., Felices, M., et al. (2021). CD16xCD33 Bispecific Killer Cell Engager (BiKE) as potential immunotherapeutic in pediatric patients with AML and biphenotypic ALL. *Cancer Immunol. Immunother.* 70 (12), 3701–3708. doi:10.1007/s00262-021-03008-0

Richardson, D. L., Moore, K. N., Vergote, I., Gilbert, L., Martin, L. P., Mantia-Smaldone, G. M., et al. (2024). Phase 1b study of mirvetuximab soravtansine, a folate receptor alpha (FRα)–targeting antibody-drug conjugate, in combination with carboplatin and bevacizumab in patients with platinum-sensitive ovarian cancer. *Gynecol. Oncol.* 185, 186–193. doi:10.1016/j.ygyno.2024.01.045

Rodriguez-Garcia, A., Minutolo, N. G., Robinson, J. M., and Powell, D. J. (2017). T-cell target antigens across major gynecologic cancers. *Gynecol. Oncol.* 145 (3), 426–435. doi:10.1016/j.ygyno.2017.03.510

Ruf, P., Bauer, H. W., Schoberth, A., Kellermann, C., and Lindhofer, H. (2021). First time intravesically administered trifunctional antibody catumaxomab in patients with recurrent non-muscle invasive bladder cancer indicates high tolerability and local immunological activity. *Cancer Immunol. Immunother.* 70 (9), 2727–2735. doi:10.1007/s00262-021-02930-7

Rui, R., Zhou, L., and He, S. (2023). Cancer immunotherapies: advances and bottlenecks. *Front. Immunol.* 14, 1212476. doi:10.3389/fimmu.2023.1212476

Saad, P., and Kasi, A. (2023). *Ipilimumab.* Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557795/ (Accessed April 10, 2023).

Sampson, J. H., Gunn, M. D., Fecci, P. E., and Ashley, D. M. (2020). Brain immunology and immunotherapy in brain tumours. *Nat. Rev. Cancer* 20 (1), 12–25. doi:10.1038/s41568-019-0224-7

Schuster, H., Peper, J. K., Bösmüller, H. C., Röhle, K., Backert, L., Bilich, T., et al. (2017). The immunopeptidomic landscape of ovarian carcinomas. *Proc. Natl. Acad. Sci.* 114 (46), E9942-E9951–E9951. doi:10.1073/pnas.1707658114

Shafabakhsh, R., Pourhanifeh, M. H., Mirzaei, H. R., Sahebkar, A., Asemi, Z., and Mirzaei, H. (2019). Targeting regulatory T cells by curcumin: a potential for cancer immunotherapy. *Pharmacol. Res.* 147, 104353. doi:10.1016/j.phrs.2019.104353

Shen, T., Huang, Z., Shi, C., Pu, X., Xu, X., Wu, Z., et al. (2020). Pancreatic cancer-derived exosomes induce apoptosis of T lymphocytes through the p38 MAPK-mediated endoplasmic reticulum stress. *FASEB J.* 34 (6), 8442–8458. doi:10.1096/fj.201902186r

Shimizu, A., Sawada, K., Kobayashi, M., Oi, Y., Oride, T., Kinose, Y., et al. (2024). Patient-derived exosomes as siRNA carriers in ovarian cancer treatment. *Cancers* 16 (8), 1482. doi:10.3390/cancers16081482

Siminiak, N., Czepczyński, R., Zaborowski, M. P., and Iżycki, D. (2022). Immunotherapy in ovarian cancer. *Archivum Immunol. Ther. Exp.* 70 (1), 19. doi:10.1007/s00005-022-00655-8

Sockolosky, J. T., Trotta, E., Parisi, G., Picton, L., Su, L. L., Le, A. C., et al. (2018). Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes. *Science* 359 (6379), 1037–1042. doi:10.1126/science.aar3246

Stump, C. T., Ho, G., Mao, C., Veliz, F. A., Beiss, V., Fields, J., et al. (2021). Remission-stage ovarian cancer cell vaccine with cowpea mosaic virus adjuvant prevents tumor growth. *Cancers* 13 (4), 627. doi:10.3390/cancers13040627

Tang, Q., Chen, Y., Li, X., Long, S., Shi, Y., Yu, Y., et al. (2022a). The role of PD-1/PD-L1 and application of immune-checkpoint inhibitors in human cancers. *Front. Immunol.* 13, 964442. doi:10.3389/fimmu.2022.964442

Tang, S., Qin, C., Hu, H., Liu, T., He, Y., Guo, H., et al. (2022b). Immune checkpoint inhibitors in non-small cell lung cancer: progress, challenges, and prospects. *Cells* 11 (3), 320. doi:10.3390/cells11030320

Tanyi, J. L., Chiang, C. L. L., Chiffelle, J., Thierry, A. C., Baumgartener, P., Huber, F., et al. (2021). Personalized cancer vaccine strategy elicits polyfunctional T cells and demonstrates clinical benefits in ovarian cancer. *npj Vaccines* 6 (1), 36. doi:10.1038/s41541-021-00332-5

Tapia-Galisteo, A., Álvarez-Vallina, L., and Sanz, L. (2023). Bi- and trispecific immune cell engagers for immunotherapy of hematological malignancies. *J. Hematol. & Oncol.* 16 (1), 83. doi:10.1186/s13045-023-01482-w

Taylor, D. D., Gerçel-Taylor, C., Lyons, K. S., Stanson, J., and Whiteside, T. L. (2003). T-cell apoptosis and suppression of T-cell receptor/CD3-zeta by Fas ligand-containing membrane vesicles shed from ovarian tumors. *Clin. Cancer Res.* 9 (14), 5113–5119.

Tchou, J., Zhao, Y., Levine, B. L., Zhang, P. J., Davis, M. M., Melenhorst, J. J., et al. (2017). Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. *Cancer Immunol. Res.* 5 (12), 1152–1161. doi:10.1158/2326-6066.CIR-17-0189

Thieblemont, C., Phillips, T., Ghesquieres, H., Cheah, C. Y., Clausen, M. R., Cunningham, D., et al. (2022). Epcoritamab, a novel, subcutaneous CD3xCD20 bispecific T-cell–engaging antibody, in relapsed or refractory large B-cell lymphoma: dose expansion in a phase I/II trial. *J. Clin. Oncol.* 41 (12), 2238–2247. doi:10.1200/jco.22.01725

Tian, W., Lei, N., Zhou, J., Chen, M., Guo, R., Qin, B., et al. (2022). Extracellular vesicles in ovarian cancer chemoresistance, metastasis, and immune evasion. *Cell death & Dis.* 13 (1), 64. doi:10.1038/s41419-022-04510-8

Tokatlian, T., Asuelime, G. E., Mock, J. Y., DiAndreth, B., Sharma, S., Toledo Warshaviak, D., et al. (2022). Mesothelin-specific CAR-T cell therapy that incorporates an HLA-gated safety mechanism selectively kills tumor cells. *J. Immunother. cancer* 10 (1), e003826. doi:10.1136/jitc-2021-003826

Tse, B. W., Collins, A., Oehler, M. K., Zippelius, A., and Heinzelmann-Schwarz, V. A. (2014). Antibody-based immunotherapy for ovarian cancer: where are we at? *Ann. Oncol.* 25 (2), 322–331. doi:10.1093/annonc/mdt405

Vallera, D. A., Ferrone, S., Kodal, B., Hinderlie, P., Bendzick, L., Ettestad, B., et al. (2020). NK-Cell-Mediated targeting of various solid tumors using a B7-H3 tri-specific killer engager *in vitro* and *in vivo*. *Cancers* 12 (9), 2659. doi:10.3390/cancers12092659

Van Coillie, S., Wiernicki, B., and Xu, J. (2020). Molecular and cellular functions of CTLA-4. *Adv. Exp. Med. Biol.* 1248, 7–32. doi:10.1007/978-981-15-3266-5_2

van de Donk, N. W. C. J., and Zweegman, S. (2023). T-cell-engaging bispecific antibodies in cancer. *Lancet* 402 (10396), 142–158. doi:10.1016/S0140-6736(23)00521-4

Wada, S., Yada, E., Ohtake, J., Fujimoto, Y., Uchiyama, H., Yoshida, S., et al. (2016). Current status and future prospects of peptide-based cancer vaccines. *Immunotherapy* 8 (11), 1321–1333. doi:10.2217/imt-2016-0063

Wang, B., Pei, J., Xu, S., Liu, J., and Yu, J. (2023a). Recent advances in mRNA cancer vaccines: meeting challenges and embracing opportunities. *Front. Immunol.* 14, 1246682. doi:10.3389/fimmu.2023.1246682

Wang, L., Luo, Y., Ren, S., Zhang, Z., Xiong, A., Su, C., et al. (2023b). A phase 1b study of ivonescimab, a PD-1/VEGF bispecific antibody, as first-or second-line therapy for advanced or metastatic immunotherapy naïve non-small-cell lung cancer. *J. Thorac. Oncol. Official Publ. Int. Assoc. Study Lung Cancer*, S1556–S0864. doi:10.1016/j.jtho.2023.10.014

Wang, Q., Chen, Y., Park, J., Liu, X., Hu, Y., Wang, T., et al. (2019). Design and production of bispecific antibodies. *Antibodies* 8 (3), 43. doi:10.3390/antib8030043

Wang, Q., Zhang, X., Li, B., Liu, X., Li, A., Li, H., et al. (2024). Tumor-derived exosomes promote tumor growth through modulating microvascular hemodynamics in a human ovarian cancer xenograft model. *Microcirculation* 31, e12876. doi:10.1111/micc.12876

Wu, B., Yu, C., Zhou, B., Huang, T., Gao, L., Liu, T., et al. (2017). Overexpression of TROP2 promotes proliferation and invasion of ovarian cancer cells. *Exp. Ther. Med.* 14 (3), 1947–1952. doi:10.3892/etm.2017.4788

Wu, Z., and Cheung, N. V. (2018). T cell engaging bispecific antibody (T-BsAb): from technology to therapeutics. *Pharmacol. & Ther.* 182, 161–175. doi:10.1016/j.pharmthera.2017.08.005

Xiao, Y., Bi, M., Guo, H., and Li, M. (2022). Multi-omics approaches for biomarker discovery in early ovarian cancer diagnosis. *EBioMedicine* 79, 104001. doi:10.1016/j.ebiom.2022.104001

Xu, L., Sun, H., Lemoine, N. R., Xuan, Y., and Wang, P. (2024). Oncolytic vaccinia virus and cancer immunotherapy. *Front. Immunol.* 14, 1324744. doi:10.3389/fimmu.2023.1324744

Yeh, J. M., Ward, Z. J., Chaudhry, A., Liu, Q., Yasui, Y., Armstrong, G. T., et al. (2020). Life expectancy of adult survivors of childhood cancer over 3 decades. *JAMA Oncol.* 6 (3), 350–357. doi:10.1001/jamaoncol.2019.5582

Yi-Ju, C., Abila, B., and Kamel, Y. M. (2023). CAR-T: what is next? *Cancers* 15 (3), 663. doi:10.3390/cancers15030663

Yim, K. H. W., Al Hrout, A., Borgoni, S., and Chahwan, R. (2020). Extracellular vesicles orchestrate immune and tumor interaction networks. *Cancers* 12 (12), 3696. doi:10.3390/cancers12123696

Zalatan, J. G., Petrini, L., and Geiger, R. (2024). Engineering bacteria for cancer immunotherapy. *Curr. Opin. Biotechnol.* 85, 103061. doi:10.1016/j.copbio.2023.103061

Zhang, X., He, T., Li, Y., Chen, L., Liu, H., Wu, Y., et al. (2021). Dendritic cell vaccines in ovarian cancer. *Front. Immunol.* 11, 613773. doi:10.3389/fimmu.2020.613773

Zhang, Z. J., Chen, X. H., Chang, X. H., Ye, X., Li, Y., and Cui, H. (2012). Human embryonic stem cells-a potential vaccine for ovarian cancer. *Asian Pac. J. cancer Prev. APJCP* 13 (9), 4295–4300. doi:10.7314/apjcp.2012.13.9.4295

Zhao, S., Mi, Y., Guan, B., Zheng, B., Wei, P., Gu, Y., et al. (2020). Tumor-derived exosomal miR-934 induces macrophage M2 polarization to promote liver metastasis of colorectal cancer. *J. Hematol. & Oncol.* 13 (1), 156. doi:10.1186/s13045-020-00991-2

Zhao, Y., Chen, G., Chen, J., Zhuang, L., Du, Y., Yu, Q., et al. (2023). AK112, a novel PD-1/VEGF bispecific antibody, in combination with chemotherapy in patients with advanced non-small cell lung cancer (NSCLC): an open-label, multicenter, phase II trial. *EClinicalMedicine* 62, 102106. doi:10.1016/j.eclinm.2023.102106

Zhou, M., Tang, Y., Xu, W., Hao, X., Li, Y., Huang, S., et al. (2023). Bacteria-based immunotherapy for cancer: a systematic review of preclinical studies. *Front. Immunol.* 14, 1140463. doi:10.3389/fimmu.2023.1140463

Zhou, W., Zhou, Y., Chen, X., Ning, T., Chen, H., Guo, Q., et al. (2021). Pancreatic cancer-targeting exosomes for enhancing immunotherapy and reprogramming tumor microenvironment. *Biomaterials* 268, 120546. doi:10.1016/j.biomaterials.2020.120546

Zhu, K., Ma, J., Tian, Y., Liu, Q., and Zhang, J. (2024). An immune-related exosome signature predicts the prognosis and immunotherapy response in ovarian cancer. *BMC women's health* 24 (1), 49. doi:10.1186/s12905-024-02881-y

Zhu, S. Y., and Yu, K. D. (2022). Breast cancer vaccines: disappointing or promising? *Front. Immunol.* 13, 828386. doi:10.3389/fimmu.2022.828386

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