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The role of immune checkpoints in gastrointestinal diseases

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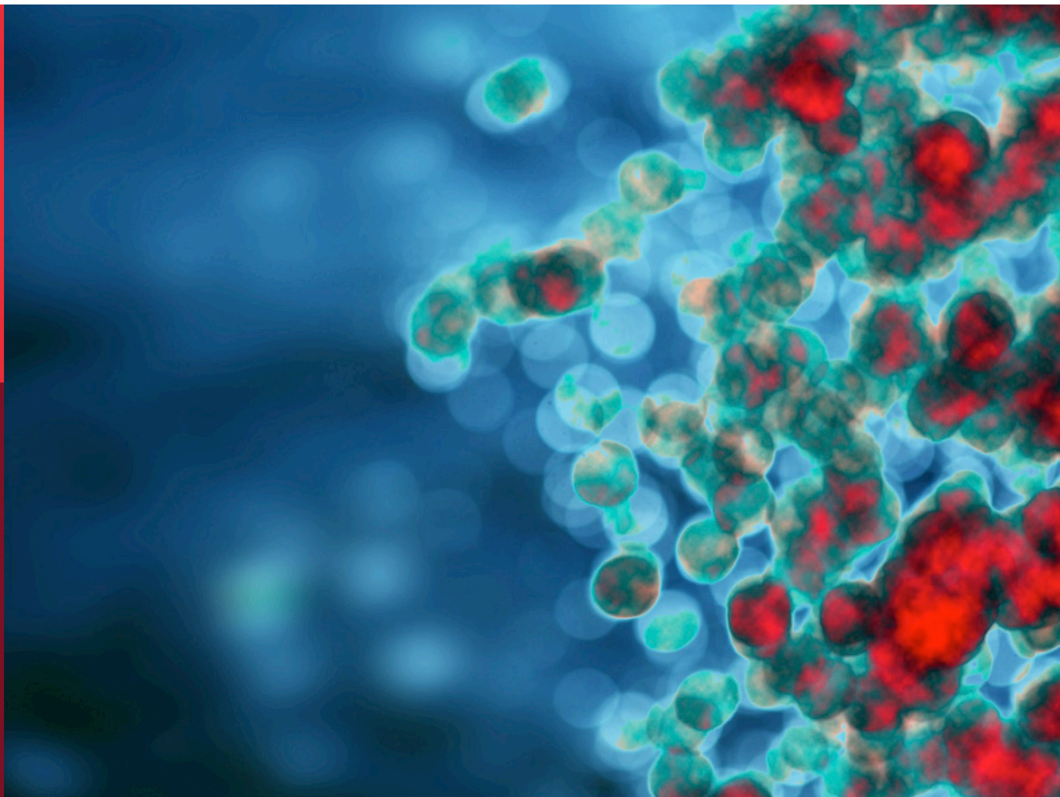

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The role of immune checkpoints in gastrointestinal diseases

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Editorial: The role of immune checkpoints in gastrointestinal diseases

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KEYWORDS

immune checkpoint inhibitor (ICI), treatment efficacy, gastrointestinal disease, immunotherapy, gut microbiota

Editorial on the Research Topic
The role of immune checkpoints in gastrointestinal diseases

Immunotherapy is one of the most cutting-edge fields in the current treatment of gastrointestinal (GI) diseases (Abdul-Latif et al., 2020). Immune checkpoint inhibitors (ICIs), as one of the most recognized immunotherapy strategies, have been gradually applied in the clinical treatment of GI tumors and have also been gradually explored in GI inflammatory diseases. However, whether ICIs can be used under the circumstances of most digestive diseases, and how to combined use of ICIs with other therapeutic drugs has still lack of clinical evidence. In addition, accumulating evidence has shown that unlike the success of cancer immunotherapies in certain cancer types like melanoma (Larkin et al., 2015), the overall response rate (ORR) of ICIs therapy in the non-selective GI patients is still not satisfactory even though these patients may have been predicted to be responsive based on the expression levels of molecules such as PD-L1 (Ganesh et al., 2019). Therefore, how to improve current prediction strategies so as to benefit more patients based on the expression patterns of immune checkpoints on different immune cells has also been an important research field. In this Research Topic, with the efforts of five guest editors, 12 articles consisting of 6 original researches, 4 reviews, and 2 case reports were collected, providing a deep understanding and new comprehensive insights of the application of immunotherapy in gastrointestinal diseases, especially in gastrointestinal cancers. These findings partly help to answer questions mentioned above in the research field of "*The role of immune checkpoints in gastrointestinal diseases*."

Most of the studies in this Research Topic were related to cancer process. Esophageal cancer (EC) is one of the deadliest malignancies due to its late-stage diagnosis, and immunotherapies, represented by ICIs, has gained promising perspectives for the treatment of patients with EC (Wadhwa et al., 2023). There is a lack of adequate evidence for the application of immunotherapies in treating patients with locally advanced EC. Qin et al. carried out a comprehensive meta-analysis to compare the efficacy and safety of the neoadjuvant use of ICIs combined with chemotherapy or chemoradiotherapy. Their results indicated that neoadjuvant immunotherapy could significantly improve the prognosis of patients with locally advanced EC, with acceptable toxicity. With regard to those with initially unresectable locally advanced EC, Huang et al. performed a real-world clinical trial and found that immunotherapy can offer patients a chance to receive a radical resection. Conversion surgery following immunochemotherapy was feasible and safe for these patients, with a better radiological and pathological response.

How about results on the application of ICIs in other locally advanced GI cancer? As one of the most common malignant tumors over the world, treatment strategy involving ICIs has already started in CRC, which has shown favorable outcomes against deficient mismatch repair (dMMR)/high levels of microsatellite instability (MSI-H) CRC (Schurch et al., 2020). Yang et al. reviewed recent findings about above achievements and proposed that adding immunotherapy into neoadjuvant therapy may change the treatment strategy of primary resectable or some metastatic CRC to reduce clinical stage but also to benefit patients to achieve a better local control. To test this hypothesis, the same group conducted a prospective, single-arm trial of long-course chemoradiotherapy combined with concurrent tislelizumab in locally advanced rectal cancer, to explore the safety and efficacy. Their results showed that long-course chemoradiotherapy combined with concurrent tislelizumab in patients with locally advanced low rectal cancer had favorable safety and efficacy, and did not increase the complication rate of surgery. Similar to these results, Chen et al. reviewed completed and ongoing clinical trials with ICIs in the area of gastroesophageal cancer (GEC). They found that ICIs combined with chemotherapy can be an effective first-line treatment and a monotherapy in second-line or more treatment and in maintenance therapy. To achieve a better response, Chen et al. also suggested that current biomarkers for predicting ICIs efficacy should be improved.

In consistent with above notion, one research group explored the expression patterns of immune checkpoints on cancer tissue and peripheral blood T cells in patients with gastric cancer. They found that the expression levels of immunosuppressive markers were significantly increased in cancer tissues and peripheral blood T cells, suggesting that peripheral blood analysis may be an important tool for prognostic assessment of patients with gastric cancer. Based on the co-expression of immune checkpoint molecules on T cells, does combined use of immune checkpoint inhibitors represent a potential promising strategy to improve current efficacy of ICIs? In one case report study, Peng et al. explored this aspect and found that patients with HER-2-positive advanced gastroesophageal junction cancer received PD-1/CTLA-4 bispecific immunotherapy combined with chemotherapy could achieve a complete remission.

Despite above aspects about the application of ICIs in treating GI cancers and strategies to predict and improve the efficacy of ICIs, toxicity is also a major problem to limit the use of ICIs (Tang et al., 2021). Zhou et al. reviewed findings about the adverse events of ICIs, especially for ICI-related colitis. They proposed that the gut microbiota acted as an important regulator in the pathogenesis of ICI-related colitis, and microbiota modulations like probiotics and fecal microbiota transplantation might be potential therapeutic strategy to treat these adverse events of ICIs.

In summary, the 12 articles in this Research Topic explore or discuss the application of ICIs in treating GI diseases, and provide potential strategies to predict and/or improve the efficacy of ICIs. Based on the importance of gut microbiota in predicting the efficacy of ICIs and their regulation in ICI-related adverse events (Lu et al., 2022), more insightful studies on the role and regulatory mechanisms of gut microbiota in participating ICIs responses are urgently needed, which may provide more promising therapeutic strategies in this area.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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

Abdul-Latif, M., Townsend, K., Dearman, C., Shiu, K. K., and Khan, K. (2020). Immunotherapy in gastrointestinal cancer: The current scenario and future perspectives. *Cancer Treat. Rev.* 88, 102030. doi:10.1016/j.ctrv.2020.102030

Ganesh, K., Stadler, Z. K., Cercek, A., Mendelsohn, R. B., Shia, J., Segal, N. H., et al. (2019). Immunotherapy in colorectal cancer: Rationale, challenges and potential. *Nat. Rev. Gastroenterol. Hepatol.* 16 (6), 361–375. doi:10.1038/s41575-019-0126-x

Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J. J., Cowey, C. L., Lao, C. D., et al. (2015). Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* 373 (1), 23–34. doi:10.1056/NEJMoa1504030

Lu, Y., Yuan, X., Wang, M., He, Z., Li, H., Wang, J., et al. (2022). Gut microbiota influence immunotherapy responses: Mechanisms and therapeutic strategies. *J. Hematol. Oncol.* 15 (1), 47. doi:10.1186/s13045-022-01273-9

Schurch, C. M., Bhate, S. S., Barlow, G. L., Phillips, D. J., Noti, L., Zlobec, I., et al. (2020). Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front. *Front. Cell.* 182 (5), 1341–1359. doi:10.1016/j.cell.2020.07.005

Tang, L., Wang, J., Lin, N., Zhou, Y., He, W., Liu, J., et al. (2021). Immune checkpoint inhibitor-associated colitis: From mechanism to management. *Front. Immunol.* 12, 800879. doi:10.3389/fimmu.2021.800879

Wadhwa, V., Patel, N., Grover, D., Ali, F. S., and Thosani, N. (2023). Interventional gastroenterology in oncology. *CA Cancer J. Clin.* 73 (3), 286–319. doi:10.3322/caac.21766

ORIGINAL RESEARCH
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Conversion Surgery Following Immunochemotherapy in Initially Unresectable Locally Advanced Esophageal Squamous Cell Carcinoma—A Real-World Multicenter Study (RICE-Retro)

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Purpose: The present study sets out to evaluate the feasibility, safety, and effectiveness of conversion surgery following induction immunochemotherapy for patients with initially unresectable locally advanced esophageal squamous cell carcinoma (ESCC) in a real-world scenario.

Materials and Methods: In this multi-center, real-world study (NCT04822103), patients who had unresectable ESCC disease were enrolled across eight medical centers in China. All patients received programmed death receptor-1 (PD-1) inhibitor plus chemotherapy every 3 weeks for at least two cycles. Patients with significant relief of cancer-related clinical symptoms and radiological responsive disease were deemed surgical candidates.

Feasibility and safety profile of immunochemotherapy plus conversion surgery, radiological and pathological tumor responses, as well as short-term survival outcomes were evaluated. Moreover, data of an independent ESCC cohort receiving induction chemotherapy (iC) were compared.

Results: One hundred and fifty-five patients were enrolled in the final analysis. Esophagectomy was offered to 116 patients, yielding a conversion rate of 74.8%. R0 resection rate was 94%. Among the 155 patients, 107 (69.0%) patients experienced at least one treatment-related adverse event (TRAE) and 45 (29.0%) patients reported grade 3 and above TRAEs. Significant differences in responsive disease rate were observed between iC cohort and induction immunochemotherapy (iIC) cohort [objective response rate: iIC: 63.2% vs. iC: 47.7%, $p = 0.004$; pathological complete response: iIC: 22.4% vs. iC: 6.7%, $p = 0.001$). Higher anastomosis fistula rate was observed in the iC group (19.2%) compared with the iIC group (4%). Furthermore, Significantly higher event-free survival was observed in those who underwent conversion surgery.

Conclusion: Our results supported that conversion surgery following immunochemotherapy is feasible and safe for patients with initially unresectable locally advanced ESCC. Both radiological and pathological response rates were significantly higher in the iIC cohort compared with those in the traditional iC cohort.

Keywords: esophageal squamous cell carcinoma, conversion surgery, immunotherapy, effectiveness, real-world study

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) could easily penetrate the esophageal wall and invade adjacent organs due to the lack of serosa (1). According to the National Comprehensive Cancer Network guideline, cT4b tumors with evident involvement of the adjacent organs (aorta, trachea, or bronchus) or had multi-station, bulky lymphadenopathy are considered unresectable (ESOPH-C, 1 of 3) (2). The current standard of care for the unresectable locally advanced ESCC is definitive chemoradiation or systemic chemotherapy alone (if local therapy is not indicated) (2); however, the treatment outcomes remain dismal (3). Limited progress has been made in treating unresectable locally advanced ESCC. Thus, novel effective therapeutics are needed.

The emergence of immune checkpoint inhibitors (ICIs) has revolutionized the treatment of advanced or metastatic gastroesophageal cancers (4–7). Recently, the largest randomized, placebo-controlled, phase 3 study (KEYNOTE-590) to date had confirmed better survival benefits of pembrolizumab plus chemotherapy over placebo plus chemotherapy in 749 patients with unresectable locally advanced or metastatic EC. The combination of ICIs and chemotherapy also demonstrated a comparable safety profile to chemotherapy alone (≥G3 TRAEs, 72% vs. 68%) (6). Because of the exciting results released by these clinical trials, NCCN recommended immunotherapy combined with chemotherapy as the first-line treatment for both unresectable locally advanced and metastatic disease (ESOPH-F, 3 of 17) (2). Further, Fan et al. reported that the initially unresectable locally advanced ESCC could be transformed into surgical candidates after receiving immunochemotherapy, and the conversion rate reached 75% (8). Furthermore, recent studies showed that patients receiving induction chemoradiotherapy or chemotherapy followed by conversion surgery could have a better prognosis than those without surgery (9). However, currently, there lacks strong evidence to support the application of conversion surgery following immunochemotherapy in initially unresectable locally advanced ESCC. Hence, this study aimed to evaluate the feasibility, safety, and effectiveness of conversion surgery following induction immunochemotherapy (iIC) for initially unresectable ESCC in a real-world scenario.

MATERIALS AND METHODS

Study Design and Participants

The study was designed to be a multi-center and real-world retrospective study (RICE-retro, real-world study of ICI and chemotherapy for advanced esophageal cancer) to investigate the feasibility, safety, and effectiveness of induction ICIs plus chemotherapy at the Guangdong Provincial People's Hospital, the First Affiliated Hospital of Shantou University Medical College, the First Affiliated Hospital of Sun Yat-sen University, the Affiliated Cancer Hospital of Guangzhou Medical University, General Hospital of Southern Theater Command, Gaozhou People's Hospital, Shenzhen People's Hospital and Shantou Central Hospital. The study protocol was reviewed and approved by the Institutional Review Board (IRB) at each participating institution and registered at ClinicalTrials.gov (NCT04822103). Eligible patients were at least 18 years old with an endoscopy-guided, histologically confirmed ESCC, who were deemed unsuitable radiotherapy candidates by radiation oncologists and have radiologically confirmed unresectable cT4b tumors with evident involvement of the adjacent organs (aorta, trachea, or bronchus) or had multi-station, bulky lymphadenopathy before treatment. Confirmed diagnosis of organ invasions was based on the previously reported criteria (1, 10). All patients were treatment-naive, with a Karnofsky Performance Scale (KPS) ≥ 80, adequate organ function, and no distant metastasis. Patients who had previously participated in other interventional clinical trials during their preoperative treatment were excluded from this study. Before the initiation of iIC, all patients received the endoscopy-guided biopsy and contrast-enhanced positron emission tomography (PET)/computed tomography (CT) for diagnostic workup. The clinical and pathologic staging were determined by the surgeons, radiologists and pathologists based on the eight edition staging system of the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC). Baseline measurement of tumor lesions and lymph nodes was based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (11).

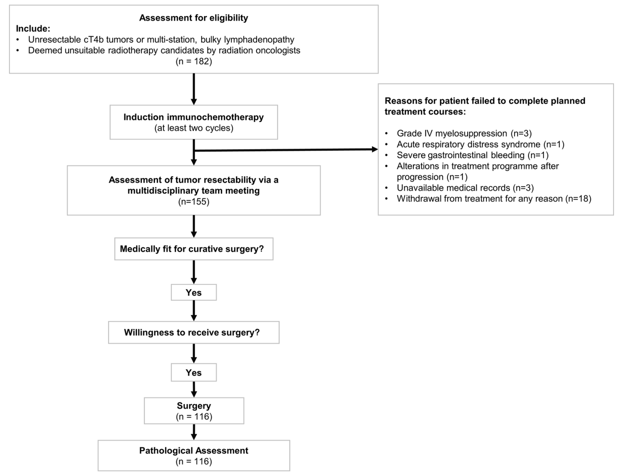

FIGURE 1 | Flowchart of the induction treatment course for initially unresectable esophageal squamous cell carcinoma patients. Patients who met the inclusion criteria received at least two cycles of immunochemotherapy. Patients who failed to complete planned cycles of treatment were excluded from the subsequent analysis. Assessment of tumor response was conducted *via* a multidisciplinary team meeting. McKeown esophagectomy and Ivor Lewis esophagectomy would be performed on medically fit individuals with willingness to receive surgery.

Treatment Regimen

The ICIs administered in the current study were PD-1 inhibitors (camrelizumab, pembrolizumab, sintilimab, tislelizumab, toripalimab, and nivolumab), which were administered intravenously at a fixed dose of 200 mg every 3 weeks. The chemotherapy regimen included platinum-based plus docetaxel- or taxane-based agents every 3 weeks intravenously with their doses adjusted by patients' general condition and the liver or renal functions. All participants enrolled were fully informed of all alternative regimens and provided written consents.

Three to four weeks after the completion of at least two cycles of iIC, contrast-enhanced thoracoabdominal CT or PET/CT was performed for disease evaluation. Tumor responses were denoted by complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

A multidisciplinary team meeting was held during each patient's radiological evaluation of tumor response. In general, patients with significant relief of cancer-related clinical symptoms and radiological CR/PR diseases were deemed surgical candidates. For patients whose condition was evaluated as SD status, conversion surgery would be performed only if the shrinkage extent of both primary tumor and lymph nodes enables the formation of clear tumor-and-adjacent organ boundary. Furthermore, surgery was deemed unsuitable for those with radiologically confirmed PD. Flowchart of the study design was presented in **Figure 1**.

Surgery and Pathological Assessments

Minimally invasive esophagectomy with two-field or three-field lymphadenectomy was performed on medically fit patients. McKeown and Ivor Lewis esophagectomy were the two primary surgical approaches. The pathological examination was performed and re-evaluated by two pathologists independently according to the standardized pathological assessment protocol adopted by all research centers to minimize the interobserver variability. Tumor regression grade (TRG) was calculated according to Becker system, a four-tier scoring system estimating the percentage of residual tumor in relationship to the macroscopically identifiable tumor bed (12). Immunohistochemistry staining was performed for PD-1 (clone: MRQ-22, Abcam, 1:50) and PD-L1 (clone: 22C3, Abcam, 1:500), with their expression levels presented as the combined positive score (CPS). CPS was defined as the number of PD-L1 staining cells (tumor cell, lymphocytes, and macrophages) divided by total number of viable tumor cells, multiplied by 100 (13).

Outcome Evaluation

Feasibility of iIC was defined as at least 80% of the patients completed all planned courses of iIC. Feasibility of conversion surgery was defined as at least 80% of the patients were medically fit for surgery after completion of iIC. Objective response rate (ORR) was defined as best overall response of complete or PR rate, per RECIST version 1.1. The safety profile was assessed by the proportion of participants with ≥ grade 3 adverse events as defined by Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (14). Confirmation of the relationship between AEs and the drugs in use was based on the WHO-UMC Causality Categories (15). The key secondary end point was pathological CR (pCR) defined as the absence of invasive/*in situ* cancer in the primary lesion site. Major pathological response (mPR) was defined as ≤10% residual viable tumor following iIC (16). R0 resection was defined as the rate of negative margins microscopically (including circumferential resection margin). Event-free survival (EFS) was calculated from the date of treatment initiation to the date of first progression (local recurrence of tumor or distant metastasis) or death from any cause (17). Patients who were lost to follow up or still alive at the time of final analysis were classified as censored data. Downstaging of primary tumor, nodal, or combined TNM stage was recorded if the stage obtained from the pathological examination was earlier than the pretreatment clinical stage (18).

Furthermore, to compare the oncological outcomes of RICE-retro with conventional preoperative chemotherapy, data of a cohort of patients with ESCC receiving induction chemotherapy (iC) from these centers were retrospectively analyzed.

Statistical Analysis

Descriptive data were reported as mean ± standard deviation (SD), median [interquartile range (IQR)], or frequency (percentage). Comparisons of continuous variables were performed using the Student's t-test or the Wilcoxon rank sum test as appropriate. Categorical clinicopathological variables were compared by using the Chi-square test or Fisher's exact test. Two-sided $P < 0.05$ was considered statistically significant in all tests. All statistical analyses were performed using the software "Statistical Package for Social Science" (SPSS) version 26 for Windows (SPSS, Inc., Chicago, Illinois) and R 4.0.0 (R Core Team 2020) (19). High-quality figures were generated using the R packages.

RESULTS

Clinicopathological *Characteristics*

From November 2019 to June 2021, 182 patients with ESCC were included at eight institutions in China and finally 155 patients completed the planned treatment courses. The date of the last follow-up was October 1, 2021. Most patients were male (121 of 155, 78.1%), and the median age was 61 years (IQR, 55–66 years). Baseline clinicopathologic information was presented in **Table 1**. Most tumors were located in the middle (48.4%) and lower (39.4%) portion of the thoracic esophagus. Twenty-one patients had clinical stage III disease before surgery, whereas stage IV disease accounted for 86.5% (n = 134) of patients.

Feasibility

The proportion of patients with successful completion of planned treatment course was 85.2% (155 of 182). Patients failed to complete induction treatment were due to grade IV myelosuppression (3 of 182, 1.6%), acute respiratory distress syndrome (1 of 182, 0.5%), severe gastrointestinal bleeding (1 of 182, 0.5%), and alterations in treatment program after progression (1 of 182, 0.5%). Other patients were excluded from final analysis due to unavailable medical records that would hamper statistical analysis (3 of 182, 1.6%) and withdrawal from treatment for any reason (18 of 182, 9.9%).

Upon the completion of induction treatment, 126 of 155 (81.3%) patients were considered suitable for conversion surgery. Ten patients were unwilling to undergo surgery. Finally, esophagectomy was then offered to the remaining 116 patients, yielding a conversion rate of 74.8%.

Safety

Among the 155 patients, 107 (69.0%) patients experienced at least one treatment-related adverse event (TRAE) and the common TRAEs included fatigue (80 of 155, 51.6%), nausea (64 of 155, 41.3%), and diarrhea (47 of 155, 30.3%). Grade 3 and above (grade ≥ 3) TRAEs were found by 29.0% (45 of 155) of the patients, including leukopenia (20 of 155, 12.9%), neutropenia (18 of 155, 11.6%), rash (12 of 155, 7.7%), diarrhea (6 of 155, 3.9%), and infection (6 of 155, 3.9%). There were immune-related skin toxicities, including pruritus (47 of 155, 30.3%) and rash (44 of 155, 28%) of any grade. The details of TRAEs observed in our study cohort were shown in **Table 2**, and a clinical heatmap was used to depict the association between clinicopathological characteristics such as radiological tumor response and each type of adverse event (**Supplementary Figure 1**).

The median postoperative time length of hospital stay (PLOS) was 11 (IQR, 8–14) days, and the median operative time was 325 min (IQR, 260–390). Intraoperative blood loss was 100 ml (IQR, 50–100). Postoperative complications are summarized in **Table 2**. Of the 116 patients, five patients (4%) experienced

TABLE 1 | Clinicopathological characteristics of the RICE cohort.

Characteristics	No. (%)
Sex	
Male	121 (78.1)
Female	34 (21.9)
Age (years)	
Median	61
IQR	55-66
KPS	
80	14 (9.0)
90	141 (91.0)
History of smoking	
Yes	85 (54.8)
No	70 (45.2)
History of drinking	
Yes	63 (40.6)
No	92 (59.4)
Family oncological history	
Yes	33 (21.3)
No	122 (78.7)
Tumor location	
Thoracic upper portion	19 (12.3)
Thoracic middle portion	75 (48.4)
Thoracic lower portion	61 (39.4)
cT	
cT2	1 (0.6)
cT3	24 (15.5)
cT4a	28 (18.1)
cT4b	102 (65.8)
cN	
cN0	60 (38.7)
cN1	65 (41.9)
cN2	24 (15.5)

(Continued)

TABLE 1 | Continued

Characteristics	No. (%)
cN3	6 (3.9)
cTNM	
III	21 (13.5)
IVA	134 (86.5)
pT	
pT0	26 (22.4)
pTis	11 (9.5)
pT1a	10 (8.6)
pT1b	20 (17.2)
pT2	17 (14.7)
pT3	32 (27.6)
pN	
pN0	84 (72.4)
pN1	21 (18.1)
pN2	10 (8.6)
pN3	1 (0.9)
pTNM	
I	68 (58.6)
II	16 (13.8)
IIIA	13 (11.2)
IIIB	18 (15.5)
IVA	1 (0.9)
Lymphovascular invasion	
Yes	9 (7.8)
No	107 (92.2)
Perineureal invasion	
Yes	9 (7.8)
No	107 (92.2)
R0	
R0	104 (94)
R1	7 (6)

Variables are described as n(%) or median [interquartile range (IQR)]. cT, clinical tumor stage; cN, clinical nodal stage; cTNM, clinical tumor-nodal-metastatic stage; pT, pathological tumor stage; pN, pathological nodal stage; pTNM, pathological tumor-nodal-metastatic stage.

anastomosis fistula, and one patient died within 30 days after surgery.

Significant differences in mean PLOS (iC vs. iIC: 18 ± 14 days vs. 12 ± 9 days, p = 0.005), mean operative time (iC vs. iIC: 395 ± 109 min vs. 332 ± 87 min, p = 0.023), and mean intraoperative blood loss (iC vs. iIC: 199 ± 156 ml vs. 110 ± 88 ml, p = 0.001) were observed between the iC and iIC groups. Higher anastomosis fistula rate was observed in the iC group (19.2%) compared with the iIC group (4%). The details of postoperative events of iC group are provided in **Supplementary Table 1**.

Effectiveness

Of the 155 patients, six patients (3.9%) achieved radiological CR, 92 patients achieved PR (59.4%), and 45 patients (29%) achieved SD. The ORR and DCR were 63.3% and 92.3%, respectively. A typical case presenting the radiological assessment before and after iIC was shown in **Figure 2**. We categorized patients into radiological responders and radiological non-responders. Responsive disease included CR and PR, whereas unresponsive disease included SD and progression disease. Significant difference in responsive disease rate was observed between the iC cohort and iIC cohort (iIC: 98 of 155, 63.2% vs. iC: 94 of 197, 47.7%, p = 0.004) (**Figure 3A**).

In terms of pathological responses, pCR of the primary tumor was observed in 22.4% (26 of 116) of the patients. mPR was observed in 57.8% (67 of 116) of the patients. R0 resection was achieved in 109 of the 116 patients. **Figure 3** showed both radiological and pathological response rates between iC cohort and iIC cohort. Statistically significant differences in pCR (iIC: 26 of 116, 22.4% vs. iC: 8 of 120, 6.7%, p = 0.001) was revealed (**Figure 3B**). The TRG scores were: TRG 1a (ypT0, 26 of 116, 22.4%), TRG1b (48 of 116, 41.4%), TRG 2 (16 of 116, 13.85%), and TRG 3 (26 of 116, 22.4%). Swimmer plot (**Supplementary Figure 2A**) and waterfall plot (**Supplementary Figure 2B**) were used to depict treatment course and treatment response of the patients. Downstaging of tumor stage was achieved in 111 (of 116, 95.7%) patients. Moreover, downstaging of clinical N stage

TABLE 2 | Adverse events during immunochemotherapy and after surgery.

Event	No. (%)
Events of any grade during immunochemotherapy	
• Nausea	64 (41)
• Vomiting	38 (25)
• Diarrhea	47 (30)
• Constipation	24 (15)
• Dyspnea	15 (10)
• Rash	44 (28)
• Pruritus	47 (30)
• Infection	11 (7)
• Pain	39 (25)
• Fatigue	80 (52)
• Leukopenia	33 (21)
• Neutropenia	32 (21)
• Lymphopenia	14 (9)
• Anemia	21 (14)
• Thrombocytopenia	5 (3)
Events of grade ≥ 3 during immunochemotherapy	
• Nausea	5 (3)
• Vomiting	4 (3)
• Diarrhea	6 (4)
• Constipation	0 (0)
• Dyspnea	1 (1)
• Rash	12 (8)
• Pruritus	3 (2)
• Infection	6 (4)
• Pain	2 (1)
• Fatigue	3 (2)
• Leukopenia	20 (13)
• Neutropenia	18 (12)
• Lymphopenia	3 (2)
• Anemia	3 (2)
• Thrombocytopenia	0 (0)
Postoperative events	
• Heart issues	3 (3)
• Pneumonia	10 (9)
• Atelectasis	10 (9)
• Pleural effusion	8 (7)
• Anastomosis fistula	5 (4)
• Wound infection	2 (2)
• Hoarseness	2 (2)
• Hypoxia	2 (2)
• Dysphagia	0 (0)
• Hemothorax	0 (0)
• Chylothorax	0 (0)
• Mediastinitis	0 (0)
• Death	1 (1)

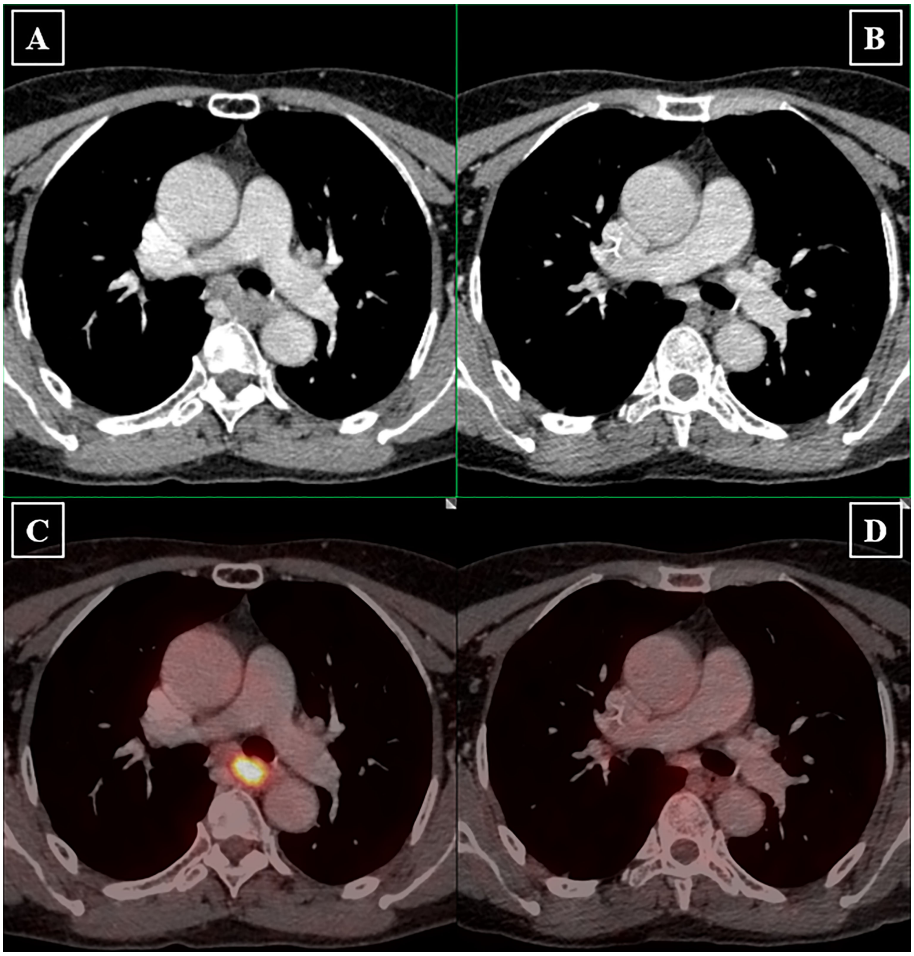

FIGURE 2 | Radiological assessment before and after induction immunochemotherapy. Longest diameters in the plane of measurement of primary lesions were recorded. Lymph nodes were considered malignant if the short axis is longer than 1.5 mm. Pretreatment clinical staging of primary tumor and lymph nodes were determined by both the physician in charge and radiologists. **(A)** Pretreatment PET-CT image shows that the primary tumor is large, irregular in shape with evident left bronchial compression. The normal esophageal lumen disappears due to extensive thickening of the esophageal wall. **(B)** Posttreatment PET-CT image shows that significant tumor shrinkage provides clear demarcation between primary tumor and the left bronchus. Esophageal lumen reappears. **(C)** Pretreatment PET-CT image presents hypermetabolic characteristic of the primary tumor. **(D)** Subsequent PET-CT image revealed tumor metabolic value reduced to background level.

was achieved in 41% (48 of 116) of patients, whereas 11.2% (13 of 116) of patients had an upstaging in N stage postoperatively.

Overall, 12 (of 155; 7.7%) patients had radiological PDs and 10 (of 155; 6.5%) patients died during follow-up. Significantly higher EFS was observed in those who underwent conversion surgery than those in the non-surgery group (**Figure 4A**). Those had a mPR status also demonstrated a significantly higher EFS than the non-mPR cohort (**Figure 4B**), and there is a statistical difference between pCR and non-pCR patients. Further, the survival plot showed a trend of better EFS in surgical candidates who actually received surgery as subsequent treatment than those who declined surgery regardless of their medical fitness upon preoperative evaluation (**Supplementary Figure 3**).

Expression of PD-L1 of Clinical Specimens

The PD-L1 CPS scores of the surgical candidates were evaluated and compared. No significant association was found among patients with different TRGs (p = 0.206). Moreover, PD-L1 expression did not correlate significantly with both pathological and radiological responses (p = 0.486).

DISCUSSION

Conversion surgery following iIC for initially unresectable locally advanced ESCC has been reported (8). However, real-world evidence is currently unavailable. The feasibility of immunochemotherapy in the present study was 85.2%, which was comparable to both induction chemoradiotherapy and chemotherapy alone. Moreover, the previously reported conversion rates in induction chemoradiotherapy ranged from 42.6% to 69% (1, 9, 20), and the conversion rates in iC fell between 32% and 65% (1, 10, 21, 22), which were lower than the

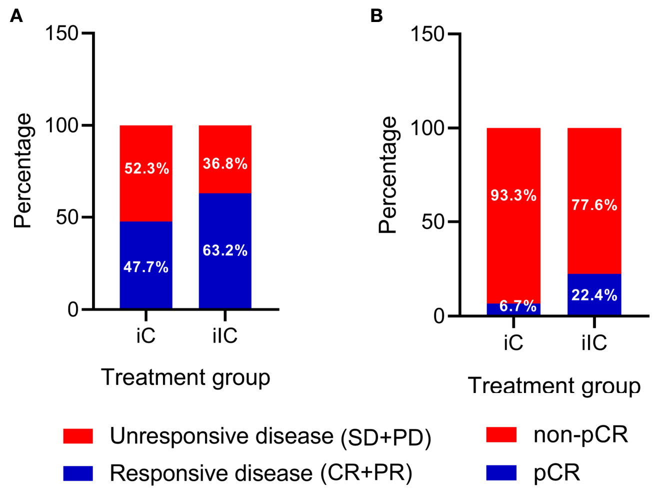

FIGURE 3 | Radiological and pathological responses between induction chemotherapy and induction immunochemotherapy. **(A)** Radiological assessment of tumor responses. Significantly higher responsive disease rate was observed in the iIC cohort. **(B)** Pathological assessment of tumor responses. Significantly higher pCR rate was observed in the iIC cohort. Responsive disease included complete response and partial response. Unresponsive disease included stable disease and progression disease. iC, induction chemotherapy; iIC, induction immunochemotherapy.

74.8% reported in the current study. This finding suggested that induction immunochemotherapy could improve the curative resection rates in patients with initially unresectable ESCC. Furthermore, it was found that conversion surgery could bring about significantly higher EFS than those without conversion surgery (**Figure 4A**). Taken together, induction immunochemotherapy plus conversion surgery may benefit more patients due to its high feasibility and potential survival benefit than the current standard-of-care approach.

Generally, the total grade ≥ 3 TRAEs of iIC incidence was relatively manageable and acceptable. A higher incidence of grade ≥ 3 TRAEs was reported in several studies in which induction therapy was adopted (1, 21). Sugimura et al. reported that in iC, the incidence of grade ≥ 3 neutropenia was 41% and the incidence of lymphopenia was 12% (1), which were higher than those in the current study. The safety profile of the current study was similar to the studies conducted by Cheng et al. (23) and Gu et al. (24), indicating that chemotherapy in combination with immunotherapy may not enhance accumulative toxicities compared with chemotherapy alone. Moreover, the safety profiles of immunochemotherapy were comparable and manageable in both the induction and the neoadjuvant settings. However, immune-related TRAEs such as rash and pruritus were not reported in the chemotherapy and radiotherapy-based cohorts. It was reported that immunotherapy could increase activation of B cells, which further release excessive inflammatory cytokines and thus leads to cutaneous adverse events (25). These results suggested that the safety profile of iIC was comparable to that of standard preoperative treatment for initially unresectable ESCC. Although these studies had heterogenous designs, sample sizes, and ethnic disparities, their consensus results indicated that immunochemotherapy was safe to use in the induction settings for advanced esophageal cancer. Despite this, the severe adverse

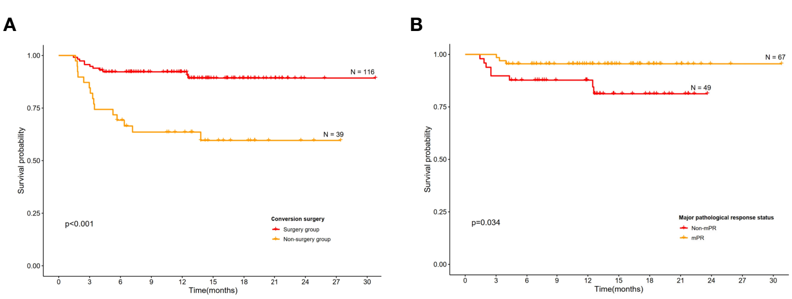

FIGURE 4 | Kaplan–Meier curves for event-free survival. **(A)** Conversion surgery group versus non-surgery group among all patients. **(B)** Event-free survival according to major pathological complete response.

events in certain individuals could not be neglected. It remains problematic to identify patients in danger of grade ≥ 3 TRAEs in advances. Larger-scale studies are needed to address this issue.

Compared with the iC cohort, intraoperative events in the RICE-retro cohort such as median operative time and blood loss were more favorable. Furthermore, the occurrences of postoperative complications in the RICE-retro cohort were also significantly lower than those in the iC cohort, indicating that the conversion surgery following immunochemotherapy did not bring about more intraoperative or postoperative burdens to both surgeons and patients. It was reported that dense fibrosis in the esophageal mesentery occurred after induction immunochemotherapy, which increased the difficulty of surgery (8). However, in this multicenter, real-world study, despite the formation of scar tissues, we discovered that the significant shrinkage of primary tumor actually lowered the surgical difficulty.

We also evaluated the effectiveness of iIC, which had achieved a promising ORR (63.3%), DCR (92.3%), and pCR (22.4%). The ORR varied from 20.2% to 72% in studies concerning iC plus radiotherapy (1, 21). The ORR derived from RICE-retro cohort falls within the upper range. The current study demonstrated that iIC had a superior radiological response rate over iC alone. The synergistic effect of chemotherapy and immunotherapy has been explored in the molecular level. Research showed that chemotherapy could downregulate coinhibitory molecules such as PD-L1 on the surface of cancer cells (26). Moreover, the combination of ICIs and chemotherapy could synergistically induce antigen-specific immunity and enhance the infiltration of $CD8^+$, and $CD4^+$FoxP3 T cells to the tumor microenvironment (27). However, the pCR rate of iIC did not have distinct advantage over that of other induction regimens, indicating that local cancer therapy such as radiotherapy, if applicable, may be needed to improve the locoregional therapeutic efficacy.

In terms of the use of immunochemotherapy in the neoadjuvant setting, Li et al. reported that ORR and pCR reached 100% and 56%, respectively, in PALACE-1 (28). Other phase II clinical trials adopting neoadjuvant immunochemotherapy reported that ORR ranged from 66.7% to 85% and pCR ranged from 16.7% to 45.4% (23, 28–36). Compared with these studies, the disease response rate reported by RICE-retro study appeared to be lower than most neoadjuvant immunochemotherapy studies. There are several possible explanations for this result. First, in this real-world study, most participants had more advanced tumor and nodal stages and therefore later clinical stages. Immunotherapy combined with chemotherapy achieved poorer effectiveness in patients with a more advanced pretreatment clinical stage (7). Even so, RICE-retro indicated that 95.7% of patients achieved T downstaging and that more than one-third of the patients achieved N downstaging. Second, the difference in sample sizes between RICE-retro and these clinical trials should be taken in consideration. The number of participants vary from 13 to 56 patients in other clinical trials, whereas 155 patients were included in RICE-retro cohort. Larger sample size may not necessarily guarantee robustness of the conclusion. However, a relatively larger amount of data generated from the multicenter studies could reduce potential bias as well as provide more generalized evidence. Third, the unstandardized pathological assessment such as incomplete specimen sampling may generate false-negative results which cause highly inflated pCR rate. It was noteworthy that the recorded pCR from RICE-retro reached 22.4% after re-evaluating the slides from the enrolled centers according to a standardized protocol. Insufficient information regarding pCR assessment process was provided by different medical centers that investigate the efficacy or effectiveness of iIC; thus, a high pCR rate should be cautiously interpreted.

To the best of our knowledge, this is the first and largest multicenter real-world study investigating the feasibility, effectiveness, and safety profiles of iIC in patients with ESCC. Despite the retrospective nature, the current study provides unique real-world data that reflected the pragmatic clinical practice differing from the ideal setting of clinical trials. Nonetheless, this study also had several limitations. First, the endoscopic ultrasonography was not applied to all patients in the pretreating assessment of tumor stage because some tumors were too bulky for the endoscope to pass through the esophageal tract. However, similar to that reported by Hashimoto et al., the pathologists observed evidence of tumor regression changes in all layers of the esophageal walls in the resected specimen (37). Second, the effectiveness or safety profiles should be cautiously interpreted due to the implementation of miscellaneous ICIs in our study and their potentially different pharmacodynamics and pharmacokinetics.

Our results supported that conversion surgery following immunochemotherapy is feasible and safe for patients with initially unresectable locally advanced ESCC. Both radiological and pathological response rates were significantly higher in the iIC cohort compared with those in the iC cohort. These findings provide new insight into the role of iIC, further larger-scale studies are needed to establish the standard-of-care use of iIC in the preoperative settings for patients with initially unresectable ESCC.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in the paper and/or the **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of Guangdong Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SH, HW, and CC: Conceptualization, Data curation, Formal analysis, Validation, Roles/Writing - original draft, Writing - review & editing. MZ, EX, WL, GW, JT, XB, DZ, LX, HYZ, GC, WZ, YT, FX, ZD, ZX, FW, ZH, HZ, XS, ZL, TS, JL, SY, and SX: Methodology, Resources, Writing - review & editing. GQ and JF:

Conceptualization, Project administration, Resources, Supervision, Writing - review & editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.935374/full#supplementary-material

Supplementary Figure 1 | Association between treatment-related adverse events and clinicopathological information. The clinical heatmap depicts the included individuals' clinicopathological features and their reported adverse events. Different color blocks were used to classify each clinicopathological features. Darkness of red color represents severity of the treatment-related adverse events.

Supplementary Figure 2 | Swimmer plot **(A)** and waterfall plot **(B)**. **(A)** The swimmer plot depicts each patient as one line. C1 represents the first cycle from initiation of the first immunochemotherapy to initiation of the second immunochemotherapy and so on. Various colors and shapes are used to represent the radiological outcomes and treatment-related adverse events. **(B)** Maximum radiological response from baseline. Color blocks represent different radiological outcome per RECIST 1.1.

Supplementary Figure 3 | Event-free survival according to willingness to undergo conversion surgery among surgical candidates.

REFERENCES

1. Sugimura K, Miyata H, Tanaka K, Makino T, Takeno A, Shiraishi O, et al. Multicenter Randomized Phase 2 Trial Comparing Chemoradiotherapy and Docetaxel Plus 5-Fluorouracil and Cisplatin Chemotherapy as Initial Induction Therapy for Subsequent Conversion Surgery in Patients With Clinical T4b Esophageal Cancer: Short-Term Results. *Ann Surg* (2021) 274(6): e465–e72. doi: 10.1097/SLA.0000000000004564
2. National Comprehensive Cancer Network. *Esophageal and Esophagogastric Junction Cancers (Version 1.2022)* (2022). Available at: https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf.
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(2):115–32. doi: 10.3322/caac.21338
4. Wang ZX, Cui C, Yao J, Zhang Y, Li M, Feng J, et al. Toripalimab Plus Chemotherapy in Treatment-Naïve, Advanced Esophageal Squamous Cell Carcinoma (JUPITER-06): A Multi-Center Phase 3 Trial. *Cancer Cell* (2022) 40(3):277–88.e3. doi: 10.1016/j.ccell.2022.02.007
5. Doki Y, Ajani JA, Kato K, Xu J, Wyrwicz L, Motoyama S, et al. Nivolumab Combination Therapy in Advanced Esophageal Squamous-Cell Carcinoma. *N Engl J Med* (2022) 386(5):449–62. doi: 10.1056/NEJMoa2111380
6. Sun JM, Shen L, Shah MA, Enzinger P, Adenis A, Doi T, et al. Pembrolizumab Plus Chemotherapy Versus Chemotherapy Alone for First-Line Treatment of Advanced Oesophageal Cancer (KEYNOTE-590): A Randomised, Placebo-Controlled, Phase 3 Study. *Lancet* (2021) 398(10302):759–71. doi: 10.1016/S0140-6736(21)01234-4
7. Luo H, Lu J, Bai Y, Mao T, Wang J, Fan Q, et al. Effect of Camrelizumab vs Placebo Added to Chemotherapy on Survival and Progression-Free Survival in Patients With Advanced or Metastatic Esophageal Squamous Cell Carcinoma: The ESCORT-1st Randomized Clinical Trial. *Jama* (2021) 326 (10):916–25. doi: 10.1001/jama.2021.12836
8. Fan M, Dai L, Yan W, Yang Y, Lin Y, Chen K. Efficacy of Programmed Cell Death Protein 1 Inhibitor in Resection Transformation Treatment of Esophageal Cancer. *Thorac Cancer* (2021) 12(15):2182–8. doi: 10.1111/1759-7714.14054
9. Miyata H, Sugimura K, Motoori M, Omori T, Yamamoto K, Yanagimoto Y, et al. Clinical Implications of Conversion Surgery After Induction Therapy for T4b Thoracic Esophageal Squamous Cell Carcinoma. *Ann Surg Oncol* (2019) 26(13):4737–43. doi: 10.1245/s10434-019-07727-8
10. Makino T, Yamasaki M, Miyazaki Y, Wada N, Takahashi T, Kurokawa Y, et al. Utility of Initial Induction Chemotherapy With 5-Fluorouracil, Cisplatin, and Docetaxel (DCF) for T4 Esophageal Cancer: A Propensity Score-Matched Analysis. *Dis Esophagus* (2018) 31(4). doi: 10.1093/dote/dox130
11. 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
12. Becker K, Mueller JD, Schulmacher C, Ott K, Fink U, Busch R, et al. Histomorphology and Grading of Regression in Gastric Carcinoma Treated With Neoadjuvant Chemotherapy. *Cancer* (2003) 98(7):1521–30. doi: 10.1002/cncr.11660
13. Shah MA, Kojima T, Hochhauser D, Enzinger P, Raimbourg J, Hollebecque A, et al. Efficacy and Safety of Pembrolizumab for Heavily Pretreated Patients With Advanced, Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus: The Phase 2 KEYNOTE-180 Study. *JAMA Oncol* (2019) 5 (4):546–50. doi: 10.1001/jamaoncol.2018.5441
14. Devision of Cancer Treatment & Diagnosis NCI. *Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0* (2017). Available at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.
15. World Health Organization. *The Use of the WHO-UMC System for Standardised Case Causality Assessment 2013*. Available at: https://www.who.int/publications/m/item/WHO-causality-assessment.
16. Hellmann MD, Chaft JE, William WNJr., Rusch V, Pisters KMW, Kalhor N, et al. Pathological Response After Neoadjuvant Chemotherapy in Resectable Non-Small-Cell Lung Cancers: Proposal for the Use of Major Pathological Response as a Surrogate Endpoint. *Lancet Oncol* (2014) 15(1):e42–50. doi: 10.1016/S1470-2045(13)70334-6
17. Zheng Y, Liu X-B, Sun H-B, Xu J, Shen S, Ba Y-F, et al. A Phase III Study on Neoadjuvant Chemotherapy Versus Neoadjuvant Toripalimab Plus Chemotherapy for Locally Advanced Esophageal Squamous Cell Carcinoma: Henan Cancer Hospital Thoracic Oncology Group 1909 (Hchtog1909). *Ann Trans Med* (2021) 9(1):73–. doi: 10.21037/atm-20-5404
18. Kamarajah SK, Navidi M, Wahed S, Immanuel A, Hayes N, Griffin SM, et al. Significance of Neoadjuvant Downstaging in Carcinoma of Esophagus and Gastroesophageal Junction. *Ann Surg Oncol* (2020) 27(9):3182–92. doi: 10.1245/s10434-020-08358-0
19. R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing (2020).
20. Pimiento JM, Weber J, Hoffe SE, Shridhar R, Almhanna K, Vignesh S, et al. Outcomes Associated With Surgery for T4 Esophageal Cancer. *Ann Surg Oncol* (2013) 20(8):2706–12. doi: 10.1245/s10434-013-2885-x
21. Chiarion-Sileni V, Corti L, Ruol A, Innocente R, Boso C, Del Bianco P, et al. Phase II Trial of Docetaxel, Cisplatin and Fluorouracil Followed by

Carboplatin and Radiotherapy in Locally Advanced Oesophageal Cancer. *Br J Cancer* (2007) 96(3):432–8. doi: 10.1038/sj.bjc.6603585

22. Abe T, Higaki E, Hosoi T, Nagao T, Bando H, Kadowaki S, et al. Long-Term Outcome of Patients With Locally Advanced Clinically Unresectable Esophageal Cancer Undergoing Conversion Surgery After Induction Chemotherapy With Docetaxel Plus Cisplatin and 5-Fluorouracil. *Ann Surg Oncol* (2021) 28(2):712–21. doi: 10.1245/s10434-020-08865-0
23. Cheng C, Yang W, Chen W, Yeung S-CJ, Xing X, Wang X, et al. Neoadjuvant PD-1 Blockade in Combination With Chemotherapy for Patients With Resectable Esophageal Squamous Cell Carcinoma. *J Clin Oncol* (2021) 39 (3_suppl):220. doi: 10.1200/JCO.2021.39.3_suppl.220
24. Gu Y, Chen X, Wang D, Ding M, Xue L, Zhen F, et al. 175p A Study of Neoadjuvant Sintilimab Combined With Triplet Chemotherapy of Lipo-Paclitaxel, Cisplatin, and S-1 for Resectable Esophageal Squamous Cell Carcinoma (ESCC). *Ann Oncol* (2020) 31:S1307–S8. doi: 10.1016/j.annonc.2020.10.196
25. Thibult ML, Mamessier E, Gertner-Dardenne J, Pastor S, Just-Landi S, Xerri L, et al. PD-1 Is a Novel Regulator of Human B-Cell Activation. *Int Immunol* (2013) 25(2):129–37. doi: 10.1093/intimm/dxs098
26. Ramakrishnan R, Gabrilovich DI. Mechanism of Synergistic Effect of Chemotherapy and Immunotherapy of Cancer. *Cancer Immunol Immunother* (2011) 60(3):419–23. doi: 10.1007/s00262-010-0930-1
27. Mkrtichyan M, Najjar YG, Raulfs EC, Abdalla MY, Samara R, Rotem-Yehudar R, et al. Anti-PD-1 Synergizes With Cyclophosphamide to Induce Potent Anti-Tumor Vaccine Effects Through Novel Mechanisms. *Eur J Immunol* (2011) 41(10):2977–86. doi: 10.1002/eji.201141639
28. Li C, Zhao S, Zheng Y, Han Y, Chen X, Cheng Z, et al. Preoperative Pembrolizumab Combined With Chemoradiotherapy for Oesophageal Squamous Cell Carcinoma (PALACE-1). *Eur J Cancer* (2021) 144:232–41. doi: 10.1016/j.ejca.2020.11.039
29. Yamamoto S, Kato K, Daiko H, Kojima T, Hara H, Abe T, et al. FRONTiER: A Feasibility Trial of Nivolumab With Neoadjuvant CF or DCF Therapy for Locally Advanced Esophageal Carcinoma (JCOG1804E)—The Short-Term Results of Cohort A and B. *J Clin Oncol* (2021) 39(3_suppl):202. doi: 10.1200/JCO.2021.39.3_suppl.202
30. Wang Z. Neoadjuvant Camrelizumab Combined With Chemotherapy and Apatinib for Locally Advanced Thoracic Esophageal Squamous Cell Carcinoma (ESCC): A Single-Arm, Open-Label, Phase Ib Study. *J Clin Oncol* (2021) 39(15_suppl):4047. doi: 10.1200/JCO.2021.39.15_suppl.4047
31. Wang F, Qi Y, Meng X, Fan Q. Camrelizumab in Combination With Preoperative Chemotherapy for Locally Advanced Esophageal Squamous Cell Carcinoma: A Single-Arm, Open-Label, Phase II Study. *J Clin Oncol* (2021) 39(3_suppl):222. doi: 10.1200/JCO.2021.39.3_suppl.222
32. Li Z, Liu J, Zhang M, Shao J, Yang Y, Li H, et al. A Phase II Study of Neoadjuvant Immunotherapy Combined With Chemotherapy (Camrelizumab Plus Albumin Paclitaxel and Carboplatin) in Resectable Thoracic Esophageal Squamous Cell Cancer (NICE Study): Interim Results. *J Clin Oncol* (2021) 39(15_suppl):4060. doi: 10.1200/JCO.2021.39.15_suppl.4060
33. Li J, Liu J, Li Z, Cui F, Zeng Y, Liang W, et al. Camrelizumab Plus Chemotherapy as Neoadjuvant Therapy for Resectable, Locally Advanced Esophageal Squamous Cell Carcinoma (NIC-ESCC2019): A Multicenter, Open-Label, Single-Arm, Phase 2 Study. *J Clin Oncol* (2021) 39 (15_suppl):4028. doi: 10.1200/JCO.2021.39.15_suppl.4028
34. Hong MH, Kim H, Park SY, Kim DJ, Lee CG, Cho J, et al. A Phase II Trial of Preoperative Chemoradiotherapy and Pembrolizumab for Locally Advanced Esophageal Squamous Cell Carcinoma (ESCC). *J Clin Oncol* (2019) 37 (15_suppl):4027. doi: 10.1200/JCO.2019.37.15_suppl.4027
35. Li K, Yang X, Luo W, Ma Q, Wang Y, Xiong Y, et al. 415 Toripalimab Plus Nab-Paclitaxel and Carboplatin as Neoadjuvant Therapy for Patients With Esophageal Squamous Cell Carcinoma at Clinical Stage T2-T4/N0-N2/M0: A Single-Arm, Single-Center Clinical Study. *J ImmunoTher Cancer* (2020) 8 (Suppl 3):A253. doi: 10.1136/jitc-2020-SITC2020.0415
36. van den Ende T, Clercq N, van Berge Henegouwen MI, Gisbertz SS, Geijsen D, Verhoeven R, et al. Neoadjuvant Chemoradiotherapy Combined With Atezolizumab for Resectable Esophageal Adenocarcinoma: A Single Arm Phase II Feasibility Trial (PERFECT). *Clin Cancer Res* (2021) 27(12):3351–3359. doi: 10.1158/1078-0432.CCR-20-4443
37. Hashimoto T, Makino T, Yamasaki M, Tanaka K, Miyazaki Y, Takahashi T, et al. The Pattern of Residual Tumor After Neoadjuvant Chemotherapy for Locally Advanced Esophageal Cancer and Its Clinical Significance. *Ann Surg* (2020) 271(5):875–84. doi: 10.1097/SLA.0000000000003129

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Current progress and future perspectives of neoadjuvant anti-PD-1/PD-L1 therapy for colorectal cancer

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Immunotherapies, especially the programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors, have revolutionized the therapeutic strategies of various cancers. As for colorectal cancer (CRC), the current clinical application of PD-1/PD-L1 inhibitors are mainly used according to the mutation pattern, which is categorized into deficient mismatch repair (dMMR)/high levels of microsatellite instability (MSI-H) and proficient mismatch repair (pMMR), or non-high levels of microsatellite instability (non-MSI-H). PD-1/PD-L1 inhibitors have been proven to have favorable outcomes against dMMR/MSI-H CRC because of more T-cell infiltration into tumor tissues. Nevertheless, the effectiveness of PD-1/PD-L1 inhibitors in pMMR/non-MSI-H CRC is still uncertain. Because of the quite-lower proportion of dMMR/MSI-H in CRC, PD-1/PD-L1 inhibitors have been reported to combine with other antitumor treatments including chemotherapy, radiotherapy, and targeted therapy for better therapeutic effect in recent clinical trials. Neoadjuvant therapy, mainly including chemotherapy and radiotherapy, not only can reduce clinical stage but also benefit from local control, which can improve clinical symptoms and the quality of life. Adding immunotherapy into neoadjuvant therapy may change the treatment strategy of primary resectable or some metastatic CRC. In this review, we focus on the development of neoadjuvant anti-PD-1/PD-L1 therapy and discuss the future perspectives in CRC.

KEYWORDS

colorectal cancer, PD-1/PD-L1 inhibitors, neoadjuvant, microsatellite instability, mismatch repair

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors all over the world, with new cases accounting for 10.0% of all cancers each year (1). At present, the treatment strategies mainly include surgical resection, chemotherapy, radiotherapy, and molecular targeted therapy (2, 3). Although a variety of therapeutic strategies have made significant progress in CRC treatment recently (4–6), the number of CRC-related deaths still reaches 915,880 each year, accounting for 9.4% of all tumor-related deaths, ranking second in all tumors worldwide (7). Consequently, the benefits of current treatment have encountered a bottleneck and novel strategies are urgent for better therapeutic effects in CRC patients.

Recently, immunotherapy has received rapid development and more attention in clinical application because of its good antitumor effect, which further provides motivation for CRC (8, 9). Compared with traditional treatments, immunotherapy could kill cancer cells by activating the antitumor immunity and is specifically targeted against cancer antigens to prevent normal cells from being attacked (10–12). Among them, programmed cell death protein 1 (PDCD1, PD-1) is the most important receptor for activating T-cell expression and mediating immunosuppression, while the programmed cell death ligand 1 (CD274, PD-L1) is involved in programmed death 1, resulting in T-cell apoptosis or anergy (13, 14). Therefore, PD-1/PD-L1 inhibitors could stop T-cell apoptosis and dysfunction, which further enhances the activation of T cells (15). Since nivolumab was firstly used in humans in 2006, PD-1/PD-L1 inhibitors were applied in many clinical trials to treat various refractory cancers, including melanoma, gastric cancer, and lung cancer (16–18). CRC is categorized into deficiency mismatch repair/high levels of microsatellite instability (dMMR/MSI-H) and proficient mismatch repair/non-high levels of microsatellite instability (pMMR/non-MSI-H) according to the mutation pattern (19, 20). Many clinical trials have proven that immune checkpoint inhibitors (ICIs) exhibited effective and stable therapeutic effects on dMMR/MSI-H CRC patients; therefore, several drugs like nivolumab and pembrolizumab are approved by the US Food and Drug Administration to treat this kind of patients (21–23).

Neoadjuvant therapy is the use of radiotherapy, chemotherapy, and a combination of various treatment methods before surgery, which can reduce the staging of tumors, thereby reducing local recurrence and acquiring better prognosis (24–26). At present, neoadjuvant therapy has been proven to be effective in the treatment of some CRC patients, especially locally advanced rectal cancer (LARC) and colorectal liver metastases (CRLMs) (27, 28). Therefore, the overall survival (OS) rate of neoadjuvant therapy is proven to be not remarkably higher than postoperative therapy (29, 30). Neoadjuvant radiotherapy could enlarge the anti-PD-1/PD-L1 treatment effect by promoting different links in the immune response such as the activation and recruitment of T cells, promotion of dendritic cell maturation, antigen exposure, and upregulation of major histocompatibility complex molecules (31, 32). Additionally, neoadjuvant chemotherapy could induce PD-1/PD-L1 expression and further profit the effect of ICI treatment (33, 34). Consequently, adding anti-PD-1/PD-L1 therapy into neoadjuvant therapy might change the treatment strategy of primary resectable or some metastatic CRC and further acquire better prognosis and survival results. Hence, this review aimed to focus on the development of neoadjuvant anti-PD-1/PD-L1 therapy and discuss the current opportunities and challenges, highlighting considerations for the upfront treatment in resectable and part of metastatic CRC.

Mechanisms of programmed cell death 1/programmed cell death ligand 1 inhibitors in deficient mismatch repair/high levels of microsatellite instability colorectal cancer

Mechanisms of anti-PD-1/PD-L1 therapy

The antitumor immune process mainly includes immune elimination, immune balance, and immune escape. PD-1 and PD-L1 are a pair of important immune checkpoint (ICs) that work as the brake on the immune system and play a crucial role in the tumor immune escaping process (35). After the binding of PD-1 and PD-L1, tumor cells take advantage of the recognition of the T-cell receptor, further suppressing immunity and evading immune surveillance (36). In 2002, the evidence that the PD-1 pathway mediating tumor immunity was first reported in that the overexpression of PD-L1 will weaken the cytolytic activity of T cells and then significantly promote the occurrence and invasion of tumors (37). Interestingly, such effect could be reversed by the application of monoclonal antibodies against PD-L1 (38). PD-L1 is highly expressed on the surface of many tumor cells, which can also induce immune cells [especially T helper lymphocytes, (Th)] to secrete immunosuppressive factors and further inhibit the killing effect of the antitumor immunity (39). As shown in Figure 1, anti-PD-1/PD-L1 therapy can bind to PD-1 and PD-L1 correspondingly, further preventing the combination of PD-1 on the surface of T cells and PD-L1 on the surface of tumor cells (40). Such function could reverse the inhibitory effect of the immune system by tumor cells and restore the antitumor immunity.

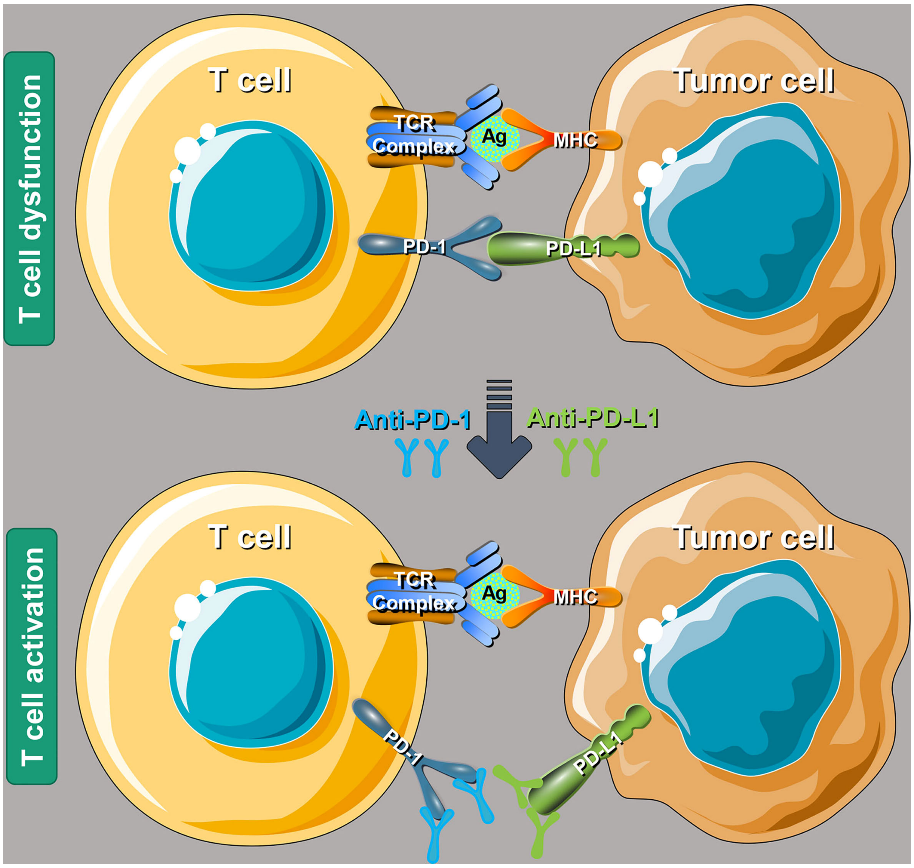

FIGURE 1
Schematic mechanism of programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors to restore T-cell functions. T-cell receptor, antigen, and major histocompatibility complex (MHC). Reproduced with permission (40).

Biological features of deficient mismatch repair/high levels of microsatellite instability colorectal cancer

A human body could maintain genomic stability by evolving sophisticated mechanisms. Mismatch repair (MMR) is an evolutionarily conserved system consisting of five key proteins: mutL homolog 1 (MLH1); postmeiotic segregation 2 (PMS2); and mutS homologs 2, 3, and 6 (MSH2, 3, and 6), which could be used to identify and repair base misinsertion, deletion, and misfusion during the progress of DNA replication, DNA recombination, and some forms of DNA damage (41, 42). In addition, MMR can also play a key role in response to DNA-damaging agents by apoptosis induction or regulating the cell cycle (43, 44). Significantly, the change of the MMR status may lead to different microsatellite lengths called microsatellite instability (MSI), which can be accurately detected by PCR or second-generation sequencing technology (45).

During the carcinogenesis of normal colorectal epithelial cells, one of the important driving factors is genomic instability, which results in unrestricted proliferation and the avoidance of immune clearance in cancer cells (46). The state of dMMR/MSI-H in CRC was firstly reported in the Lynch syndrome, which is a kind of inherited cancer syndrome and mainly resulted by the mutations of the MMR gene (most commonly MLH1 and MSH2) (47). Compared with pMMR, dMMR CRC has higher tumor mutational burden (TMB), while the mutation rate increases approximately 100–1,000 times (48). The accounts of the dMMR/MSI-H of all CRC cases are approximately 15% while approximately 85% of patients are proficient in MMR (49). Interestingly, approximately 20% of stage II and 11% of stage III tumors are dMMR/MSI-H; however, the percentage is only 5% in stage IV (50).

The prognosis of stage II or III dMMR/MSI-H CRC patients is significantly better than that of pMMR/non-MSI-H, whereas, stage IV patients were reported with a poor prognosis (51). CRC with dMMR have many noteworthy characteristics like a lymphocytic infiltrate, tendency to arise in the proximal colon, lower transfer rate, and signet ring or mucinous appearance (52). According to previous literature reports, stage II dMMR/MSI-H

CRC patients cannot benefit from adjuvant chemotherapy based on traditional cytotoxic drugs like 5-FU (53, 54). This phenomenon might be mainly because dMMR/MSI-H CRC cannot achieve the recognition of 5-FU-modified DNA, which is an important step that triggers the cytotoxic progress (55). However, the efficacy of oxaliplatin adjuvant therapy does not appear to be affected by the MMR or MSI status (56, 57). It has been mainly reported that dMMR/MSI-H CRC has a good response to ICI treatments, especially anti-PD-1/PD-L1 therapy. It is reported that dMMR/MSI-H CRC had remarkably higher levels of cytotoxic T lymphocytes (CTLs), Th1, Th2, follicular helper T cells, and T-cell markers (58). Additionally, higher TMB, tumor neoantigen burden (TNB), and more lymphocyte infiltration and PD-L1 expression in tumor tissues have also been reported (59, 60). The sufficient evidence above prompts that PD-1/PD-L1 inhibitors could enhance antitumor immunity and result in an excellent therapeutic effect when treating these individuals.

Exploration of programmed cell death 1/ programmed cell death ligand 1inhibitors in metastatic deficient mismatch repair/ high levels of microsatellite instability colorectal cancer

The KEYNOTE-016 study reported in 2016 that the overall response rate (ORR) was 0% and the disease control rate (DCR) was 16% in pMMR/non-MSI-H CRC patients who received pembrolizumab, compared with 50% and 89% for dMMR/ MSI-H, respectively (61). The subsequent phase 2 study, KEYNOTE-164, reported the median PFS of 4.1 months, 24-month OS rate of 63%, ORR of 33%, and DCR of 57% in dMMR/ MSI-H metastatic CRC (mCRC) patients (62). CheckMate-142 was a phase 2 clinical trial that evaluated the curative effect of another PD-1 inhibitor, nivolumab, in dMMR/MSI-H mCRC patients. At a median follow-up of 12 months, 31% (23 of 74) of patients reached the ORR, while the OS and progression-free survival (PFS) were 73% and 50%, correspondingly (63). In consideration of the above outcomes, nivolumab and pembrolizumab received the accelerated approval of the FDA as the second-line treatment for patients with dMMR/MSI-H mCRC in 2017 (64).

As an important milestone in the development of CRC immunotherapy, KEYNOTE-177 compared the efficacy of pembrolizumab compared to standard chemotherapy in the first-line treatment of dMMR/MSI-H mCRC. At the final analysis in 2021, median OS (the median follow-up of 44.5 months) was not reached in the pembrolizumab group while it was 36.7 months in the chemotherapy group. In addition, the median PFS was 16.5 months in the pembrolizumab group while it was 8.2 months in the chemotherapy group (65). Due to the gratifying results, pembrolizumab or nivolumab, alone or in combination with ipilimumab, was recommended as a first-line treatment option for patients with dMMR/MSI-H mCRC, whether it is eligible for intensive therapy in National Comprehensive Cancer Network (NCCN) Guidelines Version 2.2021 (66).

Programmed cell death 1/ programmed cell death ligand 1 inhibitors for neoadjuvant treatment in colorectal cancer

Neoadjuvant therapy for CRC mostly focuses on locally advanced rectal cancer and some resectable metastatic CRC. Traditional neoadjuvant therapies include chemotherapy, radiotherapy, targeted therapy, and combination therapy. At present, the neoadjuvant treatment for rectal cancer is based on radiotherapy and combined with chemotherapy drugs, while for colon cancer, it is mostly based on drugs, including chemotherapy drugs and targeted drugs.

Neoadjuvant anti- programmed cell death 1/programmed cell death ligand 1 therapy in deficient mismatch repair/high levels of microsatellite instability colorectal cancer

NCCN Guidelines Version 2.2021 changed the previous recommendation on detecting the MMR/MSI status. The guidelines recommend universal MMR or MSI testing for all patients with a personal history of colon or rectal cancer. In addition to its role as a predictive marker for immunotherapy use in the advanced CRC setting, the MSI/MMR status can also help to identify individuals with the Lynch syndrome and to inform adjuvant therapy decisions for patients with stage II CRC (66). Previous recommendations limited such testing to patients with suspected metastases. Consequently, new guidelines mean that anti-PD-1/PD-L1 therapy not only can be applicable to stage IV dMMR/MSI-H mCRC patients but also be used as part of neoadjuvant therapy. As mentioned previously, dMMR/MSI-H patients are resistant to some conventional chemotherapy. A retrospective study in 2020 involving 5,086 LARC patients between 2010 and 2015 in the National Cancer Database suggested that the postoperative pathologic complete response (pCR) rate of dMMR/MSI-H after neoadjuvant chemoradiotherapy was significantly lower than that of the pMMR/non-MSI-H group (5.9% vs. 8.9%) (67).

The encouraging results of ICIs in the treatment of dMMR/ MSI-H mCRC have greatly promoted the exploration of them in neoadjuvant therapy. The NICHE clinical trial from the Netherlands is the pioneer with the inclusion criteria of stage I,

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II, or III resectable colon adenocarcinoma (68). Patients with non-metastatic resectable dMMR or pMMR CRC received a single dose of ipilimumab and two doses of nivolumab, followed by surgery within 6 weeks. In addition, patients with pMMR tumors were randomized to receive or not receive celecoxib. Pathological responses (PR, at least 50% tumor regression) were observed in all 20 dMMR patients, including 19 major pathological responses (MPRs, ≤10% residual viable tumor) and 12 pCR. However, 4/15 of pMMR patients reached PR, with three MPRs and no pCR. A phase 2 study from China involved clinical stage T3/T4 or any T with lymph node positivity (N+) dMMR/MSI-H CRC and treated using toripalimab on day 1, with or without celecoxib 200 mg orally twice daily from day 1 to 14 of each 14-day cycle, for six cycles before surgical resection (69). The pCR rate in the toripalimab monotherapy group was 65% (11/17), while in the toripalimab-plus-celecoxib group, it even reached 89% (17/19). A very recent study reported a combination of neoadjuvant chemoradiotherapy and immunotherapy treating dMMR/MSI-H stage II or III rectal cancer (70). Patients received neoadjuvant dostarlimab every 3 weeks for 6 months (nine cycles) and then followed by standard radiation therapy with a concurrent administration of capecitabine at standard doses, and finally followed by total mesorectal excision (TME). All 12 patients who reached a clinical complete response (cCR) have undergone at least 6 months of follow-up, with no evidence of tumor according to magnetic resonance imaging (MRI), ^{18}F-fluorodeoxyglucose (FDG) PET, endoscopic evaluation, digital rectal examination, or biopsy. In summary, dMMR/MSI-H CRC receiving neoadjuvant anti-PD-1/PD-L1 therapy could obtain a higher pCR or cCR rate, which might guide clinicians to choose neoadjuvant treatment in the future.

Neoadjuvant anti-programmed cell death 1/programmed cell death ligand 1 therapy in proficient mismatch repair/non-high levels of microsatellite instability colorectal cancer

Differently, pMMR/non-MSI-H CRC could not respond well to immunotherapy. For this problem, many studies concentrated on the strategy of combined with chemotherapy or radiation therapy to improve the curative effect. Many traditional chemotherapeutic agents like oxaliplatin, 5-FU, and gemcitabine can modulate tumor-infiltrating lymphocytes (TILs) as immunogenic cell death inducers to reactivate antitumor immunity in the tumor-immunosuppressive microenvironment (71). Hence, the combination of chemotherapy and immunotherapy can promote the immune response, enhance the therapeutic effect of ICIs, and further achieve the effect of improving the clinical prognosis of patients. It has been widely demonstrated that radiotherapy combined with immunotherapy could achieve an effect of 1 + 1 > 2 in clinic. As shown in Figure 2, radiotherapy can effectively activate the antitumor effect by inducing tumour antigen release, enhancing tumour cell immunogenicity, activating immune cells, and secreting immune factors and promote tumor-related antigen presentation (72). Additionally, radiotherapy not only can upregulate the expression of PD-1 on T cells and PD-L1 on tumor cells for suppressing immunotherapy resistance but also kill tumor cells and induce the release of inflammatory cytokines, damage-associated molecular patterns, and tumor-associated antigens, achieving the synergistic antitumor effect (73, 74).

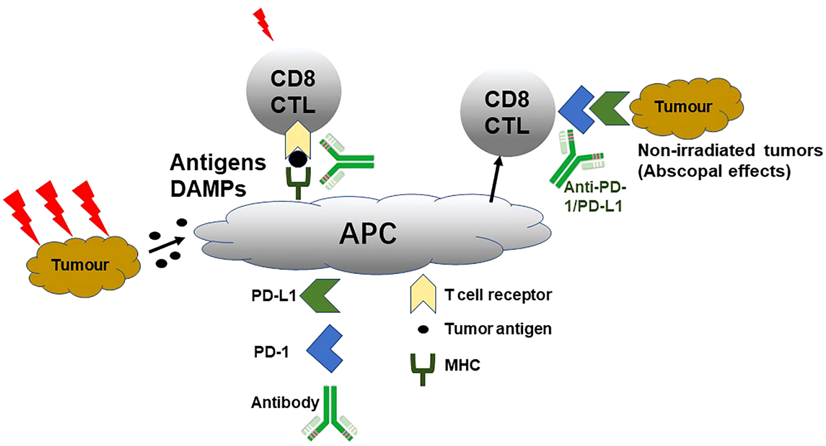

FIGURE 2
Schematic mechanism of radiotherapy enlarging anti-PD-1/PD-L1 curative effect. Damage-associated molecular patterns, cytotoxic T lymphocytes, antigen-presenting cell, and MHC. Reproduced with permission (72).

The VOLTAGE-A study from Japan reported the short-term results of $T_{3–4}N_{0-2}M_0$ LARC patients regardless of the MMR/MSI status receiving preoperative immunotherapy combined with chemoradiotherapy followed by radical surgery (75). The detailed neoadjuvant schedule was five cycles of nivolumab after 50.4 Gy with capecitabine. In this study, 11/37 (30%) of MSS patients reaching pCR and 14/37 (38%) reaching MPRs according to the American Joint Committee on Cancer guidelines for the evaluation of the tumor regression grade were observed. As of December 2020, with a median follow-up of 32.9 months, two cases of local recurrence and four cases of distant metastasis were observed in the MSS group. In addition, this study reported a combination of biomarkers (PD-L1 expression in ≥1% of tumor cells, $CD8^+$ T-cell/effector regulatory T-cell ratios ≥2.5) to predict the efficacy of neoadjuvant chemoradiotherapy combined with anti-PD-1/PD-L1 therapy in MSS LARC patients, which has good application potential in subsequent studies. A phase 2 single-arm trial from China involved $T_{3-4}N_0M_0$ or $T_{1-4}N_{0-2}M_0$ rectal adenocarcinoma (an inferior margin of 10 cm from the anal verge) patients to monitor the outcomes (76). The eligible patients received short-course radiotherapy (5 × 5 Gy over 5 days), followed 1 week later by two subsequent 21-day cycles of CAPOX (oxaliplatin day 1 and capecitabine day 1–14) plus camrelizumab (day 1), followed by radical surgery according to TME principles. The pCR (ypT0N0) rate in pMMR patients reached an amazing 46.2% (12/26). This scheme not only can shorten the preoperative treatment time but also acquired the satisfactory anal preservation rate of 88.9%. An American trial reported in 2021 assessed whether the addition of pembrolizumab to neoadjuvant chemoradiotherapy can lead to an improvement in the neoadjuvant rectal (NAR) score instead of pCR compared with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) (77). As shown in Figure 3, the NAR score is calculated according to the following formula as a predictive indicator of survival after preoperative chemoradiotherapy for rectal cancer (78). Patients with stage II/III LARC with distal location (cT_{3-4} , ≤ 5 cm from anal verge, N_{0-2}), with bulky disease (any cT_4 or tumor within 3 mm of mesorectal fascia), at high risk for metastatic disease (cN_2), and/or who were not candidates for sphincter-sparing surgery (SSS) were enrolled. A total of 185 patients were randomized (1:1) to neoadjuvant FOLFOX for 4 months and then underwent chemoradiotherapy (capecitabine, 50.4 Gy) with (n = 90) or without (n = 95) pembrolizumab (six doses every 3 weeks) before surgery. Unfortunately, this study yielded negative results with the mean NAR score being 11.53 vs. 14.08, cCR rate of 13.9% vs. 13.6%, and pCR rate of 31.9% vs. 29.4% in the pembrolizumab arm and control arm, correspondingly.

Exploration of neoadjuvant anti-programmed cell death 1/programmed cell death ligand 1 therapy in our center

Our center also initiated a prospective, multicenter, phase 2 clinical trial to explore safety and efficacy of long-course neoadjuvant chemoradiotherapy plus tislelizumab followed by TME for LARC (78). As of 30 June 2022, a total number of patients (n = 43) were enrolled, while 30 (29 pMMR/non-MSI-H and 1 dMMR/MSI-H) patients had undergone TME surgery, with the R0 resection rate of 100% and sphincter-saving resection rate of 90.0% (27/30). The objective response rate reached 100% (30/30) with the pCR rate of 43.3% (13/30) and MPR rate of 40.0% (12/30). At present, this study continues to enroll patients and is estimated to enroll 50 patients. We also expect exciting results at the final primary endpoints (pCR rate) and secondary endpoints (NAR score, ORR, R0 resection rate, anal preservation rate). According to several existing research data, the pCR rate of pMMR/non-MSI-H patients can reach up to 46.2% after combined immunotherapy, which seems to be significantly improved after combined radiotherapy. This strategy may improve the quality of life for LARC patients, especially those with ultralow rectal cancer (≤5 cm from the lower edge of the tumor to the anus), to achieve organ preservation and the use of watch and wait for the future.

Endpoint evaluation of neoadjuvant immunotherapy

As more and more patients reached pCR in neoadjuvant immunotherapy, Watch and Wait strategy is a strategy which is more and more likely to achieve the purpose of anus reservation and reduce surgical trauma without affecting the survival rate. evaluated at or near cCR can be considered for Watch and

$$NAR = \frac{[5\,pN - 3(cT - pT) + 12]^2}{9.61}$$

FIGURE 3
Calculation formula of the neoadjuvant rectal (NAR) score. NAR, pathologic nodal stage, clinical tumor stage, and pathologic tumor stage.

Waiting under the premise of close follow-up, while for patients with a clear tumor residue, radical surgery is recommended as soon as possible. Rectal MRI is currently an important staging method recommended by international guidelines for the diagnosis of primary rectal cancer. MRI can accurately display the anatomy of rectum and adjacent organs, further providing relatively accurate information on the tumor stage. However, since the measurement of the tumor site after neoadjuvant therapy is often interfered by necrosis and other factors, traditional MRI methods cannot accurately monitor the tumor response (79).

Due to immune cell infiltration and other reasons, one of the characteristics of immune neoadjuvant therapy is that the imaging and pathological evaluation results may differ greatly. Such a phenomenon is called pseudoprogression (PSPD), which is manifested in that many patients do not observe tumor remission on imaging but maintaining stability or even some enlargement, but a pathological examination may find a tumor regression in these patients (80). Thus, how to recognize and identify the different between PSPD and true progression is significant. An interesting clinical study that included 123 patients with dMMR/MSI-H mCRC treated with ICIs was reported to evaluated the PSPD frequency with the median follow-up of 22.3 months (81). A total of 29% (36/123) of patients experienced radiological progressive disease (PD) according to Response Evaluation Criteria in Solid Tumours, version 1.1 (RECIST 1.1), of which 61.1% (22/36) occurred in the first 3 months, and 80.1% of patients (29/36) continued immunotherapy. Among them, 12 cases were PSPD, accounting for 52% of the early imaging PD. The median time to PSPD was 5.7 weeks. Interestingly, the incidence of PSPD was 14.8% (9/61) in the PD-1 antibody–alone group while it was 4.8% (3/62) in the PD-1 antibody plus anticytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) antibody group. A systematic review had also reported that Immune-based Response Evaluation Criteria in Solid Tumors standards have no significant impact on ORR and DCR statistics compared with RECIST 1.1, and the prediction difference of the mean survival time is also negligible (0.46 months) (82). Therefore, the current evaluation criteria and methods of neoadjuvant anti-PD-1/PD-L1 therapy efficacy need to be improved, which should also be a key consideration in the design of relevant clinical studies.

Safety of neoadjuvant programmed cell death 1/programmed cell death ligand 1 inhibitors in colorectal cancer

With the wide application of immunotherapy in the field of cancer, more and more studies were reported concentrating on the safety in clinical practice. The immune-related adverse events (irAEs) might involve multiple organs including skin (like vitiligo), endocrine system (like hyperthyroidism), respiratory system (like pneumonia), gastrointestinal system (like diarrhea and colitis), and cardiovascular system (like myocarditis) (83, 84). The mentioned adverse events above usually occur in the first 2–3 months, while skin manifestations happen firstly (85). Even though the occurrence of irAEs might be associated with a clinical benefit for patients receiving anti-PD-1/PD-L1 therapy, grade 3–4 irAEs might be life-threatening and result in the permanent suspension of medication (86, 87). According to reported clinical trials, the incidence of grade ≥3 irAEs was 13%–22% using ICI monotherapy, while it was 22%–64% by dual ICIs (88). The overall adverse event rate reported in KEYNOTE 177 was 22% (33/153) with 9% (14/153) of grade ≥3 in the pembrolizumab group, while it was 13% (18/143) and 2% (3/143) in the chemotherapy group (65). The reported adverse events include hypothyroidism, colitis, hyperthyroidism, pneumonitis, adrenal insufficiency, hepatitis, infusion reactions, severe skin reactions, and thyroiditis after treating with pembrolizumab. Additionally, there are also literatures that support the fact that single PD-1/PD-L1 inhibitors caused fewer treatment-related adverse events than chemotherapy alone (22). At present, many academic organizations including the European Society for Medical Oncology, American Society of Clinical Oncology, and NCCN have published standards and guidelines for irAEs, which can escort the clinical use (89–91). Overall, these irAEs caused by PD-1/PD-L1 inhibitors are acceptable, predictable, and controllable. Therefore, anti-PD-1/PD-L1 therapy may play a role in all scenarios where neoadjuvant therapy can be used in the treatment of CRC, while safety is a premise and guarantee.

Controversy and challenges

There are many environmental, dietary, and lifestyle factors including diet, smoking, alcohol, obesity, sleep, exercise, and microbiome that might influence the carcinogenic mechanisms, response to therapy, biology, and clinical outcome of CRC. These factors might influence the molecular pathology, immune infiltrates, and response to therapy in each patient differentially, which is increasingly evident in patients treated with immunotherapy. Additionally, gene-by-environment interactions also influence the germline genetic variations on both the immune system and cancer. Moreover, the molecular pathological epidemiology might be related to the microbiome, molecular pathologies, immune cell infiltrates, and clinical outcomes in CRC patients, especially in immunotherapy. Therefore, the relationship between the above-mentioned factors and neoadjuvant anti-PD-1/PD-L1 therapy for CRC still needs further exploration and discussion in the future.

Anti-PD-1/PD-L1 therapy is a useful therapeutic strategy following surgical resection, chemotherapy, radiotherapy, and targeted therapy, which perform great potential in the treatment of CRC. We should fully recognize the broad application prospect of immunotherapy in the neoadjuvant therapy of CRC in the future.

However, the following points need to be noted. Firstly, the detection of the MMR/MSI status before CRC treatment is important, especially targeted detection combined with clinical characteristics, family history, and imaging features, to avoid missing the beneficiaries of immunotherapy. For pMMR/non-MSI-H patients, more novel neoadjuvant combination strategies like improving the immunogenicity and increasing the invasion ability of immune cells need to be monitored and developed. Secondly, as an emerging therapeutic method with potential, concerns about its safety still cannot be ignored. Especially, there are few studies on the evaluation of surgery-related complications after neoadjuvant anti-PD-1/PD-L1 therapy. Thirdly, the evaluation of treatment effect after neoadjuvant immunotherapy for CRC and the selection of following organ preservation and the Watch and Waiting strategy are not clear at present. Finally, it is urgent to explore the optimal mode of neoadjuvant therapy combined with immunotherapy for CRC, including the choice of radiotherapy mode (long course vs. short course), the cooperation of chemotherapy drugs, the choice of PD-1 medication timing (synchronous radiotherapy vs. sequential radiotherapy) whether total neoadjuvant therapy, and so on. In summary, it is reasonable that immunotherapy epical anti-PD-1/PD-L1 therapy may change the neoadjuvant therapeutic foreground of CRC and ultimately achieve the goal of patient benefit.

Author contributions

All authors made substantial contributions to this review. ZZ, HY, and ZB conceived and designed the review. ZY, GW, XZ, JG, CM, YL, QW, LS, and PW retrieved and reviewed literatures. ZY and GW wrote the manuscript. ZZ, HY, and ZB reviewed and edited the manuscript. All authors read and approved the manuscript.

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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. Yang Z, Deng W, Zhang X, An Y, Liu Y, Yao H, et al. Opportunities and challenges of nanoparticles in digestive tumours as anti-angiogenic therapies. *Front Oncol* (2022) 11:789330. doi: 10.3389/fonc.2021.789330

3. Akgül Ö, Çetinkaya E, Ersöz Ş, Tez M. Role of surgery in colorectal cancer liver metastases. *World J Gastroenterol* (2014) 20(20):6113–22. doi: 10.3748/wjg.v20.i20.6113

4. Frank MH, Wilson BJ, Gold JS, Frank NY. Clinical implications of colorectal cancer stem cells in the age of single-cell omics and targeted therapies. *Gastroenterology* (2021) 160(6):1947–60. doi: 10.1053/j.gastro.2020.12.080

5. Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, et al. Immunotherapy in colorectal cancer: Rationale, challenges and potential. *Nat Rev Gastroenterol Hepatol* (2019) 16(6):361–75. doi: 10.1038/s41575-019-0126-x

6. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: A review. *JAMA* (2021) 325(7):669–85. doi: 10.1001/jama.2021.0106

7. Qiu H, Cao S, Xu R. Cancer incidence, mortality, and burden in China: a time-trend analysis and comparison with the united states and united kingdom based on the global epidemiological data released in 2020. *Cancer Commun (Lond).* (2021) 41(10):1037–48. doi: 10.1002/cac2.12197

8. Du W, Frankel TL, Green M, Zou W. IFNγ signaling integrity in colorectal cancer immunity and immunotherapy. *Cell Mol Immunol* (2022) 19(1):23–32. doi: 10.1038/s41423-021-00735-3

9. Schürch CM, Bhate SS, Barlow GL, Phillips DJ, Noti L, Zlobec I, et al. Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front. *Cell* (2020) 182(5):1341–59. doi: 10.1016/j.cell.2020.07.005

10. Kalafati L, Kourtzelis I, Schulte-Schrepping J, Li X, Hatzioannou A, Grinenko T, et al. Innate immune training of granulopoiesis promotes anti-tumor activity. *Cell* (2020) 183(3):771–85. doi: 10.1016/j.cell.2020.09.058

11. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. *Science* (2018) 359(6382):1355–60. doi: 10.1126/science.aar7112

12. Leko V, Rosenberg SA. Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. *Cancer Cell* (2020) 38(4):454–72. doi: 10.1016/j.ccell.2020.07.013

13. Sugiura D, Okazaki IM, Maeda TK, Maruhashi T, Shimizu K, Arakaki R, et al. PD-1 agonism by anti-CD80 inhibits T cell activation and alleviates autoimmunity. *Nat Immunol* (2022) 23(3):399–410. doi: 10.1038/s41590-021-01125-7

14. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, et al. PD-1+ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci U S A* (2019) 116(20):9999–10008. doi: 10.1073/pnas.1822001116

15. Zhang X, Yang Z, An Y, Liu Y, Wei Q, Xu F, et al. Clinical benefits of PD-1/PD-L1 inhibitors in patients with metastatic colorectal cancer: A systematic review and meta-analysis. *World J Surg Oncol* (2022) 20(1):93. doi: 10.1186/s12957-022-02549-7

16. Olson DJ, Eroglu Z, Brockstein B, Poklepovic AS, Bajaj M, Babu S, et al. Pembrolizumab plus ipilimumab following anti-PD-1/L1 failure in melanoma. *J Clin Oncol* (2021) 39(24):2647–55. doi: 10.1200/JCO.21.00079

17. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* (2018) 24(9):1449–58. doi: 10.1038/s41591-018-0101-z

18. Zhao S, Ren S, Jiang T, Zhu B, Li X, Zhao C, et al. Low-dose apatinib optimizes tumor microenvironment and potentiates antitumor effect of PD-1/PD-L1 blockade in lung cancer. *Cancer Immunol Res* (2019) 7(4):630–43. doi: 10.1158/2326-6066.CIR-17-0640

19. Wensink E, Bond M, Kucukkose E, May A, Vink G, Koopman M, et al. A review of the sensitivity of metastatic colorectal cancer patients with deficient mismatch repair to standard-of-care chemotherapy and monoclonal antibodies, with recommendations for future research. *Cancer Treat Rev* (2021) 95:102174. doi: 10.1016/j.ctrv.2021.102174

20. Quiroga D, Lyerly HK, Morse MA. Deficient mismatch repair and the role of immunotherapy in metastatic colorectal cancer. *Curr Treat Options Oncol* (2016) 17(8):41. doi: 10.1007/s11864-016-0414-4

21. Lichtenstern CR, Ngu RK, Shalapour S, Karin M. Immunotherapy, inflammation and colorectal cancer. *Cells* (2020) 9(3):618. doi: 10.3390/cells9030618

22. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in microsatellite-Instability-High advanced colorectal cancer. *N Engl J Med* (2020) 383(23):2207–18. doi: 10.1056/NEJMoa2017699

23. Casak SJ, Marcus L, Fashoyin-Aje L, Mushti SL, Cheng J, Shen YL, et al. FDA Approval summary: Pembrolizumab for the first-line treatment of patients with MSI-H/dMMR advanced unresectable or metastatic colorectal carcinoma. *Clin Cancer Res* (2021) 27(17):4680–4. doi: 10.1158/1078-0432.CCR-21-0557

24. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* (2018) 19(1):40–50. doi: 10.1016/S1470-2045(17)30904-X

25. de Gouw DJJM, Klarenbeek BR, Driessen M, Bouwense SAW, van Workum F, Fütterer JJ, et al. Detecting pathological complete response in esophageal cancer after neoadjuvant therapy based on imaging techniques: A diagnostic systematic review and meta-analysis. *J Thorac Oncol* (2019) 14(7):1156–71. doi: 10.1016/j.jtho.2019.04.004

26. Mota FC, Cecconello I, Takeda FR, Tustumi F, Sallum RAA, Bernardo WM. Neoadjuvant therapy or upfront surgery? a systematic review and meta-analysis of T2N0 esophageal cancer treatment options. *Int J Surg* (2018) 54(Pt A):176–81. doi: 10.1016/j.ijsu.2018.04.053

27. Tie J, Wang Y, Cohen J, Li L, Hong W, Christie M, et al. Circulating tumor DNA dynamics and recurrence risk in patients undergoing curative intent resection of colorectal cancer liver metastases: A prospective cohort study. *PLoS Med* (2021) 18(5):e1003620. doi: 10.1371/journal.pmed.1003620

28. Wang Y, Yang L, Bao H, Fan X, Xia F, Wan J, et al. Utility of ctDNA in predicting response to neoadjuvant chemoradiotherapy and prognosis assessment in locally advanced rectal cancer: A prospective cohort study. *PLoS Med* (2021) 18 (8):e1003741. doi: 10.1371/journal.pmed.1003741

29. Bosset JF, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* (2006) 355(11):1114–23. doi: 10.1056/NEJMoa060829

30. Wu F, Zhou C, Wu B, Zhang X, Wang K, Wang J, et al. Adding adjuvants to fluoropyrimidine-based neoadjuvant chemoradiotherapy for locally advanced rectal cancer: An option worthy of serious consideration. *J Cancer* (2021) 12 (2):417–27. doi: 10.7150/jca.48337

31. Tang H, Liang Y, Anders RA, Taube JM, Qiu X, Mulgaonkar A, et al. PD-L1 on host cells is essential for PD-L1 blockade-mediated tumor regression. *J Clin Invest* (2018) 128(2):580–8. doi: 10.1172/JCI96061

32. Kong Y, Ma Y, Zhao X, Pan J, Xu Z, Zhang L. Optimizing the treatment schedule of radiotherapy combined with anti-PD-1/PD-L1 immunotherapy in metastatic cancers. *Front Oncol* (2021) 11:638873. doi: 10.3389/fonc.2021.638873

33. Wu SP, Liao RQ, Tu HY, Wang WJ, Dong ZY, Huang SM, et al. Stromal PD-L1-Positive regulatory T cells and PD-1-Positive CD8-positive T cells define the response of different subsets of non-small cell lung cancer to PD-1/PD-L1 blockade immunotherapy. *J Thorac Oncol* (2018) 13(4):521–32. doi: 10.1016/j.jtho.2017.11.132

34. Fournel L, Wu Z, Stadler N, Damotte D, Lococo F, Boulle G, et al. Cisplatin increases PD-L1 expression and optimizes immune check-point blockade in non-small cell lung cancer. *Cancer Lett* (2019) 464:5–14. doi: 10.1016/j.canlet.2019.08.005

35. Bailey SR, Maus MV. Gene editing for immune cell therapies. *Nat Biotechnol* (2019) 37(12):1425–34. doi: 10.1038/s41587-019-0137-8

36. He R, Lao Y, Yu W, Zhang X, Jiang M, Zhu C. Progress in the application of immune checkpoint inhibitor-based immunotherapy for targeting different types of colorectal cancer. *Front Oncol* (2021) 11:764618. doi: 10.3389/fonc.2021.764618

37. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* (2002) 99 (19):12293–7. doi: 10.1073/pnas.192461099

38. Wu X, Gu Z, Chen Y, Chen B, Chen W, Weng L, et al. Application of PD-1 blockade in cancer immunotherapy. *Comput Struct Biotechnol J* (2019) 17:661–74. doi: 10.1016/j.csbj.2019.03.006

39. Huang MY, Jiang XM, Wang BL, Sun Y, Lu JJ. Combination therapy with PD-1/PD-L1 blockade in non-small cell lung cancer: strategies and mechanisms. *Pharmacol Ther* (2021) 219:107694. doi: 10.1016/j.pharmthera.2020.107694

40. Bie F, Tian H, Sun N, Zang R, Zhang M, Song P, et al. Research progress of anti-PD-1/PD-L1 immunotherapy related mechanisms and predictive biomarkers in NSCLC. *Front Oncol* (2022) 12:769124. doi: 10.3389/fonc.2022.769124

41. Baretti M, Le DT. DNA Mismatch repair in cancer. *Pharmacol Ther* (2018) 189(218):45–62. doi: 10.1016/j.pharmthera.2018.04.004

42. Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* (2006) 7(5):335–46. doi: 10.1038/nrm1907

43. Gupta D, Heinen CD. The mismatch repair-dependent DNA damage response: Mechanisms and implications. *DNA Repair (Amst)* (2019) 78:60–9. doi: 10.1016/j.dnarep.2019.03.009

44. Zhang D, Tang B, Xie X, Xiao YF, Yang SM, Zhang JW. The interplay between DNA repair and autophagy in cancer therapy. *Cancer Biol Ther* (2015) 16 (7):1005–13. doi: 10.1080/15384047.2015.1046022

45. Fraune C, Burandt E, Simon R, Hube-Magg C, Makrypidi-Fraune G, Kluth M, et al. MMR deficiency is homogeneous in pancreatic carcinoma and associated with high density of Cd8-positive lymphocytes. *Ann Surg Oncol* (2020) 27 (10):3997–4006. doi: 10.1245/s10434-020-08209-y

46. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013

47. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* (1993) 75(5):1027–38. doi: 10.1016/0092-8674(93) 90546-3

48. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* (2015) 372 (26):2509–20. doi: 10.1056/NEJMoa1500596

49. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* (2019) 394(10207):1467–80. doi: 10.1016/S0140-6736(19)32319-0

50. Zaanan A, Shi Q, Taieb J, Alberts SR, Meyers JP, Smyrk TC, et al. Role of deficient DNA mismatch repair status in patients with stage III colon cancer treated with FOLFOX adjuvant chemotherapy: A pooled analysis from 2 randomized clinical trials. *JAMA Oncol* (2018) 4(3):379–83. doi: 10.1001/jamaoncol.2017.2899

51. Venderbosch S, Nagtegaal ID, Maughan TS, Smith CG, Cheadle JP, Fisher D, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. *Clin Cancer Res* (2014) 20(20):5322–30. doi: 10.1158/1078-0432.CCR-14-0332

52. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* (2010) 138(6):2073–87. doi: 10.1053/j.gastro.2009.12.064

53. Cevik M, Namal E, Iner-Koksal U, Dinc-Sener N, Karaalp A, Ciftci C, et al. Association of PD-1 and PDL-1 gene polymorphisms with colorectal cancer risk and prognosis. *Mol Biol Rep* (2022) 49(3):1827–36. doi: 10.1007/s11033-021-06992-9

54. Kishore C, Bhadra P. Current advancements and future perspectives of immunotherapy in colorectal cancer research. *Eur J Pharmacol* (2021) 893:173819. doi: 10.1016/j.ejphar.2020.173819

55. Lizardo DY, Kuang C, Hao S, Yu J, Huang Y, Zhang L. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: From bench to bedside. *Biochim Biophys Acta Rev Cancer.* (2020) 1874(2):188447. doi: 10.1016/j.bbcan.2020.188447

56. Tougeron D, Mouillet G, Trouilloud I, Lecomte T, Coriat R, Aparicio T, et al. Efficacy of adjuvant chemotherapy in colon cancer with microsatellite instability: A Large multicenter AGEO study. *J Natl Cancer Inst* (2016) 108(7): djv438. doi: 10.1093/jnci/djv438

57. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* (2010) 28 (20):3219–26. doi: 10.1200/JCO.2009.27.1825

58. Gelsomino F, Barbolini M, Spallanzani A, Pugliese G, Cascinu S. The evolving role of microsatellite instability in colorectal cancer: A review. *Cancer Treat Rev* (2016) 51:19–26. doi: 10.1016/j.ctrv.2016.10.005

59. Zhang X, Wu T, Cai X, Dong J, Xia C, Zhou Y, et al. Neoadjuvant immunotherapy for MSI-H/dMMR locally advanced colorectal cancer: New strategies and unveiled opportunities. *Front Immunol* (2022) 13:795972. doi: 10.3389/fimmu.2022.795972

60. Luchini C, Bibeau F, Ligtenberg MJL, Singh N, Nottegar A, Bosse T, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: A systematic review-based approach. *Ann Oncol* (2019) 30(8):1232–43. doi: 10.1093/annonc/mdz116

61. Zhou C, Cheng X, Tu S. Current status and future perspective of immune checkpoint inhibitors in colorectal cancer. *Cancer Lett* (2021) 521:119–29. doi: 10.1016/j.canlet.2021.07.023

62. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-High/Mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol* (2020) 38(1):11–9. doi: 10.1200/JCO.19.02107

63. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* (2017) 18(9):1182–91. doi: 10.1016/S1470-2045(17)30422-9

64. Fan A, Wang B, Wang X, Nie Y, Fan D, Zhao X, et al. Immunotherapy in colorectal cancer: Current achievements and future perspective. *Int J Biol Sci* (2021) 17(14):3837–49. doi: 10.7150/ijbs.64077

65. Diaz LAJr, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. *Lancet Oncol* (2022) 23 (5):659–70. doi: 10.1016/S1470-2045(22)00197-8

66. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. Colon cancer, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2021) 19(3):329–59. doi: 10.6004/jnccn.2021.0012

67. Hasan S, Renz P, Wegner RE, Finley G, Raj M, Monga D, et al. Microsatellite instability (MSI) as an independent predictor of pathologic complete response (PCR) in locally advanced rectal cancer: A national cancer database (NCDB) analysis. *Ann Surg* (2020) 271(4):716–23. doi: 10.1097/SLA.0000000000003051

68. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med* (2020) 26 (4):566–76. doi: 10.1038/s41591-020-0805-8

69. Hu H, Kang L, Zhang J, Wu Z, Wang H, Huang M, et al. Neoadjuvant PD-1 blockade with toripalimab, with or without celecoxib, in mismatch repair-deficient or microsatellite instability-high, locally advanced, colorectal cancer (PICC): a single-centre, parallel-group, non-comparative, randomised, phase 2 trial. *Lancet Gastroenterol Hepatol* (2022) 7(1):38–48. doi: 10.1016/S2468-1253(21)00348-4

70. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, et al. PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. *N Engl J Med* (2022) 386(25):2363–76. doi: 10.1056/NEJMoa2201445

71. Wang YJ, Fletcher R, Yu J, Zhang L. Immunogenic effects of chemotherapy-induced tumor cell death. *Genes Dis* (2018) 5(3):194–203. doi: 10.1016/j.gendis.2018.05.003

72. Wen L, Tong F, Zhang R, Chen L, Huang Y, Dong X. The research progress of PD-1/PD-L1 inhibitors enhancing radiotherapy efficacy. *Front Oncol* (2021) 11:799957. doi: 10.3389/fonc.2021.799957

73. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, McKenna C, Jones S, Cheadle EJ, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res* (2014) 74(19):5458–68. doi: 10.1158/0008-5472.CAN-14-1258

74. Deng L, Liang H, Burnette B, Weicheslbaum RR, Fu YX. Radiation and anti-PD-L1 antibody combinatorial therapy induces T cell-mediated depletion of myeloid-derived suppressor cells and tumor regression. *Oncoimmunology* (2014) 3:e28499. doi: 10.4161/onci.28499

75. Bando H, Tsukada Y, Inamori K, Togashi Y, Koyama S, Kotani D, et al. Preoperative chemoradiotherapy plus nivolumab before surgery in patients with microsatellite stable and microsatellite instability-high locally advanced rectal cancer. *Clin Cancer Res* (2022) 28(6):1136–46. doi: 10.1158/1078-0432.CCR-21-3213

76. Lin Z, Cai M, Zhang P, Li G, Liu T, Li X, et al. Single-arm trial of preoperative short-course radiotherapy followed by chemotherapy and camrelizumab in locally advanced rectal cancer. *J Immunother Cancer* (2021) 9 (11):e003554. doi: 10.1136/jitc-2021-003554

77. Rahma OE, Yothers G, Hong TS, Russell MM, You YN, Parker W, et al. Use of total neoadjuvant therapy for locally advanced rectal cancer: Initial results from the pembrolizumab arm of a phase 2 randomized clinical trial. *JAMA Oncol* (2021) 7(8):1225–30. doi: 10.1001/jamaoncol.2021.1683

78. Yang Z, Zhang X, Zhang J, Gao J, Bai Z, Deng W, et al. Rationale and design of a prospective, multicenter, p II clinical trial of safety and efficacy evaluation of long course neoadjuvant chemoradiotherapy plus tislelizumab followed by total mesorectal excision for locally advanced rectal cancer (NCRT-PD1-LARC trial). *BMC Cancer* (2022) 22(1):462. doi: 10.1186/s12885-022-09554-9

79. Zhao M, Zhao L, Yang H, Duan Y, Li G. Apparent diffusion coefficient for the prediction of tumor response to neoadjuvant chemo-radiotherapy in locally advanced rectal cancer. *Radiat Oncol* (2021) 16(1):17. doi: 10.1186/s13014-020-01738-6

80. Borcoman E, Kanjanapan Y, Champiat S, Kato S, Servois V, Kurzrock R, et al. Novel patterns of response under immunotherapy. *Ann Oncol* (2019) 30 (3):385–96. doi: 10.1093/annonc/mdz003

81. Colle R, Radzik A, Cohen R, Pellat A, Lopez-Tabada D, Cachanado M, et al. Pseudoprogression in patients treated with immune checkpoint inhibitors for microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer. *Eur J Cancer* (2021) 144:9–16. doi: 10.1016/j.ejca.2020.11.009

82. Park HJ, Kim GH, Kim KW, Lee CW, Yoon S, Chae YK, et al. Comparison of RECIST 1.1 and iRECIST in patients treated with immune checkpoint inhibitors: A systematic review and meta-analysis. *Cancers (Basel)* (2021) 13(1):120. doi: 10.3390/cancers13010120

83. Andrews MC, Duong CPM, Gopalakrishnan V, Iebba V, Chen WS, Derosa L, et al. Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade. *Nat Med* (2021) 27(8):1432–41. doi: 10.1038/s41591-021-01406-6

84. Wang Y, Zhou S, Yang F, Qi X, Wang X, Guan X, et al. Treatment-related adverse events of PD-1 and PD-L1 inhibitors in clinical trials: A systematic review and meta-analysis. *JAMA Oncol* (2019) 5(7):1008–19. doi: 10.1001/jamaoncol.2019.0393

85. Baxi S, Yang A, Gennarelli RL, Khan N, Wang Z, Boyce L, et al. Immune-related adverse events for anti-PD-1 and anti-PD-L1 drugs: systematic review and meta-analysis. *BMJ* (2018) 360:k793. doi: 10.1136/bmj.k793

86. Masuda K, Shoji H, Nagashima K, Yamamoto S, Ishikawa M, Imazeki H, et al. Correlation between immune-related adverse events and prognosis in patients with gastric cancer treated with nivolumab. *BMC Cancer* (2019) 19(1):974. doi: 10.1186/s12885-019-6150-y

87. Lo JA, Fisher DE, Flaherty KT. Prognostic significance of cutaneous adverse events associated with pembrolizumab therapy. *JAMA Oncol* (2015) 1(9):1340–1. doi: 10.1001/jamaoncol.2015.2274

88. Hirano H, Takashima A, Hamaguchi T, Shida D, Kanemitsu YColorectal Cancer Study Group (CCSG) of the Japan Clinical Oncology Group (JCOG). Current status and perspectives of immune checkpoint inhibitors for colorectal cancer. *Jpn J Clin Oncol* (2021) 51(1):10–9. doi: 10.1093/jjco/hyaa200

89. Haanen JBAG, Carbonnel F, Robert C, Kerr KM, Peters S, Larkin J, et al. Management of toxicities from immunotherapy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2017) 28(suppl_4):iv119–42. doi: 10.1093/annonc/mdx225.Erratumin:AnnOncol

90. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline. *J Clin Oncol* (2018) 36(17):1714–68. doi: 10.1200/JCO.2017.77.6385

91. Thompson JA, Schneider BJ, Brahmer J, Andrews S, Armand P, Bhatia S, et al. NCCN guidelines insights: Management of immunotherapy-related toxicities, version 1.2020. *J Natl Compr Canc Netw* (2020) 18(3):230–41. doi: 10.6004/jnccn.2020.0012

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PD-1 inhibitors plus anti-angiogenic therapy with or without intensity-modulated radiotherapy for advanced hepatocellular carcinoma: A propensity score matching study

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Background: Whether intensity-modulated radiotherapy (IMRT) can enhance the efficacy of the programmed death (PD)-1 inhibitors combined with anti-angiogenic therapy for hepatocellular carcinoma (HCC) is unclear. Therefore, we conducted this multicenter retrospective study to investigate the efficacy of the combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT.

Methods: From April 2019 to March 2022, a total of 197 patients with HCC [combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT (triple therapy group), 54; PD-1 inhibitors plus anti-angiogenic therapy (control group), 143] were included in our study. Propensity score matching (PSM) was applied to identify two groups with similar baselines. The objective response rate (ORR), overall survival (OS), and progression-free survival (PFS) of the two groups were compared before and after matching.

Results: Prior to PSM, the triple therapy group had higher ORR (42.6% vs 24.5%, P = 0.013) and more superior median OS (mOS) (20.1 vs 13.3 months, P = 0.009) and median PFS (mPFS) (8.7 vs 5.4 months, P = 0.001) than the control group. Following PSM, the triple therapy group still exhibited better mPFS (8.7 vs 5.4 months, P = 0.013) and mOS (18.5 vs 12.6 months, P = 0.043) than the control group. However, the ORR of the two groups was similar (40% vs 25%, P = 0.152). No significant difference was observed in the treatment-related adverse events between the two groups (P < 0.05 for all).

Conclusions: The combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT for HCC is a promising regimen.

KEYWORDS

programmed death-1 inhibitors, anti-angiogenic therapy, intensity-modulated radiotherapy, hepatocellular carcinoma, propensity score matching

Introduction

Hepatocellular carcinoma (HCC) is the most common cause of cancer-related death (1). Despite the wide use of early detection techniques to diagnose HCC, most patients are diagnosed at an advanced stage (2). The overall survival (OS) of patients with HCC is extremely short, therefore, the prognosis of patients should be urgently improved (3).

Currently, the combination of programmed death 1/ programmed death ligand 1 (PD-1/PD-L1) inhibitors and targeted drugs has become prominent in HCC research. Atezolizumab plus bevacizumab, the current first-line treatment option, extends median OS (mOS) to 19.2 months and objective response rate (ORR) to 27.3% in inoperable HCC (4, 5). Additionally, Ren et al. (6) reported an ORR of 21% and a median progression-free survival (mPFS) of 4.6 months in patients with inoperable HCC who received sintilimab plus bevacizumab. In the RESCUE study of camrelizumab plus apatinib for advanced HCC, the ORR was 34.3% and mPFS was 5.7 months (7). Despite breakthroughs in the combination therapy of PD-1/PD-L1 inhibitors and targeted drugs, its ORR was still low. The addition of other treatments that can improve local control of HCC has become a new research direction.

Intensity-modulated radiotherapy (IMRT), an external RT modality, is a local treatment method that uses radiation to irradiate malignant tumor cells. Abulimiti et al. (8) confirmed that IMRT plus sorafenib can improve the prognosis of advanced HCC, for which the mOS was observed to be 11.4 months and the mPFS was 6 months. Additionally, patients with advanced HCC who received IMRT in combination with apatinib had an mPFS of 7.8 months and an ORR of 15% (9). Radiotherapy can not only promote the generation and infiltration of T cells but also stimulate systemic anti-tumor immunity to control metastatic lesions, causing the "abscopal effect" (10). Furthermore, targeting vascular endothelial growth factor (VEGF) can normalize tumor vessels and enhance T cell infiltration, thus, providing a rationale for combining this therapy with immunotherapy (11).

Based on these results, the combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT is a promising treatment modality. We conducted this multicenter retrospective study to investigate the efficacy of triple therapy.

Materials and methods

Patients

From April 2019 to March 2022, a total of 197 patients with HCC [combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT (triple therapy group), 54; PD-1 inhibitors plus anti-angiogenic therapy (control group), 143] from three Chinese tertiary hospitals were included in our retrospective study.

The inclusion criteria were as follows: a) Pathologically diagnosed HCC; b) Barcelona Clinic Liver Cancer (BCLC) stage B/C; c) Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0–2; d) Child-Pugh class A/B; e) at least one measurable lesion according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST); f) administration of at least one cycle of PD-1 inhibitors plus anti-angiogenic therapy with or without IMRT; g) patients were able to undergo IMRT after evaluation. The exclusion criteria were as follows: a) Incomplete information; b) number of tumors >5 or diffuse lesions; c) presence of other malignancies; d) severe ascites or hepatic encephalopathy.

This study was approved by the Ethics Committee of the affiliated hospital of Southwest Medical University (approval number KY2020254). We waived individual informed consent since this was a retrospective study.

Treatment protocol

IMRT

IMRT was performed within 7 days of the administration of the first cycle of PD-1 inhibitors plus anti-angiogenic therapy. The radiologist used the radiation planning system to delineate the target volume with computed tomography (CT) guidance. Delineation of the clinical target volume (CTV) including a 4-mm margin of the primary liver tumor was accomplished through image technology. The planning target volume (PTV) was defined as a 5-10-cm peripheral expansion based on CTV. The total target radiation dose was 48 g with 3 Gy/fraction, and at least 95% of PTV received the prescribed dose. The dose constraints for the organs at risk were as follows: Spinal cord (maximum dose ≤45 Gy); normal liver (mean dose ≤30 Gy); stomach and duodenum (maximum dose ≤54 Gy); colon (maximum dose ≤55 Gy).

Administration of PD-1 inhibitors and targeted agents

All patients received PD-1 inhibitor injection once every three weeks as well as the antiangiogenic drug on daily basis until the appearance of intolerable toxic reactions or progressive disease. The doses of PD-1 inhibitors and targeted drugs were calculated based on the patient's height and weight. Dosing delays were allowed when a serious treatment-related adverse event (TRAE) occurred.

Follow-up and data collection

The efficacy of patients was assessed by CT/Magnetic Resonance Imaging (MRI) performed every 2–3 months. Treatment response was divided into complete response (CR), partial response (PR), stable disease (SD), and progressive disease according to mRECIST. The time interval from treatment initiation to progressive disease was PFS. The time interval from the initiation of treatment to the death or last follow-up was OS.

Statistical analysis

χ^2 test and McNemar analysis were used for categorical variables. Propensity score matching (PSM) was applied to identify two groups with similar baselines. Matching variables included age, sex, tumor size, alanine transaminase level, tumor number, platelet level, alkaline phosphatase level, Child-Pugh score, alpha-fetoprotein (AFP) level, leukocyte level, BCLC stage, portal vein invasion, hepatitis B virus infection, extrahepatic metastasis, and lymph node metastasis. PFS and OS were estimated using the Kaplan–Meier method and log-rank test. Cox analysis was used to identify prognostic factors affecting OS and PFS. Statistical analysis of this study was performed using SPSS for Windows version 26.0. Two-tailed *P*-value of <0.05 was considered significant.

Results

Patient characteristics prior to and following PSM

Between April 2019 and March 2022, a total of 197 patients who met the inclusion and exclusion criteria received the combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT and PD-1 inhibitors plus anti-angiogenic therapy.

Prior to PSM, there were differences in gender, leukocyte level, BCLC stage, lymph node metastasis, and extrahepatic metastases between the two groups ($P < 0.05$ for all). Eighty patients were identified through PSM. In this matched cohort, no differences in any covariates at baseline were observed between the two groups (Table 1).

The triple therapy group exhibited promising efficacy

As of April 2022, before matching, a total of 91 (63.6%) and 19 (35.2%) patients died in the control group and the triple therapy group, respectively. The median follow-up time of the control group and triple therapy group was 15.5 and 12 months, respectively. Patients who received triple therapy had longer mPFS (8.7 vs 5.4 months, $P = 0.001$, Figure 1A) and mOS (20.1 vs 13.3 months, $P = 0.009$, Figure 1B) than those who received PD-1 inhibitors plus anti-angiogenic therapy. Following PSM, 14 patients (35%) in the triple therapy group and 27 patients (67.5%) in the control group died. Patients who received triple therapy had longer mPFS (8.7 vs 5.4 months, $P = 0.013$, Figure 1C) and mOS (18.5 vs 12.6 months, $P = 0.043$, Figure 1D) than those who received PD-1 inhibitors plus anti-angiogenic therapy.

TABLE 1 Baseline characteristics of the patients before and after PSM.

Variable	Before PSM			After PSM		
	Triple therapy group	Control group	*P*	Triple therapy group	Control group	*P*
Patients	54	143		40	40	
Male sex	51 (94.4)	112 (78.3)	0.008	37 (92.5)	38 (95.0)	1.000
Age ≥ 65 years	11 (20.4)	29 (20.3)	0.989	10 (25.0)	6 (15.0)	0.424
Child–Pugh score			0.735			0.568
5	20 (37.0)	62 (43.4)		16 (40.0)	15 (37.5)	
6	20 (37.0)	39 (27.3)		13 (32.5)	13 (32.5)	
7	8 (14.8)	27 (18.9)		6 (15.0)	8 (20.0)	
8	4 (7.4)	10 (7.0)		3 (7.5)	2 (5.0)	
9	2 (3.7)	5 (3.5)		2 (5.0)	2 (5.0)	
Number of tumors ≥ 2	40 (74.1)	118 (82.5)	0.185	31 (77.5)	28 (70.0)	0.629
Tumor diameter, cm			0.243			0.937
< 3	3 (5.6)	9 (6.3)		2 (5.0)	1 (2.5)	
≥ 3, < 5	6 (11.1)	34 (23.8)		6 (15.0)	6 (15.0)	
≥ 5, < 10	30 (55.6)	69 (48.3)		21 (52.5)	20 (50.0)	
≥ 10	15 (27.8)	31 (21.7)		11 (27.5)	13 (32.5)	
Serum AFP, ng/ml			0.700			0.572
< 200	27 (50.0)	79 (55.2)		21 (52.5)	17 (42.5)	
≥ 200, < 400	2 (3.7)	7 (4.9)		2 (5.0)	4 (10.0)	
≥ 400	25 (46.3)	57 (39.9)		17 (42.5)	19 (47.5)	
ALP levels ≥ 125 U/L	26 (48.1)	87 (60.8)	0.108	23 (57.5)	23 (57.5)	1.000
Platelet count ≥ 100 × 109/L	46 (85.2)	109 (76.2)	0.171	32 (80.0)	35 (87.5)	0.581
ALT levels ≥ 40 U/L	31 (57.4)	74 (51.7)	0.478	20 (50.0)	20 (50.0)	1.000
Leukocyte ≥ 4 × 109/L	41 (75.9)	128 (89.5)	0.015	30 (75.0)	34 (85.0)	0.388
BCLC stage			0.041			1.000
B	3 (5.6)	24 (16.8)		3 (7.5)	3 (7.5)	
C	51 (94.4)	119 (83.2)		37 (92.5)	37 (92.5)	
Portal vein invasion	46 (85.2)	91 (63.6)	0.003	32 (80.0)	32 (80.0)	1.000
HBV	33 (61.1)	77 (53.8)	0.360	24 (60.0)	21 (52.5)	0.678
Lymph node metastasis	21 (38.9)	80 (55.9)	0.033	19 (47.5)	19 (47.5)	1.000
Extrahepatic metastases	11 (20.4)	59 (41.3)	0.006	9 (22.5)	11 (27.5)	0.774
Lung	4 (7.4)	33 (23.1)		3 (7.5)	6 (15.0)	
Bone	6 (11.1)	15 (10.5)		5 (12.5)	4 (10.0)	
Other	1 (1.9)	28 (19.6)		1 (2.5)	5 (12.5)	

PSM, propensity score matching; AFP, alpha fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus.

PFS and OS in different subgroups

In the subgroup of patients with child-pugh class A and tumor diameter of ≥ 5 cm, the triple therapy group had longer mOS (not reach vs 14.4 months, P = 0.042, Supplementary Figure 1C; 18.5 vs 11.4 months, P = 0.018, Supplementary Figure 1E) and mPFS (25.9 vs 5.5 months, P = 0.005, Supplementary Figure 1H; 8.7 vs 5.4 months, P = 0.009, Supplementary Figure 1J) than the control group. However, in the subgroup analysis of patients with portal vein tumor thrombus (PVTT), child B, and extrahepatic metastases, there were no significant differences in OS and PFS between the two groups (Supplementary Figure 1).

Tumor response

Prior to PSM, the ORR was 42.6% in the triple therapy group and 24.5% in the control group (P = 0.013). However, the disease control rates (DCR) of two groups were similar (90.7% vs 79.7%, P = 0.068). Following PSM, although the ORR and DCR of the triple therapy group were still slightly better than those of the

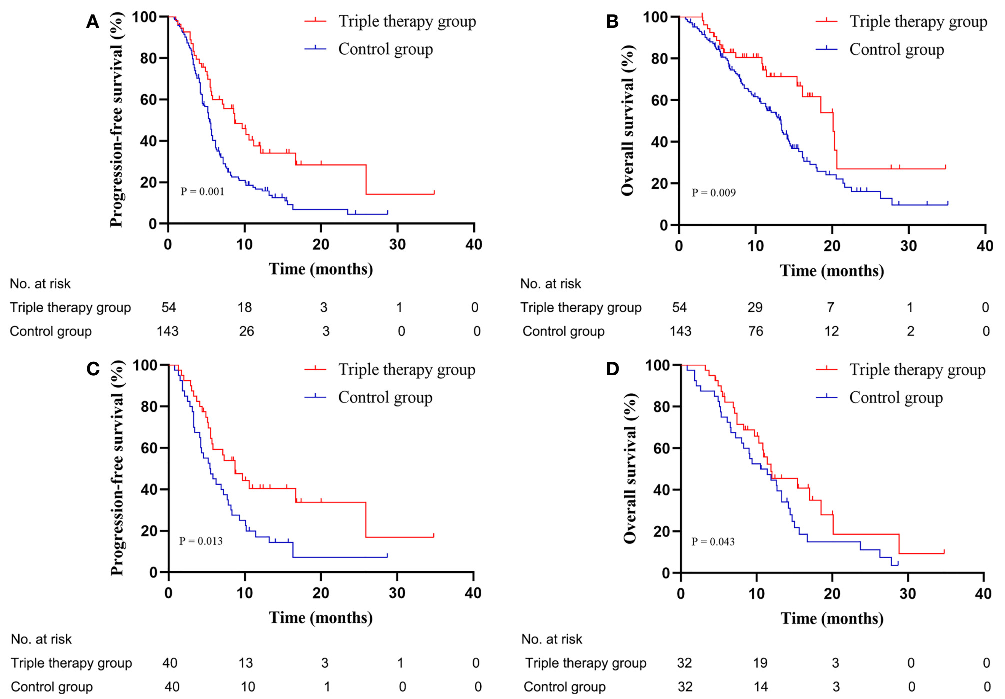

FIGURE 1
Kaplan-Meier plots: The triple therapy group exhibited longer mPFS **(A, C)** and mOS **(B, D)** than that of the control group before and after PSM. mPFS, median progression-free survival; mOS, median overall survival; PSM, propensity score matching.

control group, the differences were not significant (40% vs 25%, P = 0.152; 90% vs 77.5%, P = 0.130; respectively; Table 2).

Factors associated with PFS and OS following PSM

Univariate and multivariate Cox regression analyses were used to identify prognostic indicators affecting PFS and OS following PSM. Age, Child-Pugh class, AFP level, and triple therapy were determined to be influencing factors for PFS and OS (P < 0.05 for all). In the multivariate analysis, an AFP level of ≥400 ng/mL was an independent negative prognostic factor for PFS (Table 3), whereas child B, lymph node metastasis, and treatment method were independent prognostic factors for OS (Table 4).

Safety

We further investigated the TRAEs of the two groups. Treatment was interrupted in 55 patients (triple therapy

TABLE 2 Tumor response assessed by mRECIST.

Best response	Before PSM			After PSM		
	Triple therapy group	Control group	P	Triple therapy group	Control group	P
Objective response	23 (42.6)	35 (24.5)	0.013	16 (40.0)	10 (25.0)	0.152
Disease control	49 (90.7)	114 (79.7)	0.068	36 (90.0)	31 (77.5)	0.130
Best overall response						
Complete response	1 (1.9)	2 (1.4)		1 (2.5)	0	
Partial response	22 (40.7)	33 (23.1)		15 (37.5)	10 (25.0)	
Stable disease	26 (48.1)	79 (55.2)		20 (50.0)	21 (52.5)	
Progressive disease	5 (9.3)	29 (20.3)		4 (10.0)	9 (22.5)	

mRECIST, modified Response Evaluation Criteria in Solid Tumors.

TABLE 3 Univariate and multivariate Cox regression analysis of progression-free survival after PSM.

Variable	Univariable Cox regression			Multivariable Cox regression		
	HR	95%CI	*P*	HR	95%CI	*P*
Sex (male/female)	2.121	0.657-6.853	0.209			
Age (≥65/<65 years)	0.366	0.172-0.779	0.009	0.481	0.220-1.052	0.067
Child-Pugh class (B/A)	2.109	1.227-3.623	0.007	1.564	0.892-2.74	0.118
Number of tumors (≥2/<2)	1.584	0.859-2.922	0.141			
Tumor diameter (≥5/<5 cm)	1.334	0.672-2.648	0.409			
AFP (≥400/<400 ng/ml)	2.86	1.676-4.878	<0.001	2.043	1.158-3.605	0.014
ALP (≥125/<125 U/L)	1.202	0.716-2.016	0.487			
Platelet (<100000/≥100000/µL)	0.798	0.422-1.507	0.487			
ALT (≥40/<40U/L)	1.129	0.676-1.887	0.642			
Leukocyte (<4000/≥4000/µL)	0.730	0.400-1.333	0.305			
HBV (positive/negative)	1.044	0.622-1.751	0.870			
Portal vein invasion (yes/no)	1.291	0.669-2.490	0.446			
BCLC stage (C/B)	1.008	0.363-2.798	0.987			
Lymph node metastasis (yes/no)	1.287	0.767-2.159	0.340			
Extrahepatic metastases (yes/no)	1.090	0.605-1.962	0.774			
Triple therapy (Yes/No)	0.522	0.309-0.882	0.015	0.603	0.354-1.029	0.063

PSM, propensity score matching; HR, hazard ratio; AFP, alpha fetoprotein; ALP, alkaline phosphatase; ALT, alanine transaminase; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer.

TABLE 4 Univariate and multivariate Cox regression analysis of overall survival after PSM.

Variable	Univariable Cox regression			Multivariable Cox regression		
	HR	95%CI	*P*	HR	95%CI	*P*
Sex (male/female)	1.924	0.460-8.048	0.37			
Age (≥65/<65 years)	0.364	0.142-0.934	0.036	0.460	0.170-1.242	0.125
Child-Pugh class (B/A)	3.638	1.919-6.897	<0.001	3.114	1.538-6.305	0.002
Number of tumors (≥2/<2)	2.035	0.931-4.449	0.075			
Tumor diameter (≥5/<5 cm)	1.334	0.605-2.939	0.475			
AFP (≥400/<400 ng/ml)	2.539	1.344-4.797	0.004	1.856	0.919-3.748	0.084
ALP (≥125/<125 U/L)	1.300	0.693-2.439	0.413			
Platelet (<100000/≥100000/µL)	0.877	0.400-1.924	0.744			
ALT (≥40/<40U/L)	0.927	0.501-1.716	0.809			
Leukocyte (<4000/≥4000/µL)	0.72	0.343-1.511	0.385			
HBV (positive/negative)	1.017	0.545-1.899	0.957			
Portal vein invasion (yes/no)	2.091	0.819-5.339	0.123			
BCLC stage (C/B)	3.172	0.434-23.157	0.255			
Lymph node metastasis (yes/no)	1.928	1.014-3.665	0.045	2.002	1.036-3.871	0.039
Extrahepatic metastases (yes/no)	0.963	0.470-1.975	0.919			
Triple therapy (Yes/No)	0.520	0.272-0.993	0.048	0.511	0.262-0.996	0.049

PSM, propensity score matching; HR, hazard ratio; AFP, alpha fetoprotein; ALP, alkaline phosphatase; ALT, alanine transaminase; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer.

group, 18; control group, 37) secondary to serious TRAEs. The addition of IMRT did not significantly increase the TRAEs of PD-1 inhibitors plus anti-angiogenic therapy ($P < 0.05$ for all). There were no treatment-related deaths (Table 5).

Discussion

Currently, although atezolizumab plus bevacizumab is the first recommendation for treating advanced HCC, its ORR of 27.3% remains unsatisfactory (4, 5). Therefore, it is necessary to explore other therapeutic methods that can improve the local control of advanced HCC. This was the first study on PD-1 inhibitors with anti-angiogenic therapy and IMRT vs PD-1 inhibitors plus anti-angiogenic therapy for the treatment of advanced HCC.

Prior to PSM, the triple therapy group had higher ORR (42.6% vs 24.5%, $P = 0.013$) and longer mOS (20.1 vs 13.3 months, $P = 0.009$) and mPFS (8.7 vs 5.4 months, $P = 0.001$) than those of the control group. Following PSM, the triple therapy group revealed better efficacy than the control group. This may be owing to strong local control of radiotherapy (12, 13). It not only induces immunogenic death but also modulates the tumor microenvironment to stimulate the production of antitumor T cells (14, 15). Moreover, radiotherapy increases the production of cell adhesion molecules, and targeting VEGF can promote the normalization of the vascular endothelium. This further enhances antitumor T cell infiltration (11, 16, 17).

Currently, new techniques such as stable homogeneous iodinated formulation technology hold good potential for surgical resection after arterial embolization in clinical practice (18). However, many HCC patients have already lost the opportunity for surgery. Immunotherapy plus targeted therapy for advanced HCC has been the focus of research (4–7), whereas the research on the combination of radiotherapy and immunotherapy is in its infancy. In a retrospective study of patients with HCC receiving stereotactic body radiotherapy (SBRT) plus PD-1 inhibitors, the mPFS was 19.6 months and ORR was 71% (19). Zhong et al. (20) observed that patients with advanced HCC treated with SBRT combined with PD-1 inhibitors had a higher ORR of 40%, mPFS of 3.8 months, and mOS of 21.2 months. Additionally, Ricke et al. reported that the mOS of patients with HCC receiving selective internal radiation therapy plus sorafenib was 12.1 months (21). Further, satisfactory results were also obtained with nivolumab plus ipilimumab for advanced HCC (mOS = 22.8 months, ORR = 32%) (22). In our study, the triple therapy group revealed better efficacy than the control group.

The safety of other methods based on PD-1 inhibitors plus anti-angiogenic therapy has been questioned. Liu et al. (23) confirmed that patients with HCC treated with hepatic artery infusion chemotherapy, tyrosine kinase inhibitors, and anti-PD-1 antibodies exhibited good efficacy (mPFS = 10.6 months, ORR = 63%) and safety. Furthermore, among patients with unresectable HCC, transarterial chemoembolization-lenvatinib-pembrolizumab sequential therapy exhibited promising efficacy (mPFS = 9.2 months, mOS = 18.1 months), with a well-characterized safety profile (24). In our research, we confirmed that the addition of IMRT did not significantly increase the TRAEs of PD-1 inhibitors plus anti-angiogenic therapy. Based on these findings, combining radiotherapy with immune and targeted therapies is a promising combination modality.

TABLE 5 Treatment-related adverse events in the two groups.

Adverse Event	Triple therapy group		Control group		P
	Grade 1-2	Grade ≥3	Grade 1-2	Grade ≥3	
Leukopenia	29 (53.7)	4 (7.4)	58 (40.6)	8 (5.6)	0.173
Thrombocytopenia	24 (44.4)	3 (5.6)	52 (36.4)	7 (4.9)	0.541
Decreased appetite	15 (27.8)	3 (5.6)	32 (22.4)	7 (4.9)	0.699
Neutropenia	14 (25.9)	1 (1.9)	26 (18.2)	2 (1.4)	0.461
Fatigue	6 (11.1)	2 (3.7)	14 (9.8)	5 (3.5)	0.959
Nausea	8 (14.8)	3 (5.6)	16 (11.2)	5 (3.5)	0.612
Anemia	7 (13.0)	1 (1.9)	9 (6.3)	1 (0.7)	0.232
Increased alanine aminotransferase	10 (18.5)	2 (3.7)	18 (12.6)	1 (0.7)	0.160
Rash	4 (7.4)	2 (3.7)	8 (5.6)	1 (0.7)	0.268
Pruritus	4 (7.4)	0	9 (6.3)	1 (0.7)	0.798
Fever	3 (5.6)	0	5 (3.5)	0	0.514
Increased aspartate aminotransferase	9 (16.7)	2 (3.7)	14 (9.8)	3 (2.1)	0.314
Hypothyroidism	3 (5.6)	0	5 (3.5)	0	0.514
Hypertension	2 (3.7)	0	3 (2.1)	0	0.523
Headache	1 (1.9)	0	1 (0.7)	1 (0.7)	0.640

In the subgroups of patients with child A and tumor diameter ≥5 cm, the triple therapy group had more superior mOS and mPFS than the control group. However, in the other subgroups, there were no significant differences in OS and PFS between the two groups. Additionally, we observed that the ORR of the triple therapy group prior to PSM was better than that of the control group (42.6% vs 24.5%, $P = 0.013$) whereas the ORR of the two groups of patients following PSM was similar (40% vs 25%, $P = 0.152$). These may be owing to the smaller sample size.

Further, we explored prognostic factors affecting PFS and OS. The AFP level of ≥400 ng/mL is a risk factor for disease progression. However, for child A, without lymph node metastasis, triple therapy was an independent prognostic factor causing longer OS. Moreover, previous studies have also reported that these indicators were associated with prognosis (25–27).

This study had some limitations. First, although PSM was performed to minimize the effects of observed confounding factors, the effects of selectivity bias and various potential defects were not excluded. Second, despite this being the largest study reported to date, the number of patients in the triple therapy group remained less. Last, although our study confirms that IMRT further improves the efficacy of the combination of PD-1 inhibitors and anti-angiogenic therapy, it is still affected by the underlying heterogeneity of different therapeutic agents.

Conclusions

Conclusively, this study confirmed that the combination of PD-1 inhibitors with anti-angiogenic therapy and IMRT is a promising combination regimen. Our study provides a theoretical basis for studying combination therapy for HCC. Future prospective studies with larger sample sizes are needed to determine the efficacy of triple therapy.

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 human participants were reviewed and approved by the Ethics Committee of the affiliated hospital of Southwest Medical University (approval number KY2020254). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

KS, LG, WM, JW, YX, MR, JZ, XL, LW, BL, XY, YS, WH, HC, TG, KX, YL, JC, ZW, YJ, HL, HZ, PW, XF, SC, BY, HJ, KH, and YH collected the data. YH and KH designed the research study. KS, LG, WM, and YH wrote the manuscript and analyzed the data. All authors approved the final version of the manuscript.

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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/fimmu.2022.972503/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel R, 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. Llovet J, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* (2016) 2:16018. doi: 10.1038/nrdp.2016.18

3. Llovet J, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* (2008) 359(4):378–90. doi: 10.1056/NEJMoa0708857

4. Finn R, Qin S, Ikeda M, Galle P, Ducreux M, Kim T, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* (2020) 382 (20):1894–905. doi: 10.1056/NEJMoa1915745

5. Cheng A, Qin S, Ikeda M, Galle P, Ducreux M, Kim T, et al. Updated efficacy and safety data from IMbrave150: Atezolizumab plus bevacizumab vs. sorafenib unresectable hepatocellular carcinoma. *J Hepatol* (2022) 76(4):862–73. doi: 10.1016/j.jhep.2021.11.030

6. Ren Z, Xu J, Bai Y, Xu A, Cang S, Du C, et al. Sintilimab plus a bevacizumab biosimilar (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): a randomised, open-label, phase 2-3 study. *Lancet Oncol* (2021) 22(7):977–90. doi: 10.1016/s1470-2045(21)00252-7

7. Xu J, Shen J, Gu S, Zhang Y, Wu L, Wu J, et al. Camrelizumab in combination with apatinib in patients with advanced hepatocellular carcinoma (RESCUE): A nonrandomized, open-label, phase II trial. *Clin Cancer Res* (2021) 27(4):1003–11. doi: 10.1158/1078-0432.Ccr-20-2571

8. Abulimiti M, Li Z, Wang H, Apiziaji P, Abulimiti Y, Tan YJ. Combination intensity-modulated radiotherapy and sorafenib improves outcomes in hepatocellular carcinoma with portal vein tumor thrombosis. *J Oncol* (2021) 2021:1–10. doi: 10.1155/2021/9943683

9. Qiu H, Ke S, Cai G, Wu Y, Wang J, Shi W, et al. An exploratory clinical trial of apatinib combined with intensity-modulated radiation therapy for patients with unresectable hepatocellular carcinoma. *Cancer Med* (2022) 11(6):35. doi: 10.1002/cam4.4900

10. Herrera F, Bourhis J, Coukos GJ. Radiotherapy combination opportunities leveraging immunity for the next oncology practice. *CA Cancer J Clin* (2017) 67 (1):65–85. doi: 10.3322/caac.21358

11. Missiaen R, Mazzone M, Bergers GJ. The reciprocal function and regulation of tumor vessels and immune cells offers new therapeutic opportunities in cancer. *Semin Cancer Biol* (2018) 52:107–16. doi: 10.1016/j.semcancer.2018.06.002

12. Chen B, Wu J, Cheng S, Wang L, Rong W, Wu F, et al. Phase 2 study of adjuvant radiotherapy following narrow-margin hepatectomy in patients with HCC. *Hepatology* (2021) 74(5):2595–604. doi: 10.1002/hep.31993

13. Chen Z, Zhang X, Feng S, Feng J, Chai Z, Guo W, et al. Liver resection versus intensity-modulated radiation therapy for treatment of hepatocellular carcinoma with hepatic vein tumor thrombus: A propensity score matching analysis. *Hepatobiliary Surg Nutr* (2021) 10(5):646–60. doi: 10.21037/hbsn.2020.03.20

14. Levy A, Chargari C, Marabelle A, Perfettini J, Magné N, Deutsch EJ. Can immunostimulatory agents enhance the abscopal effect of radiotherapy? *Eur J Cancer* (2016) 62:36–45. doi: 10.1016/j.ejca.2016.03.067

15. Paris F, Fuks Z, Kang A, Capodieci P, Juan G, Ehleiter D, et al. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* (2001) 293(5528):293–7. doi: 10.1126/science.1060191

16. Hallahan D, Kuchibhotla J, Wyble CJ. Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. *Cancer Res* (1996) 56(22):5150–5. doi: 10.1002/(SICI)1097-0142(19961115)78:10<2247::AID-CNCR29>3.0.CO;2

17. Carvalho H, Villar RJC. Radiotherapy and immune response: The systemic effects of a local treatment. *Clinics* (2018) 73:e557s. doi: 10.6061/clinics/2018/e557s

18. Chen H, Cheng H, Dai Q, Cheng Y, Zhang Y, Li D, et al. A superstable homogeneous lipiodol-ICG formulation for locoregional hepatocellular carcinoma treatment. *J Control Release* (2020) 323:635–43. doi: 10.1016/j.jconrel.2020.04.021

19. Xiang Y, Wang K, Zheng Y, Feng S, Yu H, Li X, et al. Effects of stereotactic body radiation therapy plus PD-1 inhibitors for patients with transarterial chemoembolization refractory. *Front Oncol* (2022) 12:839605. doi: 10.3389/fonc.2022.839605

20. Zhong L, Wu D, Peng W, Sheng H, Xiao Y, Zhang X, et al. Safety of PD-1/PD-L1 inhibitors combined with palliative radiotherapy and anti-angiogenic therapy in advanced hepatocellular carcinoma. *Front Oncol* (2021) 11:686621. doi: 10.3389/fonc.2021.686621

21. Ricke J, Klümpen H, Amthauer H, Bargellini I, Bartenstein P, de Toni E, et al. Impact of combined selective internal radiation therapy and sorafenib on survival in advanced hepatocellular carcinoma. *J Hepatol* (2019) 71(6):1164–74. doi: 10.1016/j.jhep.2019.08.006

22. Yau T, Kang Y, Kim T, El-Khoueiry A, Santoro A, Sangro B, et al. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: The CheckMate 040 randomized clinical trial. *JAMA Oncol* (2020) 6(11):e204564. doi: 10.1001/jamaoncol.2020.4564

23. Liu B, Gao S, Zhu X, Guo J, Kou F, Liu S, et al. Real-world study of hepatic artery infusion chemotherapy combined with anti-PD-1 immunotherapy and tyrosine kinase inhibitors for advanced hepatocellular carcinoma. *Immunotherapy* (2021) 13(17):1395–405. doi: 10.2217/imt-2021-0192

24. Chen S, Wu Z, Shi F, Mai Q, Wang L, Wang F, et al. Lenvatinib plus TACE with or without pembrolizumab for the treatment of initially unresectable hepatocellular carcinoma harbouring PD-L1 expression: A retrospective study. *J Cancer Res Clin Oncol* (2022) 148(8):2115–25. doi: 10.1007/s00432-021-03767-4

25. Mehta N, Dodge J, Roberts J, Yao FJ. A novel waitlist dropout score for hepatocellular carcinoma - identifying a threshold that predicts worse post-transplant survival. *J Hepatol* (2021) 74(4):829–37. doi: 10.1016/j.jhep.2020.10.033

26. Vitale A, Burra P, Frigo A, Trevisani F, Farinati F, Spolverato G, et al. Survival benefit of liver resection for patients with hepatocellular carcinoma across different Barcelona clinic liver cancer stages: A multicentre study. *J Hepatol* (2015) 62(3):617–24. doi: 10.1016/j.jhep.2014.10.037

27. Xia F, Wu L, Lau W, Li G, Huan H, Qian C, et al. Positive lymph node metastasis has a marked impact on the long-term survival of patients with hepatocellular carcinoma with extrahepatic metastasis. *PloS One* (2014) 9(4): e95889. doi: 10.1371/journal.pone.0095889

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Interaction between gut microbiota and immune checkpoint inhibitor-related colitis

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Immune checkpoint inhibitors (ICIs) have become a promising therapeutic strategy for malignant tumors, improving patient prognosis, along with a spectrum of immune-related adverse events (irAEs), including gastrointestinal toxicity, ICI-related colitis (IRC), and diarrhea. The gut microbiota has been suggested as an important regulator in the pathogenesis of IRC, and microbiota modulations like probiotics and fecal microbiota transplantation have been explored to treat the disease. This review discusses the interaction between the gut microbiota and IRC, focusing on the potential pathogenic mechanisms and promising interventions.

KEYWORDS

immune checkpoint inhibitor, gut microbiota, colitis, diarrhea, microbiome

Introduction

Immune checkpoint inhibitors (ICIs) have received great attention as they have rapidly altered the treatment landscape for multiple tumors, including lung cancer, metastatic melanoma, and urinary epithelial carcinoma. ICIs block inhibitory molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and its ligand 1 (PD-L1) and enhance anti-tumor T-cell activity providing clinical benefits in many patients with advanced cancers (1–3). Yet, multiple organs like skin, lung, liver, and digestive tract are susceptible to the unrestrained immune response activation by the utility of ICIs, which developed to the immune-related adverse events (irAEs) ultimately, including ICI-related colitis (IRC) and diarrhea, which are major causes of ICI discontinuation (4–6).

Studies have suggested that the occurrence of diarrhea and colitis is associated with the ICI used. For example, Tandon et al. performed a meta-analysis to evaluate the risk of colitis and diarrhea in patients with advanced melanoma treated with ICIs (anti-PD-1 or anti-CTLA-4 therapy) and concluded that diarrhea and colitis are more frequent in patients treated with CTLA-4 inhibitors (7). Another study showed that patients treated with anti-CTLA-4 therapy have a higher rate of diarrhea (31.8% in anti-CTLA-4 alone versus 10.5% in anti-PD-1 alone) and colitis (7.7% in anti-CTLA-4 alone versus 0.8% in anti-PD-1 alone); also, diarrhea seems to be more common in patients treated with dual ICI therapy than in those with a single-ICI agent (8). One possible explanation for this preference is that the CTLA-4 receptor is often expressed on the surface of $CD4^+$ and $CD8^+$ cells, subsets of B cells and thymocytes, resulting in inhibition at the initial step in an immune response while the PD-1 and its ligand blockades aim at late T-cell proliferation, causing a more localized immune reaction (9, 10).

Yet, the mechanisms of IRC are still not fully understood and several key aspects have been proposed: (a) the cross-reactivity of the common antigens on tumor and healthy tissues; (b) activation of humoral immunity like elevated pre-existing autoantibodies level; (c) modulation of pro (anti)-inflammatory cytokines; (d) enhanced complement-mediated inflammation; (e) regulation of effector or suppressor immune cells (10, 11). Moreover, different management is proposed based on the IRC severity. Mild or moderate IRC is closely observed and applied with supportive treatment. Higher-grade toxicities cases may discontinue the ICI course and receive corticosteroids or immunosuppressive therapies such as tumor necrosis factor-α (TNF-α) inhibitors (e.g., infliximab) and anti-integrin agents (e.g., vedolizumab) (11). Recent studies have highlighted an indispensable role of the gut microbiota in the communication between ICI and patients. The anticancer immunotherapy relies on the immunization with some species like *Bacteroides fragilis* (12). *Bifidobacterium* and *Faecalibacterium* promote ICI efficacy with augmented dendritic cell function and T cell accumulation in the tumor microenvironment (13, 14). Fecal microbiota transplantation (FMT) has also demonstrated the ability of overcoming resistance to anti-PD-1 therapy in melanoma patients (15). Besides, emerging evidence emphasizes the critical involvement of gut microbiota in the pathogenesis of IRC, patients vulnerable to IRC development seem to have a distinct microbiota profile (Table 1) (16–22) and the microbiota modulation offers a novel alteration for the treatment. This review discusses the interaction between the gut microbiota and ICI-related colitis, focusing on the potential pathogenic mechanisms and promising interventions.

The composition of gut microbiota on ICI-related colitis

Accumulating studies indicate that the gut microbiota signature has a strong link with IRC. Chaput et al. (16) collected fecal samples from twenty-six metastatic melanoma patients before the ICI therapy and analyzed the gut microbiota 16S rRNA gene sequencing data. According to the characteristics of baseline microbiota composition, patients were divided into 3 clusters. There was a high proportion of *Faecalibacterium* and other *Firmicutes* in the microbiota composition of patients belonging to Cluster A. Cluster B was enriched in *Bacteroides*, and Cluster C, *Prevotella*. At the phyla level, patients in Cluster A were prone to develop colitis, with a preference of Firmicutes, while patients without colitis had more Bacteroidetes (like Cluster B). Specifically, *Bacteroides vulgatus*, and *Faecalibacterium prausnitzii A2-165* were detected as potential biomarkers for colitis absence during ICI therapy, whereas several OTUs in Firmicutes phylum, and *Gemmiger formicilis ATCC 27749* were detected to be with increased risk of colitis. Meanwhile, there is an overlap that gut microbiota composition associated with IRC also promotes ICI clinical response. For example, *Faecalibacterium* magnifies systemic immune response mediated by up-regulated antigen presentation and intensified effector T cell function. These overactive immune cells not only infiltrate in tumor microenvironment, strengthening ICI anti-tumor effect, but attack normal intestinal mucosal and induce IRC. In another study of advanced-stage melanoma patients undergoing ICI, stool samples were collected before, during, and after the treatment. Two natural gut microbiome clusters with distinct profiles were identified, and patients with a high proportion of *Bacteroides dorei* in gut microbiota had high risk of irAE, while the *Bacteroides vulgatus* was identified as a specific dominance strain in the low-risk cluster (18). Apart from the specific strain, it is inferred that the IRC is associated with decreased diversity of gut microbiome. The low richness of abundance in gut microbiota often refers to a fragile immune homeostasis, which are easily perturbed by ICIs intervention as observed in IRC patients. Mao et al. (22) displayed that ICI-treated hepatobiliary cancer patients with severe diarrhea tends to have lower phylogenetic diversity of gut microbiota. They also recognized several enriched taxa with significant differentiation between the severe and mild diarrhea groups. The enrichment of *Dialister* genus, which belongs to the Firmicutes phylum, was observed in the mild group. Notably, severe diarrhea patients had a higher abundance of *Prevotellamassilia timonensis*, which has been suggested as valuable biomarker. Overall, it could be speculated that a higher diversity of gut microbiome may be a protective factor against IRC.

Antibiotic use on ICI-related colitis

Patients with malignant tumor tend to experience infection due to their impaired immune system, causing higher exposure to antibiotics. In clinical practice, about 70% cancer patients receive antibiotics during the ICI treatment, how they affect IRC

TABLE 1 Gut microbiota studies for immune checkpoint inhibitor-related colitis and other irAEs.

Study	Country	Sample size	Study period	Drugs	Sample type	Incidence	Main findings
Chaput et al. (16)	France	MM (n=26)	2013.3-2014.12	Anti-CTLA-4 (n=26)	Fecal	Colitis (n=7)	Most of the baseline colitis-associated phylotypes were related to Firmicutes, whereas no colitis-related phylotypes were assigned to Bacteroidetes.
Dubin et al. (17)	the USA	MM (n=34)	Not available	Anti-CTLA-4 (n=34)	Fecal	Colitis (n=10)	Bacteroidetes phylum and three of its families (Bacteroidaceae, Rikenellaceae, Barnesiellaceae) had higher abundance in colitis-free patient.
Usyk et al. (18)	the USA	Advanced stage melanoma (n=27)	2016.9-2017.11	Anti-PD-1 (n=12); Combined (n=15)	Fecal	IrAEs: Not applicable	Patients with high abundance of *Bacteroides dorei* at baseline have high risk for severe irAEs, while patients characterized by high abundance of *Bacteroides vulgatus* have low risk.
Mohiuddin et al. (19)	the USA	Stage III and IV melanoma (n=568)	2018-2019	Anti-CTLA-4 (n=232); Anti-PD-1 (n=286); Combined (n=50)	Fecal	Antibiotic group: colitis (n=11); None-antibiotic group: colitis (n=20);	The antibiotic group had a greater incidence of colitis
Zhao et al. (20)	China	Lung cancer (n=100); Esophagus cancer (n=32); Gastrointestinal cancer (n=24); Others (n=12)	2018.8-2020.7	Nivolumab (n=52); Pembrolizumab (n=56); Camrelizumab (n=40); Toripalimab (n=20)	Fecal	IrAEs: Lung cancer (n=25); Esophagus cancer (n=8); Gastrointestinal cancer (n=6); Others (n=3)	Antibiotic exposure was associated with a higher risk of irAEs
Liu et al. (21)	China	NSCLC (n=102); Nasopharyngeal carcinoma (n=7); Melanoma (n=5); Esophagus cancer (n=5); Others (n=31)	2018.10-2021.3	Anti-PD-1 (n=150)	Fecal	Severe diarrhea (n=3); Mild diarrhea (n=10)	Patients with severe diarrhea showed a higher level of *Stenotrophomonas* and *Streptococcus* compared with patients without irAEs or with mild diarrhea
Mao et al. (22)	China	Unresectable HCC (n=30); Advanced BTC (n=35)	2018.11-2020.12	Anti-PD-1 (n=65)	Fecal	Severe diarrhea (n=8); Mild diarrhea or absence (n=57)	Patients with severe diarrhea tended to have decreased gut microbiome diversity and relative abundance; *Prevotellamassilia timonensis* was observed in more severe diarrhea patients

MM, metastatic melanoma; irAEs, immune related adverse events; NSCLC, non-small-cell lung carcinoma.
HCC, hepatocellular carcinoma; BTC, biliary tract cancer.

deserves exploration (23). Epidemiological studies emphasized that antibiotic therapy weakens ICI efficacy and shortens patient survival across malignancies (24). Antibiotics alter the composition of gut microbiota, leading a decreased bacterial-mediated secondary bile acids production and an increased inflammasome signaling, thus promotes a pro-inflammatory state, susceptible to IRC (25). As a result, the history of antibiotic use may be an indicator of IRC. Researchers established an ICI-related colitis mice model by combining dextran sulfate sodium (DSS) and anti-CTLA-4 to simulate the inflammation condition. Compared to the control group (with ICI isotype and DSS), mice with anti-CTLA-4 pretreatment showed higher mortality, more body weight loss, and worse histopathological scores, thus declaring that preprocess of ICI exaggerates the DSS-induced inflammation in mice. Moreover, pretreatment with vancomycin provoked an even more severe, largely fatal form, indicating that a Gram-positive component of the microbiota had a mitigating effect on colitis (26). Due to the limitation of mice models, they generally do not develop colitis after ICI treatment, unlike malignancy patients, in the absence of chemical damage or genetic defects. Therefore, the potential influence of additional DSS process requires to be further explored.

A clinical observational study including 832 patients with ICI treatment exhibited that antibiotic exposure is strongly correlated to grade 3 or 4 irAEs (20). Mohiuddin et al. (19) investigated 568 patients with stage III and IV melanoma receiving immunotherapy. Patients treated with antibiotics within 3 months prior to the first infusion of ICI had significantly worse overall survival and a greater incidence of colitis. The incidence and severity of colitis varies according to some factors. Anaerobic antibiotics were associated with expanded immunosuppressant use, hospitalization, intensive care unit admission due to IRC, and elevated severity grades. At the onset of colitis, the empirical antibiotic group had a higher recurrence rate and colitis severity than the group receiving antibiotics when there was positive evidence of infection. Antibiotic therapy changed the microbiome taxonomic diversity profoundly, inducing a loss of protective bacteria and an impaired immune homeostasis, thus with a worse prognosis. Therefore, it provides an implication for clinical practice that antibiotic use should be taken into consideration carefully in cancer patients.

Potential mechanisms of interaction between gut microbiota and ICI-related colitis

The species and diversity of gut microbiota influence the development of IRC; yet, the underlying mechanism is still unclear. Deciphering the biological mechanisms is critical for optimizing patient outcome. Multiple results highlighted the involvement of gut microbiota in IRC pathogenesis, not only through direct effect of bacteria, but also through indirect mechanisms like regulating metabolites, cytokines and immune cells. It provides a better understanding of the disease and some novel targets for intervention. This part depicts early evidences and hypothetical scenarios, then discusses the potential mechanisms of the interaction between gut microbiota and ICI-related colitis (Figure 1).

Direct effect of bacteria

Mounting evidences illustrated that the bacteria exert direct effect *via* extracellular enzymes, lipopolysaccharide (LPS) and

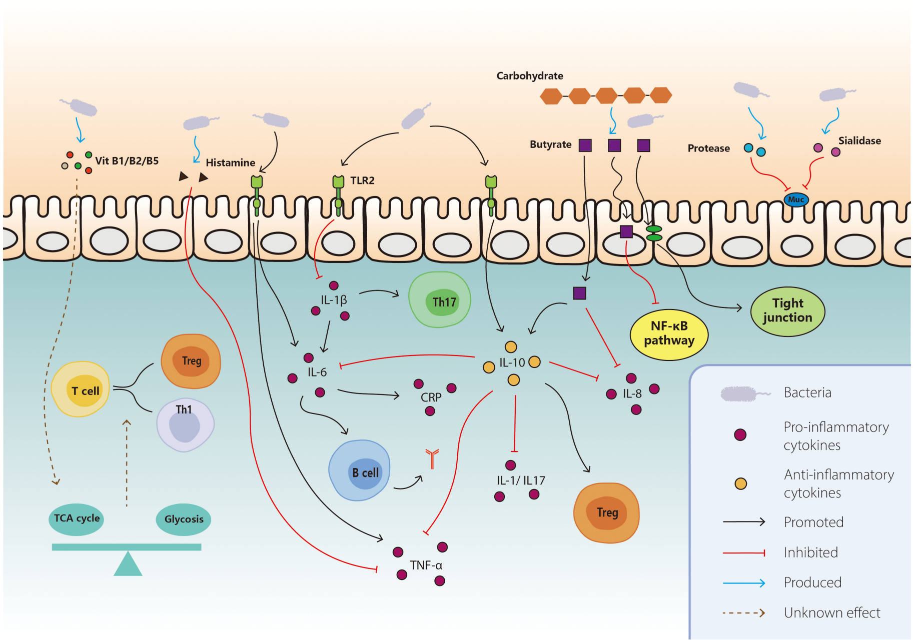

FIGURE 1
Mechanisms of interaction between gut microbiota and ICI-related colitis. On the basis of the known interaction between gut microbiota and IRC, the main mechanisms included direct effect, metabolites, cytokines, and immune cells. For protective bacteria, the pro-inflammatory pathways like IL-1b and TNF-a are inhibited, together with promoted anti-inflammatory pathways including IL-10, Th17 cells, and Treg cells. They also modulate the differentiation of T cells through vitamin B and tricarboxylic acid cycle. Butyrate produced by bacteria exerts anti-inflammatory effect via various aspect like consolidating tight junction, inducing IL-10 and suppressing NF-κB. As for harmful bacteria, they secrete some enzymes to destruct mucin and enhance the pro-inflammatory pathway like IL-6, TNF-a, CRP, and antigen production. TCA, tricarboxylic acid; NF-kB, nuclear factor kappa B; TLR, toll-like receptor; TNF-a, tumor necrosis factor-a; IL, interleukin; CRP, C-reactive protein.

others in their interaction with IRC. Higher levels of *Stenotrophomonas* have been found in severe diarrhea patients receiving ICI treatment (21). *Stenotrophomonas* is considered an environmental bacterium commonly found in the respiratory or digestive tract. It often causes pulmonary diseases like Stenotrophomonas maltophilia pneumonia and diarrhea or enteritis in some cases (27, 28). Malignancy patients with impaired immunity are predisposed to this strain and tend to experience severe diarrhea or IRC if infected (27). A range of extracellular enzymes by *Stenotrophomonas maltophilia*, including DNase, RNase, lipases, protease, and elastase, may be key factors in pathogenesis. Assisted with these enzymes, the strain breaks down the tight junction, decomposes mucin, invades tissue and causes IRC. Bacterial enzymes also play a critical role in the pathogenesis of *Prevotellmassilia timonensis*, a subspecies strain of *Prevotella*, which is associated with severe diarrhea in ICI-treated patients. It secretes sialidase, breaks sialic acid and degrades the mucin, increasing the intestinal barrier permeability (29). Dendritic cells (DCs) are also involved in its pathogenic mechanism (30). Endotoxin-like lipopolysaccharide (LPS) is another virulence factor promoting the inflammation. It actives immune cells through the toll-like-4 receptors, synthesizes and releases a variety of cytokines and inflammatory mediators, causing inflammation (31, 32). Compared to the control group, the LPS level was reduced in serum and feces of mice fed with *B. vulgatus*, which has a strong correlation with few irAEs, indicating a potential protective mechanism *via* LPS reduction (33). Microbial anti-inflammatory molecules (MAMs) have same favorable effects, which contain a series of proteins produced by *Faecalibacterium prausnitzii*. In animal models, MAMs exhibit anti-inflammatory effect by blocking the NF-κB pathway and inhibiting the pro-inflammatory Th1 and Th17 immune responses. It also consolidates the gut barrier by upregulating the tight connection gene Zo-1 (34, 35). Therefore, *Faecalibacterium prausnitzii* could prevent patients from IRC and act as a biomarker for colitis absence.

Metabolites

Short-chain fatty acid (SCFA)

The gut microbiota consumes carbohydrates and produces variable bioactive molecules, modulating the host immune system differently (36). SCFAs are one of the most extensively characterized classes of microbial metabolites (37, 38). Bacteria break complicated carbohydrates into simple fatty acids like acetate, propionate, and butyrate. These small molecules supply energy for intestinal epithelial cells and exert diverse effects on immune cell function and cytokine production (39). The anti-inflammation characteristic of butyrate is partly attributed to inhibiting the NF-κB activation and its downstream pathway, which in turn reduces the pro-inflammatory cytokines, such as IL-8, and increases anti-inflammatory factors like IL-10. The butyrate also induces tight connection protein expressions in the mucosa and consolidates the gut barrier (40). Indeed, a higher abundance of butyrate-producing *Faecalibacterium prausnitzii A2-165* was detected in colitis-absent patients with ICI therapy compared to those who experienced colitis (16). On the contrary, the reduction of SCFAs cannot supply the cell with enough energy, resulting in an impaired gut barrier and immune system. Some species of *Prevotella* genus aggravate local and systemic inflammation *via* reduction of SCFAs and IL-18 (41), which may explain their enrichment in feces of severe diarrhea patients receiving ICI treatment for malignancy.

Vitamin and polyamine

Dubin et al. (17) demonstrated that bacteria belonging to the Bacteroidaceae, Rikenellaceae, Barnesiellaceae family are enriched in patients resistant to IRC. Furthermore, according to the shotgun sequencing and metabolic pathway reconstruction, genetic pathways involved in vitamin B biosynthesis and polyamine transport are correlated with an absence of colitis.

Vitamins are necessary micronutrients generated by plants and bacteria. The gut microbiota can metabolize vitamins for humans through its relevant enzymes and transporters (42). Vitamin B1 (thiamine) is essential in energy metabolism, especially in the tricarboxylic acid (TCA) cycles (43). Accumulating evidence proved an energy supply balance between glycolysis and the TCA cycle for immune cells. Generally, quiescent or regulatory-type cells (e.g., naive T cells, Treg cells, and M2 macrophages) use the TCA cycle for energy generation, whereas activated or pro-inflammatory cells (e.g., Th1, Th2, Th17, and M1 macrophages) rely on glycolysis (44, 45). Therefore, thiamine regulates the immune cell balance and poses a potential effect on the IRC. Vitamin B2 (riboflavin) and its active forms (flavin adenine nucleotide (FAD) and flavin mononucleotide (FMN)) are cofactors in enzymatic reactions in the Krebs cycle and fatty acid oxidation (43). The oxidation process is involved in the activation, differentiation, and proliferation of immune cells *via* producing acetyl-CoA for TCA cycles and energy generation, while riboflavin deficiency inhibits acyl-CoA dehydrogenase activity in the process (46). It is speculated that riboflavin modulates immune function through fatty acid oxidation. Moreover, in the presence of NADPH oxidase 2, riboflavin induces reactive oxygen species (ROS) production, which is an essential effector and signaling molecule in inflammation and immunity (47). Pantothenate, also known as vitamin B5, is a precursor of coenzyme A (CoA). Similar to thiamine and riboflavin, pantothenate has a crucial effect on immunity *via* cell energy consumption as coenzyme A is an indispensable cofactor for the TCA cycle and fatty acid oxidation (43).

Polyamines are small cationic amines exported from bacterial cells *via* the spermidine and putrescine transport systems (pot A, B, C, and D). It resists inflammation partly by promoting colonic epithelial cell proliferation to maintain the epithelial barrier (48). Spermine, produced by amino acid decarboxylation, reduces colonic IL-18 levels and inhibits NLRP6 inflammasome assembly (49). It also suppressed the secretion of pro-inflammatory cytokines like TNF-α and lymphocyte function-associated antigen-1 (LFA-1), which is a regulator of immune cell adhesion and migration (50).

Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a group of 18 carbon conjugated dienoic acids. It is reported to benefit local immunomodulatory activity through up-regulating anti-inflammation factors, inhibiting pro-inflammation factors, and improving the tight junctions. Some studies displayed that human commensal bacteria like *Bifidobacterium* possess CLA-production ability and exhibit anti-inflammation ability (51). Wall et al. (52) found that some isomers of CLA are elevated in murine fed with *Bifidobacterium breve NCIMB 702258*, meanwhile, some pro-inflammatory cytokines like TNF-α and IFN-γ were decreased. Another subtype of *Bifidobacterium breve* ameliorated mice colitis through CLA accumulation, along with advanced tight conjunction, elevated mucin and decreased IL-1 and IL-6 (53).

Cytokines

Cytokines are a series of small molecules mainly produced by immune cells. They modulate cell growth, differentiation, development and apoptosis, regulate immunity and contribute greatly to multiple bio-active responses including inflammation. Microorganisms induce human cell to generate considerable cytokines, which mainly consists of two types, the pro-inflammatory and anti-inflammatory cytokines. For unfavorable bacteria, they promote the level of inflammation-promotion cytokines like IL-6, TNF-α and IL-1β, exaggerating the IRC. Meanwhile, some favorable bacteria support the anti-inflammatory production like IL-10, beneficial for IRC.

IL-6

IL-6 is one of the most essential and well-studied pro-inflammatory cytokines, enabling B cells to proliferate, differentiate, and secrete antibodies, and inducing a series of acute-phase reaction proteins such as C reactive protein, serum amyloid A, thrombopoietin, and complement C3. In mice models, pretreatment of ICI process enhanced the susceptibility of DSS-Induced colitis, accompanied by exacerbated hyperplasia and ulceration. It also raised inflammatory leukocyte infiltration in colonic sections, as well as the levels of inflammatory cytokines, IL-6, TNF-α, and IFN-γ in the circulation (54). Mounting evidences highlight the strong association among IL-6, bacteria and colitis. The relative abundance of *Streptococcus* in feces has a positive correlation with serum IL-6 level in mice models of colitis and with colonic mucosal TLR2 receptor expression in ulcerative colitis patients, respectively (55, 56). Moreover, another study manifested elevated levels of IL-6 and TNF-α in the serum of mice infected with *Streptococcus via* a TLR2 receptor-dependent pathway (57). Compared to control mice, *Bacteroides*-treated mice exhibited suppressed inflammation response and significantly lower plasma levels of pro-inflammatory cytokines, such as IL-6, IFN-γ, and TNF-α (33). Overall, it is believed that IL-6 meditates pathogenicity of bacteria on colitis and the reduction of IL-6 might contribute to the resistance to the IRC.

TNF-α

Apart from IL-6, another possible pathogenic mechanism of *Streptococcus* on IRC is TNF-α induction, meditated by primary bile acid and its receptors (58). TNF-α regulates multiple cellular responses such as vasodilation, edema formation, and leukocyte-epithelial cell adhesion. It also meditates blood coagulation and promotes oxidative stress, causing fever and inflammation indirectly (59). Conversely, the reduction of TNF-α contributes to recovery from colitis. In children with active distal ulcerative colitis, rectal infusion of *Lactobacillus reuteri* reduces TNF-α mucosal expression (60). The bacteria decompose dietary L-histidine to generate histamine, stimulate intracellular cAMP production through H2 receptors, inhibit TNF-α production in a PKA-MEK/ERK-MAPK-dependent pathway and relieve mucosal inflammation effectively (61).

IL-1β

As a key pro-inflammatory cytokine, IL-1β is engaged in various autoimmune inflammatory responses and cellular activities, including cell proliferation, differentiation, and apoptosis. It is confirmed that *Prevotella* aggravates the colitis *via* meditating the maturity of IL-1β (62). *Bacteroides intestinalis* was also proved to induce IRC *via* up-regulating IL-1β mucosal transcription (63). This cytokine activates the release of other pro-inflammatory cytokines like IL-6 and induces the differentiation of the Th17 cells. It also promotes monocytes differentiation to conventional DCs and M1-like macrophages and supports the activated B lymphocytes to proliferate and differentiate into plasma cells (64, 65). Meanwhile, the inhibition of IL-1β might contribute to the anti-inflammatory effect of *Bifidobacterium breve* through the interaction with TLR2 receptor and NF-κB pathway blocking (66).

IL-10

As for anti-inflammation cytokines, IL-10 suppresses the expression of major histocompatibility complex II (MHC II) on the surface of monocytes, restrains its antigen presentation, impairs the activity of T lymphocytes, and prohibits the activation, migration, and adhesion of inflammatory cells. Moreover, it strongly depresses the synthesis of IL-1, IL-6, IL-8, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) at the transcriptional level, leading an anti-inflammatory effect (67, 68). IL-10 also antagonizes the IL-17 and increases the proportion of Foxp3$^+$ Treg cells in CD4$^+$ T cells (69). The special cytokine contributes greatly to bacteria protection against colitis. After supplementation with *Bifidobacterium breve* for mice, the expression of IL-10 and IL-10Ra expanded in Treg cells in the lamina propria of the intestinal mucosa, which prevents effector T cell proliferation. However, the colitis-relieving effects of *B. breve* were reduced after IL-10 receptor knockout in mice, emphasizing the role of IL-10 in the anti-inflammatory effects of *B. breve* (70). The strain activates intestinal CD103$^+$ DCs through the TLR2/MyD88 pathway to generate IL-10 and induce IL-10-secreting type 1 regulatory T cells in the colon, which in turn induces IL-10 and TGF-β, weakening Th1 and Th2 cells function and ameliorating the colitis (71). Other studies pointed out that *F.prausnitzii A2-165* attenuates mice colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) or dinitrobenzene sulfonic acid (DNBS) and modulates the T cell response *via* inducing IL-10 in human and murine dendritic cells (72–74). Increased IL-10 levels were also observed in mice fed with *Lactobacillus reuteri*, accompanied by inflammation remission and IL-17 and IL-23 reduction (54). In the future, the level of serum IL-10 may predict patients' risk for IRC and reflect the efficacy of treatment.

Immune cells

Normally, immune checkpoint inhibitors raise the T cell activity against antigen presented in tumor. Sometimes, the activated immune cells target healthy tissues which have the same antigen causing inflammation like IRC. In general, the enrichment of pathogenic bacteria in IRC patient is usually accompanied with effector T cell accumulation. For those favorable strains for IRC, the immunosuppressive properties of Treg cell enable them to exert fundamental impact on anti-inflammation, partly contributing to their protection. Treg cells are necessary component of immune cells, responsible for maintaining self-tolerance and avoiding excessive immune response damage to the body. Treg cells moderate immunity partly by blocking the induction of IL-2 production in responder T cells and that both IL-10 and TGF-β are engaged in the process (75). Another mechanism of regulation is cytolysis of target cells mediated by Treg cells, which relies on granzyme A and B in human (76). Wang et al. (26) found that the supplementation of *bifidobacterium* mixture reduces the IRC inflammation and this effect seems to be dependent on Treg cells. Further research identified the effective specific strain, *Bifidobacterium breve*, and proved that the immune modulation of the strain on IRC has a close association with Treg cell energy metabolism (70). After gavage with *B. breve*, the circulation level of suberic acid in mice was significantly increased, reflecting the enhanced mitochondrial activity, along with elevated mitochondrial volume and stress level of Treg cells in the lamina propria. Consistent with this finding, multiple genes related to mitochondrial structural components and function were obviously upregulated (70). The relative increase in the proportion of Treg cells within the colonic mucosa was also presented in a refractory IRC patient who achieved recovery after receiving FMT therapy (77). Therefore, the relative abundance of Treg cells could be a predictor for colitis absence and a therapy target in the future.

A promising therapy for ICI-related colitis

The gut microbiota occupies a substantial place in the pathogenesis of IRC, which presents an applicable therapy through modulating its composition. Recently, probiotic supplementation has been recommended for IRC. *B.breve* exhibited anti-inflammatory effect in mice models, it ameliorates their immunopathological condition and rescues them from weight loss without apparent influence on anti-tumor immunity. *Lactobacillus reuteri* and *Lactobacillus rhamnosus GG* both abrogated IRC by inhibiting group 3 innate lymphoid cells (ILC3s) or regulating T cells (54, 78). FMT was introduced into the management as it manipulated the gut microbiota of recipients from donor microorganisms and small molecules like SCFAs. Recently, the therapy has been utilized on two refractory IRC patients (77). Two patients both received systemic corticosteroids, infliximab, and vedolizumab but had no settlement of symptoms. After the transfusion from an unrelated donor, they achieved marked improvements both in clinical symptoms and on endoscopic evaluation, with reduced inflammation and resolved ulcerations. Further analyses of patient's microbial composition revealed a tendency towards that of donor. The proportion of immune cells infiltrated in the colonic mucosa changed after the transplantation, such as the reduction in CD8$^+$T cells, providing a plausible explanation of FMT treatment on ICI-related colitis. Additional cases encouraged the idea that FMT appears to be a promising option for ICI-related colitis patients resistant to corticosteroids and monoclonal antibody therapies (79, 80). Besides, a clinical trial is undergoing about FMT in treating ICI induced-diarrhea or colitis in genitourinary cancer patients (NCT04038619). However, further investigations are required to verify the efficacy and safety of FMT on ICI-related colitis, like the donor selection and transplant frequency.

Conclusion

Alterations and dysbiosis of gut microbiota have strong association with immune-related adverse events caused by ICIs, particularly the ICI-related colitis. Several strains have been proposed as valuable biomarkers of IRC. Studies have also suggested that microbiome dysbiosis caused by antibiotics may be an indicator of IRC. Moreover, multiple factors have been identified as involved in this pathogenesis, including metabolites, cytokines, and immune cells. Until now, there is no consensus about the exact role of one strain on IRC and different results are presented based on small sample studies. Therefore, studies with large sample and detailed mechanism are required. Regarding potential treatments, microbiota modulations such as probiotics and fecal microbiota transplantation have been explored as a promising therapy for ICI-related colitis.

Author contributions

FP contributed to the conception and design of the review. The first draft of the manuscript was written by GZ. GZ created all the Figures and tables. NZ, KM, and FP revised the manuscript. All authors contributed to the article and approved the submitted version.

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. Tang L, Wang J, Lin N, Zhou Y, He W, Liu J, et al. Immune checkpoint inhibitor-associated colitis: From mechanism to management. *Front Immunol* (2021) 12:800879. doi: 10.3389/fimmu.2021.800879

2. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Sci (New York NY)* (2018) 359(6382):1350–5. doi: 10.1126/science.aar4060

3. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *New Engl J Med* (2015) 373(1):23–34. doi: 10.1056/NEJMoa 1504030

4. Abu-Sbeih H, Ali FS, Luo W, Qiao W, Raju GS, Wang Y. Importance of endoscopic and histological evaluation in the management of immune checkpoint inhibitor-induced colitis. *J immunother Cancer* (2018) 6(1):95. doi: 10.1186/ s40425-018-0411-1

5. Wang Y, Abu-Sbeih H, Mao E, Ali N, Qiao W, Trinh VA, et al. Endoscopic and histologic features of immune checkpoint inhibitor-related colitis. *Inflamm bowel Dis* (2018) 24(8):1695–705. doi: 10.1093/ibd/izy104

6. Marin-Acevedo JA, Harris DM, Burton MC. Immunotherapy-induced colitis: An emerging problem for the hospitalist. *J Hosp Med* (2018) 13(6):413–8. doi: 10.12788/jhm.2925

7. Tandon P, Bourassa-Blanchette S, Bishay K, Parlow S, Laurie SA, McCurdy JD. The risk of diarrhea and colitis in patients with advanced melanoma undergoing immune checkpoint inhibitor therapy: A systematic review and meta-analysis. *J immunother (Hagerstown Md: 1997)* (2018) 41(3):101–8. doi: 10.1097/cji.0000000000000213

8. El Osta B, Hu F, Sadek R, Chintalapally R, Tang SC. Not all immune-checkpoint inhibitors are created equal: Meta-analysis and systematic review of immune-related adverse events in cancer trials. *Crit Rev oncol/hematol* (2017) 119:1–12. doi: 10.1016/j.critrevonc.2017.09.002

9. Khoja L, Day D, Wei-Wu Chen T, Siu LL, Hansen AR. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: A systematic review. *Ann oncol: Off J Eur Soc Med Oncol* (2017) 28 (10):2377–85. doi: 10.1093/annonc/mdx286

10. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *New Engl J Med* (2018) 378 (2):158–68. doi: 10.1056/NEJMra1703481

11. Chang AE, Golob JL, Schmidt TM, Peltier DC, Lao CD, Tewari M. Targeting the gut microbiome to mitigate immunotherapy-induced colitis in cancer. *Trends Cancer* (2021) 7(7):583–93. doi: 10.1016/j.trecan.2021.02.005

12. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by ctla-4 blockade relies on the gut microbiota. *Sci (New York NY)* (2015) 350(6264):1079–84. doi: 10.1126/science.aad1329

13. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal bifidobacterium promotes antitumor immunity and facilitates anti-Pd-L1 efficacy. *Sci (New York NY)* (2015) 350(6264):1084–9. doi: 10.1126/ science.aac4255

14. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-Pd-1 immunotherapy in melanoma patients. *Sci (New York NY)* (2018) 359(6371):97–103. doi: 10.1126/ science.aan4236

15. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti-Pd-1 therapy in melanoma patients. *Sci (New York NY)* (2021) 371(6529):595–602. doi: 10.1126/ science.abf3363

16. Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann oncol: Off J Eur Soc Med Oncol* (2019) 30(12):2012. doi: 10.1093/annonc/mdz224

17. Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-Blockade-Induced colitis. *Nat Commun* (2016) 7:10391. doi: 10.1038/ncomms10391

18. Usyk M, Pandey A, Hayes RB, Moran U, Pavlick A, Osman I, et al. Bacteroides vulgatus and bacteroides dorei predict immune-related adverse events in immune checkpoint blockade treatment of metastatic melanoma. *Genome Med* (2021) 13(1):160. doi: 10.1186/s13073-021-00974-z

19. Mohiuddin JJ, Chu B, Facciabene A, Poirier K, Wang X, Doucette A, et al. Association of antibiotic exposure with survival and toxicity in patients with melanoma receiving immunotherapy. *J Natl Cancer Instit* (2021) 113(2):162–70. doi: 10.1093/jnci/djaa057

20. Zhao L, Li Y, Jiang N, Song X, Xu J, Zhu X, et al. Association of blood biochemical indexes and antibiotic exposure with severe immune-related adverse events in patients with advanced cancers receiving pd-1 inhibitors. *J immunother (Hagerstown Md: 1997)* (2022) 45(4):210–6. doi: 10.1097/cji.0000000000000415

21. Liu W, Ma F, Sun B, Liu Y, Tang H, Luo J, et al. Intestinal microbiome associated with immune-related adverse events for patients treated with anti-Pd-1 inhibitors, a real-world study. *Front Immunol* (2021) 12:756872. doi: 10.3389/fimmu.2021.756872

22. Mao J, Wang D, Long J, Yang X, Lin J, Song Y, et al. Gut microbiome is associated with the clinical response to anti-Pd-1 based immunotherapy in hepatobiliary cancers. *J immunother Cancer* (2021) 9(12):e003334. doi: 10.1136/jitc-2021-003334

23. Abu-Sbeih H, Herrera LN, Tang T, Altan M, Chaftari AP, Okhuysen PC, et al. Impact of antibiotic therapy on the development and response to treatment of immune checkpoint inhibitor-mediated diarrhea and colitis. *J immunother Cancer* (2019) 7(1):242. doi: 10.1186/s40425-019-0714-x

24. Pinato DJ, Gramenitskaya D, Altmann DM, Boyton RJ, Mullish BH, Marchesi JR, et al. Antibiotic therapy and outcome from immune-checkpoint inhibitors. *J immunother Cancer* (2019) 7(1):287. doi: 10.1186/s40425-019-0775-x

25. Hagan T, Cortese M, Rouphael N, Boudreau C, Linde C, Maddur MS, et al. Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. *Cell* (2019) 178(6):1313–28.e13. doi: 10.1016/j.cell.2019.08.010

26. Wang F, Yin Q, Chen L, Davis MM. Bifidobacterium can mitigate intestinal immunopathology in the context of ctla-4 blockade. *Proc Natl Acad Sci United States America* (2018) 115(1):157–61. doi: 10.1073/pnas.1712901115

27. Kaito S, Sekiya N, Najima Y, Sano N, Horiguchi S, Kakihana K, et al. Fatal neutropenic enterocolitis caused by stenotrophomonas maltophilia: A rare and underrecognized entity. *Internal Med (Tokyo Japan)* (2018) 57(24):3667–71. doi: 10.2169/internalmedicine.1227-18

28. Hellmig S, Ott S, Musfeldt M, Kosmahl M, Rosenstiel P, Stüber E, et al. Life-threatening chronic enteritis due to colonization of the small bowel with stenotrophomonas maltophilia. *Gastroenterology* (2005) 129(2):706–12. doi: 10.1053/j.gastro.2005.01.011

29. Ilhan ZE, Łaniewski P, Tonachio A, Herbst-Kralovetz MM. Members of prevotella genus distinctively modulate innate immune and barrier functions in a human three-dimensional endometrial epithelial cell model. *J Infect Dis* (2020) 222 (12):2082–92. doi: 10.1093/infdis/jiaa324

30. van Teijlingen NH, Helgers LC, Zijlstra-Willems EM, van Hamme JL, Ribeiro CMS, Strijbis K, et al. Vaginal dysbiosis associated-bacteria megasphaera elsdenii and prevotella timonensis induce immune activation *Via* dendritic cells. *J Reprod Immunol* (2020) 138:103085. doi: 10.1016/j.jri.2020.103085

31. Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, et al. Variation in microbiome lps immunogenicity contributes to autoimmunity in humans. *Cell* (2016) 165(4):842–53. doi: 10.1016/j.cell.2016.04.007

32. d'Hennezel E, Abubucker S, Murphy LO, Cullen TW. Total lipopolysaccharide from the human gut microbiome silences toll-like receptor signaling. *mSystems* (2017) 2(6):e00046–17. doi: 10.1128/mSystems.00046-17

33. Yoshida N, Emoto T, Yamashita T, Watanabe H, Hayashi T, Tabata T, et al. Bacteroides vulgatus and bacteroides dorei reduce gut microbial lipopolysaccharide production and inhibit atherosclerosis. *Circulation* (2018) 138(22):2486–98. doi: 10.1161/circulationaha.118.033714

34. Breyner NM, Michon C, de Sousa CS, Vilas Boas PB, Chain F, Azevedo VA, et al. Microbial anti-inflammatory molecule (Mam) from faecalibacterium prausnitzii shows a protective effect on dnbs and dss-induced colitis model in mice through inhibition of nf-κb pathway. *Front Microbiol* (2017) 8:114. doi: 10.3389/fmicb.2017.00114

35. Xu J, Liang R, Zhang W, Tian K, Li J, Chen X, et al. Faecalibacterium prausnitzii-derived microbial anti-inflammatory molecule regulates intestinal integrity in diabetes mellitus mice *Via* modulating tight junction protein expression. *J Diabetes* (2020) 12(3):224–36. doi: 10.1111/1753-0407.12986

36. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol* (2016) 16(6):341–52. doi: 10.1038/nri.2016.42

37. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* (2016) 7(3):189–200. doi: 10.1080/19490976.2015.1134082

38. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* (2013) 504(7480):451–5. doi: 10.1038/nature12726

39. Fessler J, Matson V, Gajewski TF. Exploring the emerging role of the microbiome in cancer immunotherapy. *J immunother Cancer* (2019) 7(1):108. doi: 10.1186/s40425-019-0574-4

40. Liu H, Wang J, He T, Becker S, Zhang G, Li D, et al. Butyrate: A double-edged sword for health? *Adv Nutr (Bethesda Md)* (2018) 9(1):21–9. doi: 10.1093/advances/nmx009

41. Iljazovic A, Roy U, Gálvez EJC, Lesker TR, Zhao B, Gronow A, et al. Perturbation of the gut microbiome by prevotella spp. enhances host susceptibility to mucosal inflammation. *Mucosal Immunol* (2021) 14(1):113–24. doi: 10.1038/s41385-020-0296-4

42. Putnam EE, Goodman AL. B vitamin acquisition by gut commensal bacteria. *PloS Pathog* (2020) 16(1):e1008208. doi: 10.1371/journal.ppat.1008208

43. Peterson CT, Rodionov DA, Osterman AL, Peterson SN. B vitamins and their role in immune regulation and cancer. *Nutrients* (2020) 12(11):3380. doi: 10.3390/nu12113380

44. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: Insights into metabolism and lymphocyte function. *Sci (New York NY)* (2013) 342 (6155):1242454. doi: 10.1126/science.1242454

45. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic instruction of immunity. *Cell* (2017) 169(4):570–86. doi: 10.1016/j.cell.2017.04.004

46. Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. *Semin Immunol* (2016) 28(5):514–24. doi: 10.1016/j.smim.2016.10.009

47. Schramm M, Wiegmann K, Schramm S, Gluschko A, Herb M, Utermöhlen O, et al. Riboflavin (Vitamin B2) deficiency impairs nadph oxidase 2 (Nox2) priming and defense against listeria monocytogenes. *Eur J Immunol* (2014) 44 (3):728–41. doi: 10.1002/eji.201343940

48. Rao JN, Xiao L, Wang JY. Polyamines in gut epithelial renewal and barrier function. *Physiol (Bethesda Md)* (2020) 35(5):328–37. doi: 10.1152/physiol.00011.2020

49. Levy M, Thaiss CA, Zeevi D, Dohnalová L, Zilberman-Schapira G, Mahdi JA, et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating Nlrp6 inflammasome signaling. *Cell* (2015) 163(6):1428–43. doi: 10.1016/j.cell.2015.10.048

50. Sánchez-Jiménez F, Medina M, Villalobos-Rueda L, Urdiales JL. Polyamines in mammalian pathophysiology. *Cell Mol Life sci: CMLS* (2019) 76(20):3987–4008. doi: 10.1007/s00018-019-03196-0

51. Mei Y, Chen H, Yang B, Zhao J, Zhang H, Chen W. Research progress on conjugated linoleic acid bio-conversion in bifidobacterium. *Int J Food Microbiol* (2022) 369:109593. doi: 10.1016/j.ijfoodmicro.2022.109593

52. Wall R, Ross RP, Shanahan F, O'Mahony L, O'Mahony C, Coakley M, et al. Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. *Am J Clin Nutr* (2009) 89(5):1393–401. doi: 10.3945/ajcn.2008.27023

53. Chen Y, Yang B, Ross RP, Jin Y, Stanton C, Zhao J, et al. Orally administered CLA ameliorates DSS-induced colitis in mice *via* intestinal barrier improvement, oxidative stress reduction, and inflammatory cytokine and gut microbiota modulation. *J Agric Food Chem* (2019) 67(48):13282–98. doi: 10.1021/acs.jafc.9b05744

54. Wang T, Zheng N, Luo Q, Jiang L, He B, Yuan X, et al. Probiotics lactobacillus reuteri abrogates immune checkpoint blockade-associated colitis by inhibiting group 3 innate lymphoid cells. *Front Immunol* (2019) 10:1235. doi: 10.3389/fimmu.2019.01235

55. Xu N, Bai X, Cao X, Yue W, Jiang W, Yu Z. Changes in intestinal microbiota and correlation with tlrs in ulcerative colitis in the coastal area of northern China. *Microb pathogen* (2021) 150:104707. doi: 10.1016/j.micpath.2020.104707

56. Liu JL, Gao YY, Zhou J, Tang XY, Wang P, Shen LW, et al. Changes in serum inflammatory cytokine levels and intestinal flora in a self-healing dextran sodium sulfate-induced ulcerative colitis murine model. *Life Sci* (2020) 263:118587. doi: 10.1016/j.lfs.2020.118587

57. Tomlinson G, Chimalapati S, Pollard T, Lapp T, Cohen J, Camberlein E, et al. Tlr-mediated inflammatory responses to streptococcus pneumoniae are highly dependent on surface expression of bacterial lipoproteins. *J Immunol (Baltimore Md: 1950)* (2014) 193(7):3736–45. doi: 10.4049/jimmunol.1401413

58. Yang ZH, Liu F, Zhu XR, Suo FY, Jia ZJ, Yao SK. Altered profiles of fecal bile acids correlate with gut microbiota and inflammatory responses in patients with ulcerative colitis. *World J Gastroenterol* (2021) 27(24):3609–29. doi: 10.3748/wjg.v27.i24.3609

59. Zelová H, Hošek J. Tnf-α signalling and inflammation: Interactions between old acquaintances. *Inflammation res: Off J Eur Histam Res Soc [et al]* (2013) 62 (7):641–51. doi: 10.1007/s00011-013-0633-0

60. Oliva S, Di Nardo G, Ferrari F, Mallardo S, Rossi P, Patrizi G, et al. Randomised clinical trial: The effectiveness of lactobacillus reuteri atcc 55730 rectal enema in children with active distal ulcerative colitis. *Aliment Pharmacol Ther* (2012) 35(3):327–34. doi: 10.1111/j.1365-2036.2011.04939.x

61. Thomas CM, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, et al. Histamine derived from probiotic lactobacillus reuteri suppresses tnf *Via*

modulation of pka and erk signaling. *PloS One* (2012) 7(2):e31951. doi: 10.1371/journal.pone.0031951

62. Lukens JR, Gurung P, Vogel P, Johnson GR, Carter RA, McGoldrick DJ, et al. Dietary modulation of the microbiome affects autoinflammatory disease. *Nature* (2014) 516(7530):246–9. doi: 10.1038/nature13788

63. Andrews MC, Duong CPM, Gopalakrishnan V, Iebba V, Chen WS, Derosa L, et al. Gut microbiota signatures are associated with toxicity to combined ctla-4 and pd-1 blockade. *Nat Med* (2021) 27(8):1432–41. doi: 10.1038/s41591-021-01406-6

64. Xu D, Mu R, Wei X. The roles of il-1 family cytokines in the pathogenesis of systemic sclerosis. *Front Immunol* (2019) 10:2025. doi: 10.3389/fimmu.2019.02025

65. Schenk M, Fabri M, Krutzik SR, Lee DJ, Vu DM, Sieling PA, et al. Interleukin-1β triggers the differentiation of macrophages with enhanced capacity to present mycobacterial antigen to T cells. *Immunology* (2014) 141(2):174–80. doi: 10.1111/imm.12167

66. Tomosada Y, Villena J, Murata K, Chiba E, Shimazu T, Aso H, et al. Immunoregulatory effect of bifidobacteria strains in porcine intestinal epithelial cells through modulation of ubiquitin-editing enzyme A20 expression. *PloS One* (2013) 8(3):e59259. doi: 10.1371/journal.pone.0059259

67. Ouyang W, O'Garra A. Il-10 family cytokines il-10 and il-22: From basic science to clinical translation. *Immunity* (2019) 50(4):871–91. doi: 10.1016/j.immuni.2019.03.020

68. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *New Engl J Med* (2009) 361(21):2033–45. doi: 10.1056/NEJMoa0907206

69. Heo YJ, Joo YB, Oh HJ, Park MK, Heo YM, Cho ML, et al. Il-10 suppresses Th17 cells and promotes regulatory T cells in the Cd4+ T cell population of rheumatoid arthritis patients. *Immunol Lett* (2010) 127(2):150–6. doi: 10.1016/j.imlet.2009.10.006

70. Sun S, Luo L, Liang W, Yin Q, Guo J, Rush AM, et al. Bifidobacterium alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. *Proc Natl Acad Sci United States America* (2020) 117(44):27509–15. doi: 10.1073/pnas.1921223117

71. Jeon SG, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, et al. Probiotic bifidobacterium breve induces il-10-Producing Tr1 cells in the colon. *PloS Pathog* (2012) 8(5):e1002714. doi: 10.1371/journal.ppat.1002714

72. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of crohn disease patients. *Proc Natl Acad Sci United States America* (2008) 105(43):16731–6. doi: 10.1073/pnas.0804812105

73. Martín R, Chain F, Miquel S, Lu J, Gratadoux JJ, Sokol H, et al. The commensal bacterium faecalibacterium prausnitzii is protective in dnbs-induced chronic moderate and severe colitis models. *Inflamm bowel Dis* (2014) 20(3):417–30. doi: 10.1097/01.Mib.0000440815.76627.64

74. Rossi O, van Berkel LA, Chain F, Tanweer Khan M, Taverne N, Sokol H, et al. Faecalibacterium prausnitzii A2-165 has a high capacity to induce il-10 in human and murine dendritic cells and modulates T cell responses. *Sci Rep* (2016) 6:18507. doi: 10.1038/srep18507

75. Scheinecker C, Göschl L, Bonelli M. Treg cells in health and autoimmune diseases: New insights from single cell analysis. *J Autoimmun* (2020) 110:102376. doi: 10.1016/j.jaut.2019.102376

76. Freen-van Heeren JJ. Post-transcriptional control of T-cell cytokine production: Implications for cancer therapy. *Immunology* (2021) 164(1):57–72. doi: 10.1111/imm.13339

77. Wang Y, Wiesnoski DH, Helmink BA, Gopalakrishnan V, Choi K, DuPont HL, et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med* (2018) 24(12):1804–8. doi: 10.1038/s41591-018-0238-9

78. Tan B, Tang H, Xu Y, Chen MJ, Wang MZ, Qian JM. [Protective effect and mechanism of lactobacillus rhamnosus on immune checkpoint inhibitors related colitis in mice]. *Zhonghua yi xue za zhi* (2020) 100(42):3332–7. doi: 10.3760/cma.j.cn112137-20200520-01598

79. Fasanello MK, Robillard KT, Boland PM, Bain AJ, Kanehira K. Use of fecal microbial transplantation for immune checkpoint inhibitor colitis. *ACG Case Rep J* (2020) 7(4):e00360. doi: 10.14309/crj.0000000000000360

80. Dai C, Liu WX. Refractory immune checkpoint inhibitor-induced colitis improved by fecal microbiota transplantation: A case report. *Inflamm bowel Dis* (2022) 28(3):e43–e4. doi: 10.1093/ibd/izab265

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Recent developments in PD-1/ PD-L1 blockade research for gastroesophageal malignancies

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Gastroesophageal cancers (GECs) comprise malignancies in the stomach, esophagus, and gastroesophageal junction. Despite ongoing improvements in chemoradiotherapy, the clinical outcomes of GEC have not significantly improved over the years, and treatment remains challenging. Immune checkpoint inhibitors (ICIs) have been the subject of clinical trials worldwide for several years. Encouraging results have been reported in different countries, but further research is required to apply ICIs in the clinical care of patients with GEC. This review summarizes completed and ongoing clinical trials with programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway blockers in GEC and current biomarkers used for predicting PD-1/PD-L1 blockade efficacy. This review captures the main findings of PD-1/PD-L1 antibodies combined with chemotherapy as an effective first-line treatment and a monotherapy in second-line or more treatment and in maintenance therapy. This review aims to provide insight that will help guide future research and clinical trials, thereby improving the outcomes of patients with GEC.

KEYWORDS
PD-1, PD-L1, immune checkpoint inhibitors, gastric cancer, esophageal cancer, biomarkers

Introduction

Among gastroesophageal cancers (GECs), gastric cancer (GC) ranks fifth in incidence and fourth in mortality worldwide, and the median survival for advanced GC is less than 12 months (1, 2). Esophageal cancer (EC), another GEC, ranks seventh in incidence and sixth in overall mortality worldwide (1). In 2018, an estimated 570,000 individuals were diagnosed with EC worldwide, representing 3.2% of all cancer diagnoses and 5.3% of all cancer-related deaths (3). Over the past 30 years, the clinical benefits of conventional and emerging therapies have reduced GC mortality but have not improved EC survival (4, 5).

In certain western nations, adenocarcinoma has overtaken squamous cell carcinoma as the most prevalent type of EC, and its incidence continues to increase in other nations (6).

During the initiation of cellular immunity, antigens presented by the major histocompatibility complex on the surface of antigen-presenting cells (APCs) can selectively bind to cell receptors of the T-lymphocyte membrane, triggering further T-cell activation, proliferation, and differentiation. Activated T cells serve a vital function in the immune system (7). Under normal physiological conditions, programmed death 1 (PD-1), a negative costimulatory immune molecule also known as an immune checkpoint, is found on the surface of T, B, and myeloid cells. PD-1 specifically connects to programmed death-ligand 1 (PD-L1) on the surface of APCs to trigger immunosuppressive signal transduction, resulting in a decrease in T-cell activity. As cancer develops, tumor cells attach to vascular endothelial or perivascular cells, fibroblasts, and lymphocytes in the surrounding tissue, constituting the tumor microenvironment (TME) in combination with the extracellular matrix (8, 9). The TME can disrupt the dynamic balance of the organism by blocking cell apoptosis and promoting angiogenesis and cell proliferation, leading to continued tumor cell development, immune escape, and distant metastasis. Tumor cells highly express PD-L1 to strengthen the PD-1/PD-L1 pathway, thereby exhausting T cells and permitting tumor cells to evade immune surveillance. Based on this principle, PD-1/PD-L1 antibodies were established to constrain the PD-1/PD-L1 signaling pathway by binding to receptors on the surface of T lymphocytes or tumor cells in the late stages of peripheral tissue regulation of T-lymphocyte function, thereby disrupting the immune response, preventing tumor cell immune escape, and ensuring a normal immune response (10). The combined positive score (CPS), the most accepted PD-L1 scoring method, refers to the count of PD-L1-positive cells (including tumor cells, lymphocytes, and macrophages) divided by the total count of live tumor cells, multiplied by 100. The National Comprehensive Care Network (NCCN) recommends PD-L1 testing (i.e., CPS) for metastatic/advanced EC and GC.

Immunotherapy for GEC includes targeted blockade against immune checkpoints such as PD-1/PD-L1, cytotoxic T lymphocyte antigen-4 (CTLA-4), T cell immunoglobulin-3 (Tim-3), lymphocyte activation gene-3 (Lag-3) and chimeric antigen receptor T-cell (CAR-T) cell therapy; and therapeutic cancer vaccines. Among immune checkpoint inhibitors (ICIs), PD-1/PD-L1 antibodies have shown well applicability in EC and GC, thus dramatically changing the treatment outlook for these patients. An increasing number of PD-1/PD-L1 blockers have been authorized for use in EC and GC treatment. Exploring the administration conditions of known PD-1/PD-L1 inhibitors and developing new antibodies are key directions of current research, as well as evaluating and predicting PD-1/PD-L1 blockade efficacy. Although considerable research through clinical trials has been conducted in EC and GC, much less is known concerning the proper indication of the medicine and the patient selection criteria in these trials, which are often among the potential limitations of the study design. The assessment of the efficacy of PD-1/PD-L1 antibodies frequently employs biomarkers that could be used to select GC and EC patients; however, much work is yet to be discovered in this area. In this review, we present an update on and evaluate the results of current clinical trials with PD-1/PD-L1 antibodies in EC and GC and briefly describe the progress in developing common predictive biomarkers. By comparing previous clinical trials, we also highlight study design limitations that warrant consideration prior to establishing future clinical trials, with the hope of assisting patients in reaching a greater survival outcome.

Molecular and immunological basis of esophageal cancer and gastric cancer

EC does not have clear molecular typing, but one study classified EC into low- and high-risk subtypes, which might be used as independent prognostic factors (11). The Cancer Genome Atlas (TCGA) classifies four molecular subtypes of GC: Epstein–Barr virus (EBV)-positive, high microsatellite instability (MSI-H), genomically stable (GS), and chromosomal instability (CIN) (12). PD-L1 and PD-L2 expression levels are amplified in EBV-positive GC. MSI in cancer genomes is caused by DNA mismatch repair (MMR) system deficiencies. High MSI in tumors leads to the accumulation of mutational load, which affects the tumor response to anti-PD-1 antibodies (13). The United States Food and Drug Administration (FDA) has authorized pembrolizumab for the treatment of previously treated MSI-H/mismatch repair-deficient (dMMR) solid tumors, including EC and GC (14).

EC and GC are highly immunogenic, and multiple tumor neoantigens have been identified (15, 16). Owing to characteristics such as MSI and tumor mutational burden (TMB), tumor cells are highly susceptible to multiple genetic mutations, resulting in the production of specific neoantigens (17). These neoantigens can be taken up by APCs, which deliver the neoantigen to CD8+ T lymphocytes, initiating cytotoxic T lymphocytes (CTLs) and generating a key mechanism of antitumor immunity by killing tumor cells. In the TME, inflammatory factors, lymphocytes, monocytes, macrophages, and histiocytes comprise the tumor immune microenvironment. Tumor-infiltrating lymphocytes (TILs), consisting of T, B, and natural killer (NK) cells, infiltrate heavily in esophageal squamous cell carcinoma (ESCC) and gastric adenocarcinoma (18). TILs have been confirmed to be effective and independent prognostic factors during the antitumor immune response, and PD-1 expression on TILs correlates with adverse clinical outcomes in EC (19). Increased CD8+ TIL levels have been consistently detected in PD-L1-positive EC (20). Increased

CD8+ TIL levels were closely associated with better survival, lower lymph node metastases, and higher PD-L1 expression levels; the combined evaluation of CD8+ TIL and PD-L1 expression has been used to predict patient responses to PD-1/PD-L1 antibody treatment in a range of malignancies (21). Large numbers of CD20+ B cells are significantly correlated with both modest lymph node involvement and lower TNM stage as independent factors for GC prognosis (22). Moreover, tumor-associated macrophages (TAMs) can release cytokines that promote cancer cell motility and invasion (23–25). Overall, high TAM density is considered to be a negative prognostic factor in GC (26). TAMs often differentiate into M1-like TAMs with pro-inflammatory and tumor-suppressive functions and M2-like TAMs with anti-inflammatory and tumor-promoting functions (27). M1-like TAMs are an independent prognostic factor in GC, and CD68+CD163-macrophages, a group of representative M1-like TAMs, can be used as predictive biomarkers to guide PD-1/PD-L1 antibody treatment in GC (28). M2-like TAMs are involved in the inhibition of antitumor immune responses by increasing PD-L1 expression in tumors (29). Patients with EC who have high levels of M2-like TAMs had shorter overall survival (OS) (30, 31). Thus, certain TAM subgroups could have prognostic value in gastric adenocarcinoma and esophageal adenocarcinoma (EAC) (32). Finally, through a variety of cytokines, cancer-associated fibroblasts (CAFs), valuable stromal cells in the TME, contribute to the growth, progression, and metastasis of EC (33, 34). CAFs upregulate PD-L1 expression, thereby promoting cancer cell proliferation in GC (35). Furthermore, a study investigating CAFs in GC reported that extracellular matrix CAFs recruited M2-like macrophages and were associated with poor prognosis (36).

Clinical trials exploring PD-1/PD-L1 blockade in gastroesophageal cancers

PD-1/PD-L1 inhibitors have been approved for clinical use in several countries. For example, the US FDA granted pembrolizumab, nivolumab, and dostarlimab-Gxly approval for the treatment of EC and GC under certain conditions in 2022. As a first-line therapy for ESCC, camrelizumab + chemotherapy has been approved by China in 2021. However, the findings of the few clinical trials that have tested PD-1/PD-L1 antibodies as first-line monotherapies so far are not encouraging. Chemotherapy combined with PD-1/PD-L1 antibodies is currently being investigated in clinical studies as the first-line therapeutic option. This section presents the outcomes of clinical trials with PD-1/PD-L1 antibodies in EC and GC, emphasizing progress and comparing application conditions.

PD-1/PD-L1 blockade as first-line treatment in esophageal cancer

Radical resection is the conventional first-line treatment for EC, with or without perioperative chemotherapy (37). Advanced EC is treatable with first-line chemotherapy, with an overall poor prognosis (38). Therefore, research has concentrated on the development of inhibitors for immune checkpoints. This section focuses on clinical trials exploring PD-1/PD-L1 antibodies combined with chemotherapy and introduces the application of new PD-1 antibodies as first-line treatments for EC (Table 1).

KEYNOTE-590 was the first clinical trial to evaluate the combination of PD-1 inhibition with chemotherapy as a first-line treatment for EC with significant survival benefits. In March 2021, pembrolizumab plus fluoropyrimidine- and platinum-based chemotherapy was authorized by the FDA for the first-line treatment of patients with ESCC and EAC with CPS ≥10 (category 1, requires combination with cisplatin) and CPS <10 (category 2B) (39). The KEYNOTE-590 phase 3 trial enrolled 749 patients with advanced EC or Siewert type 1 gastroesophageal junction cancer (GEJC), among which 51% of the study population had CPS ≥10. The interventions included pembrolizumab or placebo plus chemotherapy (5-fluorouracil plus cisplatin). Compared to the placebo arm, the pembrolizumab arm showed a considerably enhanced survival advantage and sustained antitumor response in the total population, advanced ESCC subgroup, and CPS ≥10 subgroup. In all three populations, the pembrolizumab arm maintained an advantage in Kaplan–Meier (KM) curves for OS, and pembrolizumab + chemotherapy treatment was roughly twice as effective as placebo + chemotherapy treatment at 24-month OS. Progression-free survival (PFS), 12-month PFS, and 18-month PFS remained superior in all three populations treated with pembrolizumab plus chemotherapy. Additionally, the pembrolizumab + chemotherapy group had approximately 15% greater overall response rate (ORR), 2.3-month greater duration of response (DoR), and a nearly 3-fold increase in 24-month DoR than the placebo + chemotherapy group. No additional adverse events (AEs) were detected, indicating the safety of pembrolizumab combined with chemotherapy (40, 41).

The CheckMate-648 study evaluated PD-1 antibody combination therapy, delivering three types of drugs to patients with ESCC (n = 970): nivolumab + chemotherapy (intravenous fluorouracil), nivolumab + ipilimumab (CTLA-4 antibody), and chemotherapy alone. In the randomized population and tumor-cell PD-L1 expression of ≥1% subgroup, the nivolumab + chemotherapy group maintained higher complete response (CR) rates and longer-lasting responses at the 13-month follow-up than the other treatment groups. The median overall survival (mOS) for >12 months of the nivolumab + ipilimumab group was 2.0–6.3 months longer than that of the chemotherapy group. In patients

TABLE 1 Clinical trials of PD-1/PD-L1 blockade as first-line treatment.

Trial	Phase	Enroll	Arm	N	mOS (m)	12mOS (%)	mPFS (m)	12mFPS (%)	ORR (%)	TRAEs (%)
First-line treatment in EC										
KEYNOTE-590/ NCT03189719	3	749	Pembrolizumab + 5-fluorouracil + cisplatin	373	12.4	NA	6.3	NA	45	98
			Placebo + 5-fluorouracil + cisplatin	376	9.8	NA	5.8	NA	29.3	97
CheckMate 648/ NCT03143153	3	970	Nivolumab + cisplatin + fluorouracil	321	13.2	54	5.8	24	47	96
			nivolumab + ipilimumab	325	12.7	54	2.9	23	28	80
			cisplatin + fluorouracil	324	10.7	44	5.6	16	27	90
ESCORT-1st/ NCT03691090	3	596	camrelizumab + paclitaxel + cisplatin	298	15.3	61.5	6.9	NA	72.1	99.3
			Placebo + paclitaxel + cisplatin	297	12	49.8	5.6	NA	62.1	97
JUPITER-06/ NCT03829969	3	514	Toripalimab + TP	257	17	66	5.7	27.8	69.3	99.2
			Placebo + TP	257	11	43.7	5.5	6.1	52.1	99.2
ORIENT-15/ NCT03748134	3	659	Sintilimab + (paclitaxel + cisplatin)/(5-fluorouracil + cisplatin)	327	16.7	64	7.2	38	66	98
			Placebo + (paclitaxel + cisplatin)/(5-fluorouracil + cisplatin)	332	12.5	52	5.7	15	45	98
NCT03603756	2	30	Camrelizumab + liposomal paclitaxel + nedaplatin + apatinib	30	19.43	NA	6.85	NA	80	100
NCT03222440	1b	20	Camrelizumab + radiotherapy	20	16.7	63.2	11.7	47.4	74	100
NCT03732508	2	23	SHR-1316 + liposomal irinotecan + 5-fluorouracil	23	11.6	NA	8.5	NA	52.2	100
First-line treatment in GC										
CheckMate 649/ NCT02872116	3	1581	Nivolumab + XELOX/FOLFOX	789	13.8	55	7.7	33	60	NA
			XELOX/FOLFOX	792	11.6	48	6.9	23	45	NA
ATTRACTION-4/ NCT02746796	3	724	Nivolumab + SOX/CAPOX	362	17.45	NA	10.45	NA	57	98
			Placebo + SOX/CAPOX	362	17.15	NA	8.34	NA	48	97
KEYNOTE-062/ NCT02494583	3	763	pembrolizumab	256	10.6	46.9	2	NA	14.8	54.3
			pembrolizumab + cisplatin + fluorouracil/ capecitabine	257	12.5	52.9	6.9	NA	48.6	94
			placebo + cisplatin + fluorouracil/capecitabine	250	11.1	45.6	6.4	NA	37.2	91.8
KEYNOTE-659/ NCT03382600	2b	100	Pembrolizumab + SOX	54	16.9	NA	9.4	NA	72.2	100
			Pembrolizumab + SP	46	17.1	NA	8.3	NA	80.4	100
NCT03472365	2	48	camrelizumab + CAPOX, subsequent camrelizumab + apatinib	48	14.9	68.8	6.8	NA	58.3	100

XELOX, capecitabine and oxaliplatin; FOLFOX, leucovorin, fluorouracil, and oxaliplatin; SOX, oxaliplation + S-1; CAPOX, oxaliplation +capecitabine; SP, S-1 + cisplatin; TP, paclitaxel plus cisplatin; N, number of patients; OS, overall survival; PFS, progression-free survival; ORR, object response rate; TRAEs, treatment-related adverse events; NA, not available.

with tumor-cell PD-L1 expression of ≥1%, the nivolumab + chemotherapy group had a substantial PFS advantage over the chemotherapy group (6.9 vs. 4.4 months). In patients with CPS ≥1 (91%), both the nivolumab + chemotherapy [hazard ratio (HR), 0.69] and nivolumab + ipilimumab (HR, 0.76) groups achieved prolonged mOS compared with that in the chemotherapy group. The survival advantage of the nivolumab-based regimen was demonstrated in subgroups with tumor-cell PD-L1 expression of ≥1% thresholds of 1%, 5%, and 10%, all with HR <1. The AEs were mainly caused by chemotherapy (nausea, loss of appetite, and stomatitis) (42). Notably, the KEYNOTE-590 and CheckMate-648 clinical trials employed similar chemotherapy drug intensities (both included fluoropyrimidine) but did not use the same evaluation criteria for PD-L1 expression and subgroup analysis.

Camrelizumab, a monoclonal antibody against PD-1, has also been researched as a first-line combination treatment in EC. Patients enrolled in the ESCORT-1st trial received camrelizumab or placebo plus chemotherapy (paclitaxel-cisplatin). The camrelizumab arm showed a longer OS tendency than the placebo arm (mOS, 15.3 vs. 12.0 months). Fewer grade 3–4 treatment-related adverse events (TRAEs) in the camrelizumab + chemotherapy group compared with the placebo + chemotherapy group (63.4% vs. 67.7%) indicated lower toxicity, with the former group experiencing adverse immune reactions mainly due to reactive capillary endothelial proliferation often associated with camrelizumab (43). The findings of this clinical trial supported the approval of camrelizumab in China for first-line treatment of unresectable, locally advanced/recurrent, or metastatic ESCC.

Toripalimab, an immunoglobulin G (IgG) PD-1 antibody, was evaluated in the JUPITER-06 trial, which enrolled 514 Chinese patients with advanced ESCC who received either toripalimab or placebo plus chemotherapy (paclitaxel plus cisplatin). PD-L1 expression was categorized as CPS ≥1 (PD-L1-positive) or CPS ≥10 (PD-L1 high expression). The toripalimab arm showed improved median progression-free survival (mPFS) (HR, 0.58) and mOS (HR, 0.58) compared to the placebo arm. The KM curves for PFS diverged early, with toripalimab retaining an advantage over the placebo. The 12-month PFS was nearly four times greater in the toripalimab + chemotherapy arm than in the placebo + chemotherapy arm. In terms of the antitumor response, the ORR (69.3% vs. 52.1%, p = 0.001) and DoR (5.6 vs. 4.2 months) were considerably higher in the toripalimab arm than in the placebo arm. The safety profile of toripalimab was considered to be acceptable. The OS and PFS benefits of toripalimab with chemotherapy were statistically significant and independent of PD-L1 expression levels (44). Both the JUPITER-06 and ESCORT-1st trials enrolled Chinese ESCC patients only. However, the survival benefit in the ESCORT-1st trial corresponded with PD-L1 expression levels, in contrast to the JUPITER-06 trial. Different PD-L1 detection methods and scoring criteria may have affected the results.

Sintilimab is a human IgG4 anti-PD-1 monoclonal antibody. In the multicenter ORIENT-15 trial, patients with ESCC received either sintilimab or placebo plus chemotherapy (93% cisplatin and paclitaxel, 7% cisplatin and 5-fluorouracil). Chinese patients made up 97% (n = 640) of the patients. The sintilimab arm had markedly better OS (16.7 vs. 12.5 months), PFS (7.2 vs. 5.7 months), and ORR (66% vs. 45%) than those in the placebo arm. The KM curves of OS remained distinct for the two groups from the beginning. The sintilimab arm outperformed the placebo arm by 13% and 23% for 1- and 2-year OS, respectively. Both tumor proportion score (TPS) and CPS for PD-L1 scoring were employed in the study. In the subgroup analysis, the survival advantage of sintilimab + chemotherapy was independent of PD-L1 expression levels (HR, 0.55 for TPS ≥10%; HR, 0.67 for TPS <10%; HR, 0.64 for CPS ≥10; HR, 0.62 for CPS <10) (45).

In the above clinical trials, PD-1 antibodies + chemotherapy were administered as a first-line combination therapy for EC. Although PD-1/PD-L1 antibody monotherapy has demonstrated good outcomes as a second- and third-line treatment, many challenges for its use as first-line treatment persist. The choice of the chemotherapeutic drug, patient distribution, inclusion criteria, and drug dose are factors that remain to be elucidated.

PD-1/PD-L1 blockade as first-line treatment in gastric cancer

The most common first-line treatment for metastatic and incurable GC is systemic therapy, with oxaliplatin frequently favored over cisplatin due to its reduced toxicity (46). Targeted therapies have also been used as first-line treatments for patients with specific types of GC. Patients with Human epidermal growth factor receptor 2 (HER2)-overexpressed gastric adenocarcinoma are recommended to receive pembrolizumab in combination with trastuzumab and chemotherapy (fluoropyrimidine and platinum) as first-line therapy. This recommendation is according to the results of the KEYNOTE-811 clinical trial. This ongoing international phase 3 trial is evaluating HER2-positive GC/GEJC in 692 patients treated with pembrolizumab or placebo plus trastuzumab and chemotherapy (capecitabine + oxaliplatin or fluorouracil + cisplatin). The trial employs MSI-H and PD-L1 as biomarkers. In the study population, 84.1% of patients had CPS ≥1, and large differences in ORR were reported. In the first interim analysis of 260 patients after an 8.5-month follow-up, the pembrolizumab arm had approximately 20% greater ORR than the placebo arm (74.4% vs. 51.9%) and maintained certain advantages in CR, disease control rate (DCR), and DoR, suggesting a more robust and durable response. Among the 433 patients examined for safety, the pembrolizumab group showed a lower incidence of grade 3–5 AEs and AEs leading to death than the placebo group. We look forward to updates from this trial (47, 48).

Based on the excellent clinical benefits and durable response achieved by nivolumab in combination with fluoropyrimidine- and platinum-containing chemotherapy in patients suffering from unresectable HER2-negative GC, GEJC, and EAC, the FDA approved this therapy in April 2021 for first-line treatment of tumors with CPS ≥5 (category 1) and CPS <5 under certain circumstances (category 2B) (49). In the CheckMate-649 trial, the analysis of survival status and antitumor response was divided into CPS ≥1 and CPS ≥5 subgroups. The nivolumab arm achieved a more pronounced OS benefit than the chemotherapy arm in the CPS ≥5 cohort (mOS, 14.4 vs. 11.1 months), CPS ≥1 cohort (HR, 0.77), and in all random patients (HR, 0.80). In patients with CPS ≥5, the nivolumab arm had 1.7-month longer PFS than the chemotherapy arm (7.7 vs. 6.0 months) and 14% longer 1-year PFS. The follow-up study determined that the survival benefit of nivolumab + chemotherapy increased with higher CPS cutoff value. In patients with CPS ≥5, the nivolumab + chemotherapy group had 15% greater ORR and 2.5-month longer response duration than the chemotherapy group. The advantage of an intense and prolonged response was also reflected in the randomized population. Meanwhile, as per the number needed to treat (NNT) analysis, the nivolumab + chemotherapy group maintained a consistent advantage over the chemotherapy group on the basis of OS, PFS, and ORR in the whole population and the CPS ≥5 subgroup. The prevalence of TRAEs was considerably higher in the nivolumab + chemotherapy group than in the chemotherapy alone group (22% vs. 12%) with more grade 3–4 TRAEs (59% vs. 44%). However, the nivolumab arm showed a lower risk of deteriorating symptoms than the chemotherapy arm (CPS ≥5, HR, 0.64; overall patients, HR, 0.77). Additionally, the nivolumab + chemotherapy group was associated with improved quality-adjusted time without symptoms or toxicity (Q-TWiST) compared to the chemotherapy group. Improving quality of life (QOL) also helps clinicians better manage patients (50–52).

A similar trial, ATTRACTION-4, enrolled 724 Asian patients with GC/GEJC from Japan, Korea, and Taiwan. The trial evaluated either nivolumab or placebo plus chemotherapy (oxaliplatin + capecitabine or fluoropyrimidine S-1). Although the OS between the two arms did not differ significantly (p = 0.26), the mPFS of the nivolumab arm was nearly 2 months longer than that of the placebo arm (10.45 vs. 8.34 months; HR, 0.68). The KM curves for PFS separated early, and the nivolumab arm consistently had superior PFS rates than the placebo arm. Additionally, regardless of PD-L1 expression levels, the nivolumab arm had a better antitumor response. The ORR was nearly 10% greater in the nivolumab arm than that in the placebo arm (57% vs. 48%). The nivolumab arm was associated with improved survival and 4-month longer DoR than the placebo arm (12.91 vs. 8.67 months). Although the nivolumab + chemotherapy group had more frequent TRAEs than the placebo + chemotherapy group, including grade ≥3 TRAEs, serious TRAEs, and TRAEs leading to treatment discontinuation, the types of TRAEs were consistent with those previously associated with chemotherapy and nivolumab treatment. The researchers determined that the toxicity of chemotherapy plus nivolumab was manageable, and that nivolumab combined with chemotherapy helped maintain QOL (53, 54). Compared to the CheckMate 649 trial, the ATTRACTION-4 trial enrolled Asian patients only and had more patients receiving subsequent anticancer drugs, which may be one of the reasons for the mOS difference between trials. Both trials added oxaliplatin as a chemotherapeutic agent and achieved good results, indicating that oxaliplatin works well in combination with nivolumab.

Pembrolizumab monotherapy was also explored as a first-line treatment for GC. The KEYNOTE-062 trial was established based on the positive outcomes of the KEYNOTE-059 and KEYNOTE-060 trials; however, KEYNOTE-062 did not achieve the desired results. The GC/GEJC population with CPS ≥1 was allocated to three arms: pembrolizumab or placebo plus chemotherapy (cisplatin combined with fluorouracil/capecitabine) and pembrolizumab alone. Analyses were performed based on CPS ≥10 (n = 281) and MSI-H (n = 50) subgroups. Among the overall study population with CPS ≥1, the pembrolizumab arm showed a lower OS compared with the chemotherapy arm (HR, 0.91) but approximately 1% and 6% higher 1- and 2-year OS, respectively. Pembrolizumab had a survival advantage over chemotherapy (HR, 0.91) and induced a longer DoR (13.7 vs. 6.8 months), suggesting that pembrolizumab had a long-term beneficial effect. In the CPS ≥10 cohort (n = 281), the pembrolizumab monotherapy arm seemed to have a clinical advantage over the chemotherapy arm, although the difference was not tested statistically (mOS, 17.4 vs. 10.8 months; HR, 0.62). The pembrolizumab arm had fewer TRAEs (54.3% vs. 91.8%) and grade ≥3 TRAEs (16.9% vs. 69.3%) than the chemotherapy arm. The overall population with CPS ≥1 was able to maintain health-related quality of life (HRQOL) when treated with pembrolizumab alone or pembrolizumab plus chemotherapy. A correlation between clinical efficacy and TMB in the pembrolizumab arm was proposed at a later stage of the study. The findings remained consistent at the 54.3-month follow-up, with the CPS ≥1 and CPS ≥10 subgroups treated with pembrolizumab having 8% and 18% greater 2-year OS than those treated with chemotherapy, respectively (55–58). Despite the lack of survival benefits compared to chemotherapy, pembrolizumab achieved better clinical benefit in the CPS ≥10 cohort than in the CPS ≥1 subgroup, suggesting that increased PD-L1 expression levels may improve OS for patients with GC. These findings seemed comparable to those in the CheckMate 649 trial. In contrast to the KEYNOTE-811 and ATTRACTION-4 trials, the KEYNOTE-062 trial used cisplatin rather than oxaliplatin, which may have led to differences in outcomes. In the ongoing KEYNOTE-859 trial, researchers are exploring the clinical effectiveness of pembrolizumab in combination with chemotherapy using 5-fluorouracil + cisplatin or capecitabine + oxaliplatin as the chemotherapeutic agents (59).

More trials investigating the combination of PD-1/PD-L1 antibodies and chemotherapy for GC/GEJC treatment are ongoing. The ORIENT-16 trial is exploring the clinical efficacy of sintilimab + oxaliplatin + capecitabine (60). The BGBA317305 trial (NCT03777657) is investigating the clinical efficacy of tislelizumab in combination with oxaliplatin + capecitabine or cisplatin + 5 fluorouracil (61). The above clinical trial results highlight that chemotherapy remains the mainstream first-line combination treatment for EC and GC for the time being. Studies exploring PD-1 antibody monotherapies have not yet demonstrated clinical advantages; however, the impact of different PD-L1 expression cutoffs on patient outcomes may influence future ICI studies.

PD-1/PD-L1 blockade as second-line or more treatment in esophageal cancer

Abundant PD-1/PD-L1 antibodies are involved in second-line treatment studies of EC and GC. Both monotherapies and combination therapies have demonstrated good applicability, and research is now focused on the possible applications of PD-1 antibody monotherapy as second-line or more treatments. Many of these agents have been approved by the FDA, including pembrolizumab, which has been approved for previously treated unresectable/metastatic MSI-H/dMMR or TMB-H solid tumors, including EC and GC (62, 63). Dostarlimab-Gxly is a second-line or more therapeutic option for MSI-H/dMMR GEC (64). Meanwhile, nivolumab is recommended for advanced

ESCC (category 1), and pembrolizumab is also recommended for advanced ESCC with CPS ≥10 (category 1) (Table 2).

Based on the positive outcomes of the KEYNOTE-180 and KEYNOTE-181 trials, the FDA approved pembrolizumab in 2019 as a second-line treatment for locally advanced/metastatic ESCC with CPS ≥10 (65). The phase II KEYNOTE-180 trial enrolled patients with advanced ESCC (n = 63) or EAC who had undergone second-line or more treatment, and patients were administered pembrolizumab for subsequent treatment. PD-L1-positive expression was defined as CPS ≥10. Antitumor responses were observed in the overall population (ORR, 9.9%), CPS ≥10 subgroup (ORR, 13.8%), and CPS <10 subgroup (ORR, 6.3%). Pembrolizumab conferred a significant survival advantage (OS, 5.8 months; 6-month OS, 49%; 12-month OS, 28%) and was deemed to be safe (TRAEs, 12.4%). The results suggested that PD-L1 expression levels may enhance the response to pembrolizumab in patients with ESCC or EAC (66, 67). In the subsequent multicenter KEYNOTE-181 trial, 528 patients (63.9%) were treated with pembrolizumab or chemotherapy (irinotecan, paclitaxel, or docetaxel). The survival advantage of pembrolizumab was more pronounced than that of chemotherapy for Asian patients. Additionally, pembrolizumab did not prolong mOS in all patients but presented a notable survival benefit in the CPS ≥10 subgroup. Among the CPS ≥10 cohort, the pembrolizumab arm had an OS advantage of almost 2.6 months over the chemotherapy arm (9.3 vs. 6.7 months), 20% greater 1-year OS (43.0% vs. 20.4%), and reduced risk of death (PFS, HR, 0.73). Among patients with ESCC, the 12-month PFS increased by 7% (16.7% vs. 7.4%). The most significant improvement in survival was observed in patients with ESCC with CPS ≥10 (HR, 0.64). An antitumor response advantage was reported in the pembrolizumab arm over the chemotherapy arm in the patients with ESCC (ORR, 16.7% vs. 7.4%), CPS ≥10 subgroup (ORR, 21.5% vs. 6.1%), and the randomized population (ORR, 13.1% vs. 6.9%). The 9-month response rate to pembrolizumab was higher than that to chemotherapy (53.5% vs. 38.1%), indicating a longer duration of response. The pembrolizumab arm had almost 20% fewer TRAEs and grade ≥3 TRAEs than the chemotherapy arm, and both sets of patients had similar HRQOL values, suggesting that pembrolizumab had a superior safety profile. However, the cost of pembrolizumab treatment far exceeded that of chemotherapy by $37,201.68. Health practitioners may value the application of pembrolizumab as a second-line therapy for EC (68–70). Both trials supported pembrolizumab monotherapy as a second-line treatment for EC. Furthermore, pembrolizumab showed greater efficacy in ESCC.

A growing number of newly developed PD-1 antibody single agents are being investigated in ESCC, and most trials have been conducted in China, where ESCC is the major subtype of EC. In the multicenter RATIONALE-302 trial, tislelizumab or chemotherapy (irinotecan, docetaxel, or paclitaxel) were administered to patients with metastatic or advanced ESCC. Tislelizumab is a specific antibody designed to target PD-1. PD-L1 expression was estimated using tumor area positivity (TAP), with TAP ≥10% set as the criterion for positive PD-L1 expression. In the overall population, the tislelizumab arm displayed an OS advantage over the chemotherapy arm (8.6 vs. 6.3 months; HR, 0.70). The mPFS was shorter in the tislelizumab arm than in the chemotherapy arm, but the KM curves for PFS began to separate at 3 months and the PFS rates for the tislelizumab arm remained progressively higher than those of the chemotherapy arm (6-month PFS, 21.9% vs. 14.9%; 12-month PFS, 12.7% vs. 1.9%). The tislelizumab arm had an OS advantage over the chemotherapy arm in the TAP ≥10% subgroup (10.3 vs. 6.8 months; HR, 0.54), TAP <10% subgroup (HR, 0.82) and TAP unknown subgroup (HR, 0.67). The OS advantage was demonstrated regardless of PD-L1 expression levels, as determined by *post-hoc* interaction analysis. The ORR of the tislelizumab arm was 10% higher than that of the chemotherapy arm (20.3% vs. 9.8%), indicating a longer-lasting antitumor response. The tislelizumab arm experienced fewer TRAEs and grade ≥ 3 TRAEs than the chemotherapy arm. Patients with advanced ESCC treated with tislelizumab demonstrated clinical improvement in OS (HR, 0.70) and a lower decline in physical function, leading to extended HRQOL (71, 72).

The phase 2 ORIENT-2 trial explored sintilimab as a second-line monotherapy for ESCC. The trial enrolled 190 patients with metastatic or advanced ESCC who were randomly assigned to the sintilimab or chemotherapy (paclitaxel or irinotecan) arms of the study. The mOS of the sintilimab arm was 1 month longer than that of the chemotherapy arm (7.2 vs. 6.2 months; HR, 0.70). The survival advantage of sintilimab over chemotherapy showed a longer tendency in the 12-month OS (37.4% vs. 21.4%) and 12-month PFS (10.7% vs. 1.9%). The sintilimab arm also had a superior safety profile than the chemotherapy arm (grade ≥3 TRAEs, 20.2% vs. 39.1%). The restricted mean survival time (RMST) and Fleming–Harrington tests led to the conclusion that sintilimab treatment for ESCC was associated with prolonged response and possible long-term survival. Biomarker analysis revealed that patients with a low neutrophil-to-lymphocyte ratio (NLR) (NLR <3) 6 weeks after sintilimab treatment had a substantial survival benefit over those with NLR >3 (OS, 14.0 vs. 6.2 months; PFS, 2.9 vs. 1.5 months). Moreover, low molecular tumor burden index (mTBI) in peripheral blood was associated with PFS (HR, 0.55), demonstrating the clinical significance of mTBI in sintilimab-treated patients. Based on these findings, researchers recommended the combination of low mTBI with high T-cell receptor clonality and NLR <3 at 6 weeks after treatment as biomarkers for predicting survival outcomes (OS and PFS) of sintilimab-treated patients with ESCC (73).

In addition to these trials, the ESCORT trial investigated camrelizumab monotherapy as a second-line treatment for advanced/metastatic ESCC in China (74), while the ATTRACTION-3 trial explored nivolumab monotherapy as a second-line therapy for advanced/metastatic ESCC (75). The above trials supported the popularity of PD-1 antibodies as monotherapies in second-line or more therapy studies in EC because Asian patients

TABLE 2 Clinical trials of PD-1/PD-L1 blockade in first-maintenance or second-line treatment.

trail	phase	enroll	arm	N	mOS (m)	12mOS (%)	mPFS (m)	12mFPS (%)	ORR (%)	TRAEs (%)
second-line treatment or more in EC										
KEYNOTE-180/NCT02559687	2	121	Pembrolizumab	121	5.8	28	2	NA	9.9	57.9
KEYNOTE-181/NCT02564263	3	628	pembrolizumab	314	7.1	32.4	2.1	NA	13.1	64
			paclitaxel/docetaxel/irinotecan	297	7.1	24.2	3.4	NA	6.7	86
RATIONALE-302/NCT03430843	3	512	tislelizumab	256	8.6	37.4	1.6	12.7	20.3	73.3
			paclitaxel/docetaxel/irinotecan	256	6.3	23.7	2.1	1.9	9.8	93.8
ORIENT-2/NCT03116152	2	190	sintilimab	95	7.2	37.4	1.6	10.4	12.6	54.3
			paclitaxel/irinotecan	95	6.2	21.4	2.9	1.7	6.3	90.8
ESCORT/NCT03099382	3	457	camrelizumab	228	8.3	34	1.9	10	NA	94
			docetaxel/irinotecan	220	6.2	22	1.9	NA	NA	90
ATTRACTION-3/NCT02569242	3	419	nivolumab	210	10.9	47	1.7	12	NA	65
			paclitaxel/docetaxel	209	8.4	34	3.4	7	NA	95
ATTRACTION-1/ONO-4538-;07	2	65	nivolumab	64	10.8	45.2	1.5	10.3	17.2	63.1
NCT02971956	2	49	Pembrolizumab	49	5.8	31.9	1.84	4.1	8	78
first-line maintenance treatment in GC										
JAVELIN Gastric 100/ NCT02625610	3	499	avelumab	249	10.4	NA	3.2	NA	13.3	61.3
			continued chemotherapy	250	10.9	NA	4.4	NA	14.4	77.3
JAVELIN Solid Tumor trial/ NCT01772004	1b	150	1 L-mn avelumab	90	11.1	46.2	2.8	13	6.7	63.3
			1 L chemotherapy		18.7	31.7	NA	NA	6.7	
			2 L avelumab	60	6.6	25.6	1.4	2	6.7	46.7
second-line treatment or more in GC										
KEYNOTE-059/NCT02335411	2	259	pembrolizumab	259	5.6	23.4	2	NA	11.6	60.2
KEYNOTE-061/NCT02370498	3	592	pembrolizumab	296	9.1	40	1.5	14	NA	53
			paclitaxel	296	8.3	27	4.1	9	NA	84
KEYNOTE-063 /NCT03019588	3	94	pembrolizumab	47	8	NA	2	NA	13	60
			paclitaxel	47	8	NA	4	NA	19	96
ATTRACTION-2/ONO-4538-12/ NCT02267343	3	493	nivolumab	330	5.26	26.2	1.61	7.6	11.2	43
			placebo	163	4.14	10.9	1.45	1.5	0	27

(Continued)

TABLE 2 Continued

trail	phase	enroll	arm	N	mOS (m)	12mOS (%)	mPFS (m)	12mFPS (%)	ORR (%)	TRAEs (%)
JAVELIN Gastric 300/ NCT02625623	3	371	avelumab	185	4.6	NA	1.4	NA	2.2	48.9
			chemotherapy	186	5	NA	2.7	NA	4.3	74
CheckMate-032/NCT01928394	1/2	160	Nivolumab 3mg/kg	59	6.2	39	1.4	8	12	69
			Nivolumab 1mg/kg plus ipilimumab 3mg/kg	49	6.9	35	1.4	17	24	84
			Nivolumab 3mg/kg plus ipilimumab 1mg/kg	52	4.8	24	1.6	10	8	75

1 L, First-Line; 1L-mn, First-Line Maintenance; 2 L, Second-Line; N, Number of patients; OS, Overall Survival; PFS, Progression Free Survival; ORR, Object Response Rate; TRAEs, Treatment-Related Adverse Events; NA, Not Available.

accounted for the majority of participants in these studies. In addition, regional differences were reflected in the KEYNOTE-181 study with Asian patients benefiting more from PD-1 blockade treatment than non-Asian patients, although the RATIONALE-302 trial did not report the same results. Additionally, different trials used different PD-L1 expression criteria, and the ORIENT-2 trial did not predict the absolute benefit of sintilimab treatment despite the use of both TPS and CPS. The exploration of appropriate predictive markers remains a pending issue.

PD-1/PD-L1 blockade as first-line maintenance therapy and second-line or more treatment in gastric cancer

Unlike EC, nivolumab and pembrolizumab monotherapies have not been authorized by the FDA as second-line treatments for GC. The conventional second-line treatment for GC is ramucirumab alone or in combination with paclitaxel (76); single-agent paclitaxel, docetaxel, and irinotecan are also suggested as category 1 therapies.

The phase 3 JAVELIN Gastric 100 trial explored the clinical effectiveness of avelumab applied to GC/GEJC as a maintenance therapy after primary induction chemotherapy. Avelumab did not markedly improve OS in either the PD-L1 expression on ≥1% of tumor cells (defined as PD-L1-positive) subgroup or randomized population. The KM curves for OS were lower in the avelumab arm than in the chemotherapy arm until 12 months. However, once the two curves crossed over, the avelumab arm preserved a trend toward higher OS, outperforming the chemotherapy arm by approximately 6% at 24-month OS (22.1% vs. 15.4%). The 1-year DoR and 2-year responses for the avelumab arm were approximately two and four times longer than those for the chemotherapy arm, respectively. In the CPS ≥1 subgroup, the mOS was comparatively higher in the avelumab arm than in the chemotherapy arm (HR, 0.72). Grade ≥3 AEs, TRAEs, and severe TRAEs occurred less frequently in the avelumab arm than in the chemotherapy arm. Although the JAVELIN Gastric 100 trial did not reach the primary endpoint of OS improvement, the potential survival benefits and excellent safety profile of avelumab in long-term treatment are informative (77). The JAVELIN Solid Tumor trial (78) also investigated the efficacy of avelumab as a first-line maintenance therapy for tumors. Although the trial data did not show a significant advantage over chemotherapy, the favorable 12-month OS and PFS in the JAVELIN Solid Tumor trial suggest a lasting effect of avelumab in long-term first-line maintenance treatment for patients with GC.

As a second-line treatment, pembrolizumab monotherapy in the phase 2 KEYNOTE-059 trial demonstrated good efficacy in advanced GC/GEJC. The phase 3 KEYNOTE-061 trial enrolled 395 patients with GC/GEJC with CPS ≥1 for subsequent administration of pembrolizumab or chemotherapy (paclitaxel). In the overall population, pembrolizumab did not demonstrate superiority in terms of OS (HR, 0.82). In the long-term follow-up, the KM curves separated at 8 months, after which the pembrolizumab arm had greater 12-month (13%) and 18-month (11%) OS than that in the chemotherapy arm. The superior response time of the pembrolizumab arm compared to the chemotherapy arm (18.0 vs. 5.3 months) suggests a survival advantage in long-term therapy. In the CPS ≥10 cohort, the OS of the pembrolizumab arm was 2.4 months longer than that of the chemotherapy arm (HR, 0.64). Pembrolizumab was associated with fewer toxic events than paclitaxel, including TRAEs, grade ≥3 TRAEs, and AEs leading to treatment discontinuation. The pembrolizumab and paclitaxel arms had comparable HRQOL scores. In the CPS ≥1 subgroup, the pembrolizumab arm had prolonged mOS compared to the paclitaxel arm (HR, 0.81), and the pembrolizumab arm had approximately 15% greater ORR than the paclitaxel arm in the CPS ≥10 cohort. The difference in 2-year OS between the pembrolizumab and paclitaxel arms increased with increasing CPS cutoff values (CPS ≥5, 15.4%; CPS ≥10, 21.1%). Additionally, the efficacy of pembrolizumab (PFS and ORR) progressively improved with increasing PD-L1 expression levels. In the CPS ≥1 subgroup, patients with Eastern Cooperative Oncology Group performance status (ECOG PS) 0 fared better when treated with pembrolizumab than with paclitaxel (OS, 12.3 vs. 9.3 months), with different results observed for patients with ECOG PS 1 (OS, 5.4 vs. 7.5 months). These results suggest that patients with better ECOG PS may respond more favorably to pembrolizumab treatment. In the follow-up biomarker analysis, tissue TMB was suggested as a predictor of pembrolizumab treatment in GC, but there are also conflicting views (79–85). Both the KEYNOTE-061 and KEYNOTE-062 trials achieved good and durable survival benefits in the CPS ≥10 subgroup, suggesting that patients with GC with high levels of PD-L1 expression may better respond to pembrolizumab, further supporting the use of PD-1 antibodies for patients with GC. The newly launched phase 3 KEYNOTE-063 trial was conducted after the KEYNOTE-061 trial. The KEYNOTE-063 trial enrolled 94 patients with advanced GC/GEJC with CPS ≥1 in Asia. This trial revealed superior results for the safety of pembrolizumab, although no definitive conclusions were reached regarding survival status and antitumor response (86).

The use of PD-1 antibodies as second-line or more treatments in GC is worth further exploration. Both the ATTRACTION-2 and CheckMate-032 trials included nivolumab, and the results were of relative clinical value, while nivolumab in the CheckMate-032 had better clinical value than nivolumab plus ipilimumab, suggesting that nivolumab-related studies are deserving of future exploration. Nevertheless, further consideration needs to be given to appropriate control treatments, since conventional second-line chemotherapy drugs may be more comparable than placebo treatments.

PD-1/PD-L1 blockade as perioperative treatment

Combined treatment improves patient survival more than resection alone in patients with localized EC or esophagogastric junction cancer (EGJC) (87, 88). Both perioperative and preoperative chemotherapy are routine regimens (89, 90). Based on the findings of the CheckMate 577 trial, nivolumab monotherapy was licensed by the FDA in May 2021 for patients with residual disease following preoperative chemoradiation and R0 resection (category 1) (91). In the CheckMate 577 trial, patients with EC/GEJC who received neoadjuvant radiotherapy were recruited and given either nivolumab or switched to a placebo treatment schedule. PFS was roughly twice as long in the nivolumab arm as that in the placebo arm (22.4 vs. 11.0 months; HR for disease recurrence or death, 0.69). The two arms continued to diverge in the KM curves, with nivolumab being continuously superior to the placebo. More AEs were associated with nivolumab treatment than with placebo treatment, but the safety profile was consistent with that of earlier trials. In the subgroup analysis, similar HR values for disease recurrence or mortality were observed for tumor-cell PD-L1 expression ≥1% (HR 0.75) and <1% (HR, 0.73), indicating that the efficacy of adjuvant nivolumab treatment was independent of PD-L1 expression levels (92). According to the CheckMate 577 trial, the European Society of Molecular Oncology recommends nivolumab as standard therapy for patients with EC/GEJC undergoing neoadjuvant chemoradiotherapy, regardless of histologic subtype (93).

Localized GC can also be treated with combination therapy to improve survival. Clinical trials exploring PD-1 antibodies combined with chemotherapy as a neoadjuvant therapy in GC have been conducted. A phase 2 study explored neoadjuvant treatment with capecitabine, sintilimab, and oxaliplatin in locally advanced GC/GEJC before surgical resection. A pathological complete response (pCR) was considered to be a predictor of the long-term benefit of neoadjuvant treatment and was set as the primary endpoint of the study. pCR and major pathological response (MPR) was achieved in 19.4% and 47.2% of the study population, respectively. The researchers attributed the results to the multiple drug combination and a high proportion of the study population with CPS ≥1. The CPS ≥1 subgroup had higher pCR (28.6%) and MPR (57.1%) than the overall population, supporting the use of CPS as a predictive biomarker to screen those who might best benefit from neoadjuvant anti-PD-1 therapy (94). Although not as much attention has been given to PD-1 antibodies in neoadjuvant studies as in first- and second-line treatment studies, many trials are underway. For instance, the KEYNOTE-585 trial has confirmed the effectiveness of perioperative chemotherapy in combination with pembrolizumab in GC (95).

Predictive biomarkers of PD-1/PD-L1 blockade efficacy

As seen from the above clinical trials, many conditions limit the ability of PD-1/PD-L1 blockade to achieve good results, and a considerable number of patients do not respond to therapy. Predictive biomarkers are essential for screening patients before the start of treatment and avoiding adverse effects. This section presents a short summary of common biomarkers used in clinical trials and briefly introduces those that may predict the effectiveness of PD-1/PD-L1 antibodies.

PD-L1 and MSI-H are recommended by the NCCN as common biomarkers in GC and EC. As shown in multiple clinical trials, patients with different PD-L1 expression levels often exhibit differences in response to PD-1 antibodies. In the CheckMate 032 trial, the beneficial effects of nivolumab in combination with ipilimumab increased with higher CPS levels, suggesting the superiority of CPS as a biomarker (96). Although the effectiveness of PD-1 antibodies in some trials was independent of PD-L1 expression levels, this difference may stem from different PD-L1 detection methods, evaluation criteria, and location of the patient. As common molecular subtypes, EBV-positive GC and MSI-H GC were both associated with enhanced ORR and PD-L1/PD-1 antibody efficacy, with EBV-positive GC having close to 100% ORR (28). Patients with MSI-H GC may have shorter PFS and lower ORR when receiving first-line chemotherapy, but higher ORR and PFS was achieved after subsequent PD-1 antibody treatment, supporting the early use of ICIs in MSI-H GC (97). Genome sequencing demonstrated that both EBV-positive GC and MSI-H GC were associated with high PD-L1 expression levels and favorable response to pembrolizumab (98).

Other common biomarkers have also been explored in GC and EC. TMB is associated with better response to PD-1 antibody treatment in EC (99). NLR is one of the leading predictive indicators of nivolumab efficacy in GC, providing a straightforward, easily acquired, and cost-effective biomarker (100). Changes in the gut microbiome were found in the DELIVER trial, in which the mechanism for bacterial invasion of epithelial cells was related to nivolumab clinical outcomes and progressive disease, suggesting a potential novel biomarker for predicting treatment response to nivolumab in advanced GC (101). Numerous predictive biomarkers have been investigated in clinical trials of GC and EC, but practical biomarkers need to be validated by credible findings.

Conclusions and perspectives

The standard of care for EC and GC has long revolved around chemotherapy and surgery. Along with research progress in targeted therapies, PD-1/PD-L1 antibodies continue to be investigated in clinical trials as reliable ICIs. This review presents an overview of the molecular and immunological background of PD-1/PD-L1 antibody applications, summarizes recent clinical trials investigating PD-1/PD-L1 blockade in EC and GC/GEJC, and briefly introduces common predictive biomarkers that could be further investigated. However, the clinical trials described herein have various potential problems that complicate the evaluation of their results. For example, some trials specified PD-L1 expression levels as an inclusion criterion, whereas other trials only explored PD-L1 expression in subgroup analyses. Furthermore, subgroups with different CPS cutoff values yielded varied CPS scores for survival results, while different PD-L1 expression detection methods might further skew conclusions when comparing trial results. Moreover, small disparities between patient locations, cancer types, and control groups affected trial outcomes and the ability to draw meaningful conclusions across trials. Indeed, the proportion of Asian patients in the study population may affect study outcomes. In addition, some chemotherapeutic drugs may affect the TME and impact the effectiveness of PD-1/PD-L1 antibodies (102, 103). Although PD-1/PD-L1 antibody treatment can prolong the life of some patients with GEC, the increased incidence of adverse effects when combined with chemotherapy cannot be ignored, and patients may develop a reduced tolerance to the drug, thereby risking treatment discontinuation. Finally, PD-1/PD-L1 antibodies are more expensive than conventional treatments, and both PD-L1 testing and dosing portals increase the cost of patient treatment. The above issues should be considered by investigators when designing future trials.

As immunotherapy research continues to advance, we believe that modalities of PD-1/PD-L1 blockade in EC and GC will further evolve. Here, we review and advise on common related issues (Table 3). First-line treatment in EC and GC has been extensively studied in combination with chemotherapy, and the choice of chemotherapeutic agents has been compared for effectiveness, while treatment alone has not yielded good results. Along with radiotherapy (104), CTLA-4 (ipilimumab), HER2 [trastuzumab (105) and margetuximab], and vascular endothelial growth factor receptor-2 (VEGFR-2) (106) antibodies are also being explored in clinical trials; studies on PD-1/PD-L1 in combination with other therapeutic modalities are promising. In response to the poor results of classical PD-1 antibody in a first-line trial, it is possible to investigate the application of PD-1 monotherapy in a strictly screened range of patients, such as PD-L1 CPS cutoffs, molecular subtypes, pathological types, and immune cell levels. Moreover, studies of biomarker detection can be performed in parallel with trials on subgroup analysis. Many PD-1 antibodies have been used in clinical studies for second-line therapy, but only pembrolizumab is used as the first choice in CPS ≥10 ESCC, with the others suggested as second-line treatment options. Other PD-1 antibodies might be tested in trials to determine their suitability in a range of patients through subgroup analysis. The new PD-1 antibody tislelizumab/sintilimab monotherapy study focused mainly on Asian ESCC patients, and the new drug could be considered for validation in a large clinical trial, including EC patients worldwide. Non-Asian regions have different pathology type proportions. How to control the balance of patient proportions needs to be considered when enrolling patients in future studies. Considering that avelumab has not achieved a clear advantage in first-line maintenance therapy, conventional PD-1 antibodies could be taken into consideration. Perioperative therapy emphasizes the importance of PD-1/PD-L1 antibodies in neoadjuvant therapy, while PD-1 antibodies in neoadjuvant therapy are typically administered as a combination or monotherapy following chemotherapy. Future studies must focus on the effect of PD-1 antibodies alone and apply PD-1 antibodies to other stages of perioperative therapy. As PD-1/PD-L1 antibodies in the CPS ≥1 subgroup are analyzed effectively in neoadjuvant therapy, whether PD-L1 routine testing is applicable to patients who could receive neoadjuvant therapy should be further investigated. In terms of biomarkers, HER2, MSI-H, and PD-L1 are currently used in testing, but new potential biomarkers are needed for HER2-, MSI-H-, and PD-L1-negative patients. Bioinformatics analysis to screen tumor cell gene expression characteristics or molecular pathways, as well as cellular and cytokine changes in the TME, may provide suitable

TABLE 3 Overview of clinical trials through comparison.

Source	Cancer types	PD-L1 scoring method and setting cut-offs	PD-1/PD-L1 antibody combined-agent or monotherapy	Results
KEYNOTE-590	EC/Siewert type 1 GEJC	CPS of 10	combined with 5-fluorouracil + cisplatin	better mOS and mPFS in patients with ESCC, patients with CPS of 10 or more and all patients
CheckMate-648	ESCC	CPS of 1 and tumor-cell PD-L1 expression of 1%	combined with cisplatin + fluorouracil	better mOS in patients with ESCC
ESCORT-1st	ESCC, all patients were Chinese	TPS of 1,5,10%	combined with paclitaxel + cisplatin	better mOS and mPFS in patients with ESCC
JUPITER-06	ESCC, all patients were Chinese	CPS of 1,10	combined with TP	better mOS and PFS benefits in patients with ESCC independent of PD-L1 expression levels
ORIENT-15	ESCC, 97% of patients was Chinese	TPS of 1,5,10% and CPS 1,5,10	combined with (paclitaxel + cisplatin)/(5-fluorouracil + cisplatin)	better mOS and PFS benefits in patients with ESCC independent of PD-L1 expression levels
KEYNOTE-811	HER2-overpressed GC/GEC	CPS of 1, 84.1% of patients had CPS of 1 or more	combined with trastuzumab + (5-fluorouracil and cisplatin)/(capecitabine and oxaliplatin)	ongoing
CheckMate 649	HER2-negative GC/GEJC/EAC	CPS of 1,5	combined with XELOX/FOLFOX	better mOS and mPFS in patients with CPS of 5 or more and all patients
ATTRACTION-4	GC/GEJC, all patients were Asian	tumor-cell PD-L1 expression of 1%	combined with SOX/CAPOX	better mPFS in all patients
KEYNOTE-062	GC/GEJC with CPS of 1 or more	CPS of 1,10	combined with cisplatin + fluorouracil/ capecitabine	not-positive results
KEYNOTE-180	EC	CPS of 10	monotherapy	PD-L1 expression levels may enhance the response to pembrolizumab in patients with ESCC or EAC
KEYNOTE-181	EC	CPS of 10	monotherapy	better mOS in patients with ESCC and patients with CPS of 10 or more
RATIONALE-302	ESCC	TAP of 10%	monotherapy	better mOS in all patients independent of PD-L1 expression levels
ORIENT-2	ESCC, all patients were Chinese	TPS of 1,10% and CPS 1,10	monotherapy	better mOS in all patients
JAVELIN Gastric 100	GC/GEJC	tumor-cell PD-L1 expression of 1%	monotherapy	not-positive results
KEYNOTE-061	GC/GEJC with CPS of 1 or more	CPS of 1	monotherapy	not-positive results, but high levels of PD-L1 expression may better respond to pembrolizumab
CheckMate 577	EC/GEJC	tumor-cell PD-L1 expression of 1%	monotherapy	better disease-free survival in all patients

ESCC, esophageal squamous cell carcinoma; GEJC, gastroesophageal junction cancer; GEC, gastroesophageal cancer; GC, gastric cancer; EC, esophageal cancer; CPS, combined positive score; TPS, tumor proportion score; TAP, tumor area positivity; XELOX, capecitabine and oxaliplatin; FOLFOX, leucovorin, fluorouracil, and oxaliplatin; SOX, oxaliplatin + S-1; CAPOX, oxaliplatin +capecitabine; SP, S-1 + cisplatin; TP, paclitaxel plus cisplatin.

combinatorial biomarkers. Overcoming the abovementioned drawbacks and exploring the best therapeutic outcomes in patients with complex EC and GC will help future investigators design valuable clinical trials, yielding beneficial outcomes.

Author contributions

MC: Conceptualization, Methodology, Investigation, Writing – Original Draft. CL: Supervision. MS: Supervision. YL: Supervision. XS: Supervision, Writing – Review and Editing, Project administration. All authors contributed to the article and approved the submitted version.

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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: A Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660

2. Zhang X, Zhang P. Gastric cancer: somatic genetics as a guide to therapy. *J Med Genet* (2017) 54:305–12. doi: 10.1136/jmedgenet-2016-104171

3. 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) 68:394–424. doi: 10.3322/caac.21492

4. *Cancer of the esophagus - cancer stat facts* . SEER. Available at: https://seer.cancer.gov/statfacts/html/esoph.html (Accessed August 17, 2022).

5. *Cancer of the stomach - cancer stat facts* . SEER. Available at: https://seer.cancer.gov/statfacts/html/stomach.html (Accessed August 17, 2022).

6. Huang J, Koulaouzidis A, Marlicz W, Lok V, Chu C, Ngai CH, et al. Global burden, risk factors, and trends of esophageal cancer: An analysis of cancer registries from 48 countries. *Cancers* (2021) 13:141. doi: 10.3390/cancers13010141

7. Smith-Garvin JE, Koretzky GA, Jordan MS. T Cell activation. *Annu Rev Immunol* (2009) 27:591–619. doi: 10.1146/annurev.immunol.021908.132706

8. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. *Cancer Res* (2019) 79:4557–66. doi: 10.1158/0008-5472.CAN-18-3962

9. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* (2013) 19:1423–37. doi: 10.1038/nm.3394

10. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* (2012) 12:252–64. doi: 10.1038/nrc3239

11. Cancer Genome Atlas Research Network, Analysis Working Group, Asan University, BC Cancer Agency, Brigham and Women's Hospital, Broad Institute and Brown University, et al. Integrated genomic characterization of oesophageal carcinoma. *Nature* (2017) 541:169–75. doi: 10.1038/nature20805

12. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* (2014) 513:202–9. doi: 10.1038/nature13480

13. Mandal R, Samstein RM, Lee K-W, Havel JJ, Wang H, Krishna C, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response. *Science* (2019) 364:485–91. doi: 10.1126/science.aau0447

14. KEYTRUDA® (pembrolizumab) injection, for intravenous use initial U.S. approval: 2014. (2014). Available at: https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf

15. Nicholas B, Bailey A, McCann KJ, Wood O, Walker RC, Ternette N, et al. Identification of neoantigens in esophageal adenocarcinoma. *Immunology* (2022). doi: 10.1111/imm.13578

16. Chen C, Zhou Q, Wu R, Li B, Chen Q, Zhang X, et al. A comprehensive survey of genomic alterations in gastric cancer reveals recurrent neoantigens as potential therapeutic targets. *BioMed Res Int* (2019) 2019:e2183510. doi: 10.1155/2019/2183510

17. Dhupar R, van der Kraak L, Pennathur A, Schuchert MJ, Nason KS, Luketich JD, et al. Targeting immune checkpoints in esophageal cancer: A high mutational load tumor. *Ann Thorac Surg* (2017) 103:1340–9. doi: 10.1016/j.athoracsur.2016.12.011

18. Oya Y, Hayakawa Y, Koike K. Tumor microenvironment in gastric cancers. *Cancer Sci* (2020) 111:2696–707. doi: 10.1111/cas.14521

19. Nomoto D, Baba Y, Okadome K, Yagi T, Kalikawe R, Kiyozumi Y, et al. Prognostic impact of PD-1 on tumor-infiltrating lymphocytes in 433 resected esophageal cancers. *Ann Thorac Surg* (2022) 113:286–94. doi: 10.1016/j.athoracsur.2021.01.013

20. Kelly RJ. The emerging role of immunotherapy for esophageal cancer. *Curr Opin Gastroenterol* (2019) 35:337–43. doi: 10.1097/MOG.0000000000000542

21. Kiyozumi Y, Baba Y, Okadome K, Yagi T, Ishimoto T, Iwatsuki M, et al. IDO1 expression is associated with immune tolerance and poor prognosis in patients with surgically resected esophageal cancer. *Ann Surg* (2019) 269:1101–8. doi: 10.1097/SLA.0000000000002754

22. Ni Z, Xing D, Zhang T, Ding N, Xiang D, Zhao Z, et al. Tumor-infiltrating b cell is associated with the control of progression of gastric cancer. *Immunol Res* (2021) 69:43–52. doi: 10.1007/s12026-020-09167-z

23. Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, et al. Macrophages promote the invasion of breast carcinoma cells *via* a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res* (2005) 65:5278–83. doi: 10.1158/0008-5472.CAN-04-1853

24. Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, et al. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* (2004) 64:7022–9. doi: 10.1158/0008-5472.CAN-04-1449

25. Qian B-Z, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell* (2010) 141:39–51. doi: 10.1016/j.cell.2010.03.014

26. Cardoso AP, Pinto ML, Pinto AT, Oliveira MI, Pinto MT, Gonçalves R, et al. Macrophages stimulate gastric and colorectal cancer invasion through EGFR Y(1086), c-src, Erk1/2 and akt phosphorylation and smallGTPase activity. *Oncogene* (2014) 33:2123–33. doi: 10.1038/onc.2013.154

27. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* (2010) 11:889–96. doi: 10.1038/ni.1937

28. Zhao R, Wan Q, Wang Y, Wu Y, Xiao S, Li Q, et al. M1-like TAMs are required for the efficacy of PD-L1/PD-1 blockades in gastric cancer. *OncoImmunology* (2021) 10:1862520. doi: 10.1080/2162402X.2020.1862520

29. Yagi T, Baba Y, Okadome K, Kiyozumi Y, Hiyoshi Y, Ishimoto T, et al. Tumour-associated macrophages are associated with poor prognosis and programmed death ligand 1 expression in oesophageal cancer. *Eur J Cancer* (2019) 111:38–49. doi: 10.1016/j.ejca.2019.01.018

30. Yamamoto K, Makino T, Sato E, Noma T, Urakawa S, Takeoka T, et al. Tumor-infiltrating M2 macrophage in pretreatment biopsy sample predicts response to chemotherapy and survival in esophageal cancer. *Cancer Sci* (2020) 111:1103–12. doi: 10.1111/cas.14328

31. Li J, Xie Y, Wang X, Li F, Li S, Li M, et al. Prognostic impact of tumor-associated macrophage infiltration in esophageal cancer: a meta-analysis. *Future Oncol* (2019) 15:2303–17. doi: 10.2217/fon-2018-0669

32. Svensson MC, Svensson M, Nodin B, Borg D, Hedner C, Hjalmarsson C, et al. High infiltration of CD68+/CD163– macrophages is an adverse prognostic factor after neoadjuvant chemotherapy in esophageal and gastric adenocarcinoma. *JIN* (2022) 14:614–27. doi: 10.1159/000524434

33. Qin Y, Wang F, Ni H, Liu Y, Yin Y, Zhou X, et al. Cancer-associated fibroblasts in gastric cancer affect malignant progression *via* the CXCL12-CXCR4 axis. *J Cancer* (2021) 12:3011–23. doi: 10.7150/jca.49707

34. Hassan MS, Cwidak N, Awasthi N, von Holzen U. Cytokine interaction with cancer-associated fibroblasts in esophageal cancer. *Cancer Control* (2022) 29:10732748221078470. doi: 10.1177/10732748221078470

35. Mu L, Yu W, Su H, Lin Y, Sui W, Yu X, et al. Relationship between the expressions of PD-L1 and tumour-associated fibroblasts in gastric cancer. *Artif Cells Nanomedicine Biotechnol* (2019) 47:1036–42. doi: 10.1080/21691401.2019.1573741

36. Li X, Sun Z, Peng G, Xiao Y, Guo J, Wu B, et al. Single-cell RNA sequencing reveals a pro-invasive cancer-associated fibroblast subgroup associated with poor clinical outcomes in patients with gastric cancer. *Theranostics* (2022) 12:620–38. doi: 10.7150/thno.60540

37. Huang F-L, Yu S-J. Esophageal cancer: Risk factors, genetic association, and treatment. *Asian J Surg* (2018) 41:210–5. doi: 10.1016/j.asjsur.2016.10.005

38. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *New Engl J Med* (2008) 358:36–46. doi: 10.1056/NEJMoa073149

39. Research C for DE and. *FDA Approves pembrolizumab for esophageal or GEJ carcinoma* (2021). FDA. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-esophageal-or-gej-carcinoma (Accessed October 13, 2022).

40. Sun J-M, Shen L, Shah MA, Enzinger P, Adenis A, Doi T, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): A randomised, placebo-controlled, phase 3 study. *Lancet* (2021) 398:759–71. doi: 10.1016/S0140-6736(21)01234-4

41. Kato K, Shah MA, Enzinger P, Bennouna J, Shen L, Adenis A, et al. KEYNOTE-590: Phase III study of first-line chemotherapy with or without pembrolizumab for advanced esophageal cancer. *Future Oncol* (2019) 15:1057–66. doi: 10.2217/fon-2018-0609

42. Doki Y, Ajani JA, Kato K, Xu J, Wyrwicz L, Motoyama S, et al. Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *N Engl J Med* (2022) 386:449–62. doi: 10.1056/NEJMoa2111380

43. Xu R, Luo H, Lu J, Bai Y, Mao T, Wang J, et al. ESCORT-1st: A randomized, double-blind, placebo-controlled, phase 3 trial of camrelizumab plus chemotherapy versus chemotherapy in patients with untreated advanced or metastatic esophageal squamous cell carcinoma (ESCC). *JCO* (2021) 39:4000–0. doi: 10.1200/JCO.2021.39.15_suppl.4000

44. Wang Z-X, Cui C, Yao J, Zhang Y, Li M, Feng J, et al. Toripalimab plus chemotherapy in treatment-naïve, advanced esophageal squamous cell carcinoma (JUPITER-06): A multi-center phase 3 trial. *Cancer Cell* (2022) 40:277–288.e3. doi: 10.1016/j.ccell.2022.02.007

45. Lu Z, Wang J, Shu Y, Liu L, Kong L, Yang L, et al. Sintilimab versus placebo in combination with chemotherapy as first line treatment for locally advanced or metastatic oesophageal squamous cell carcinoma (ORIENT-15): multicentre, randomised, double blind, phase 3 trial. *BMJ* (2022) 377:e068714. doi: 10.1136/bmj-2021-068714

46. NCCN clinical practice guidelines in oncology (NCCN guidelines ®). *Gastric Cancer* (2022). Available at: https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf

47. Janjigian YY, Kawazoe A, Yañez P, Li N, Lonardi S, Kolesnik O, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. *Nature* (2021) 600:727–30. doi: 10.1038/s41586-021-04161-3

48. Janjigian Y, Kawazoe A, Weber P, Luo S, Lonardi S, Kolesnik O, et al. LBA-4 initial data from the phase 3 KEYNOTE-811 study of trastuzumab and chemotherapy with or without pembrolizumab for HER2-positive metastatic gastric or gastroesophageal junction (G/GEJ) cancer. *Ann Oncol* (2021) 32:S227. doi: 10.1016/j.annonc.2021.06.011

49. Research C for DE and. *FDA Approves nivolumab in combination with chemotherapy for metastatic gastric cancer and esophageal adenocarcinoma* (2021). FDA. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-nivolumab-combination-chemotherapy-metastatic-gastric-cancer-and-esophageal (Accessed October 13, 2022).

50. Janjigian YY, Shitara K, Moehler M, Garrido M, Salman P, Shen L, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet* (2021) 398:27–40. doi: 10.1016/S0140-6736(21)00797-2

51. Sugarman R, Nunna S, Betts KA, Nie X, Nguyen H. Number needed to treat (NNT) analysis of patients in CheckMate 649 (CM 649): Nivolumab plus chemotherapy versus chemotherapy as first-line (1L) treatment for advanced gastric cancer, gastroesophageal junction cancer, and esophageal adenocarcinoma (GC/GEJ/EAC). *JCO* (2022) 40:307–7. doi: 10.1200/JCO.2022.40.4_suppl.307

52. Sugarman R, Botteman M, Rusibamayila N, Nguyen H, Lin D. A quality-adjusted time without symptoms or toxicity (Q-TWiST) analysis of patients in CheckMate 649: Nivolumab plus chemotherapy versus chemotherapy as first-line treatment for advanced gastric cancer/gastroesophageal junction cancer/esophageal adenocarcinoma (GC/GEJC/EAC). *JCO* (2022) 40:273–3. doi: 10.1200/JCO.2022.40.4_suppl.273

53. Boku N, Ryu M-H, Kato K, Chung HC, Minashi K, Lee K-W, et al. Safety and efficacy of nivolumab in combination with s-1/capecitabine plus oxaliplatin in patients with previously untreated, unresectable, advanced, or recurrent gastric/gastroesophageal junction cancer: interim results of a randomized, phase II trial (ATTRACTION-4). *Ann Oncol* (2019) 30:250–8. doi: 10.1093/annonc/mdy540

54. Kang Y-K, Chen L-T, Ryu M-H, Oh D-Y, Oh SC, Chung HC, et al. Nivolumab plus chemotherapy versus placebo plus chemotherapy in patients with HER2-negative, untreated, unresectable advanced or recurrent gastric or gastro-oesophageal junction cancer (ATTRACTION-4): A randomised, multicentre, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* (2022) 23:234–47. doi: 10.1016/S1470-2045(21)00692-6

55. Wainberg ZA, Shitara K, Van Cutsem E, Wyrwicz L, Lee KW, Kudaba I, et al. Pembrolizumab with or without chemotherapy versus chemotherapy alone for patients with PD-L1–positive advanced gastric or gastroesophageal junction adenocarcinoma: Update from the phase 3 KEYNOTE-062 trial. *JCO* (2022) 40:243–3. doi: 10.1200/JCO.2022.40.4_suppl.243

56. Lee K-W, Van Cutsem E, Bang Y-J, Fuchs CS, Kudaba I, Garrido M, et al. Association of tumor mutational burden with efficacy of Pembrolizumab ±Chemotherapy as first-line therapy for gastric cancer in the phase III KEYNOTE-062 study. *Clin Cancer Res* (2022) 28:3489–98. doi: 10.1158/1078-0432.CCR-22-0121

57. Shitara K, Van Cutsem E, Bang Y-J, Fuchs C, Wyrwicz L, Lee K-W, et al. Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with first-line, advanced gastric cancer: The KEYNOTE-062 phase 3 randomized clinical trial. *JAMA Oncol* (2020) 6:1571–80. doi: 10.1001/jamaoncol.2020.3370

58. Van Cutsem E, Valderrama A, Bang Y-J, Fuchs CS, Shitara K, Janjigian YY, et al. Quality of life with first-line pembrolizumab for PD-L1-positive advanced gastric/gastroesophageal junction adenocarcinoma: results from the randomised phase III KEYNOTE-062 study. *ESMO Open* (2021) 6:100189. doi: 10.1016/j.esmoop.2021.100189

59. Tabernero J, Bang Y-J, Van Cutsem E, Fuchs CS, Janjigian YY, Bhagia P, et al. KEYNOTE-859: a phase III study of pembrolizumab plus chemotherapy in gastric/gastroesophageal junction adenocarcinoma. *Future Oncol* (2021) 17:2847–55. doi: 10.2217/fon-2021-0176

60. Xu J, Jiang H, Pan Y, Gu K, Cang S, Han L, et al. LBA53 sintilimab plus chemotherapy (chemo) versus chemo as first-line treatment for advanced gastric or gastroesophageal junction (G/GEJ) adenocarcinoma (ORIENT-16): First results of a randomized, double-blind, phase III study. *Ann Oncol* (2021) 32:S1331. doi: 10.1016/j.annonc.2021.08.2133

61. Xu R, Arkenau H-T, Bang Y-J, Denlinger CS, Kato K, Tabernero J, et al. Tislelizumab plus chemotherapy versus placebo plus chemotherapy as first-line therapy in patients with locally advanced unresectable or metastatic gastric or gastroesophageal junction (G/GEJ) adenocarcinoma. *J Clin Oncol* (2020) 38 (4_suppl). doi: 10.1200/JCO.2020.38.4_suppl.TPS458

62. Research C for DE and *FDA Grants accelerated approval to pembrolizumab for first tissue/site agnostic indication* (2019). FDA. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-pembrolizumab-first-tissuesite-agnostic-indication (Accessed October 13, 2022).

63. Research C for DE and *FDA Approves pembrolizumab for adults and children with TMB-h solid tumors* (2020). FDA. Available at: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors (Accessed October 13, 2022)

64. Research C for DE and *FDA Grants accelerated approval to dostarlimab-gxly for dMMR advanced solid tumors* (2022). FDA. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-dostarlimab-gxly-dmmr-advanced-solid-tumors (Accessed October 14, 2022).

65. Research C for DE and *FDA Approves pembrolizumab for advanced esophageal squamous cell cancer* (2019). FDA. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-advanced-esophageal-squamous-cell-cancer (Accessed October 13, 2022).

66. Shah MA, Kojima T, Hochhauser D, Enzinger P, Raimbourg J, Hollebecque A, et al. Efficacy and safety of pembrolizumab for heavily pretreated patients with advanced, metastatic adenocarcinoma or squamous cell carcinoma of the esophagus: The phase 2 KEYNOTE-180 study. *JAMA Oncol* (2019) 5:546–50. doi: 10.1001/jamaoncol.2018.5441

67. Shah M, Kojima T, Hochhauser D, Enzinger P, Raimbourg J, Hollebecque A, et al. 261 association of t-cell–inflamed gene expression profile and PD-L1 status with efficacy of pembrolizumab in patients with esophageal cancer from KEYNOTE-180. *J Immunother Cancer* (2020) 8. doi: 10.1136/jitc-2020-SITC2020.0261

68. Kojima T, Shah MA, Muro K, Francois E, Adenis A, Hsu C-H, et al. Randomized phase III KEYNOTE-181 study of pembrolizumab versus chemotherapy in advanced esophageal cancer. *J Clin Oncol* (2020) 38:4138–48. doi: 10.1200/JCO.20.01888

69. Zhan M, Xu T, Zheng H, He Z. Cost-effectiveness analysis of pembrolizumab in patients with advanced esophageal cancer based on the KEYNOTE-181 study. *Front Public Health* (2022) 10:790225. doi: 10.3389/fpubh.2022.790225

70. Adenis A, Kulkarni AS, Girotto GC, de la Fouchardiere C, Senellart H, van Laarhoven HWM, et al. Impact of pembrolizumab versus chemotherapy as second-line therapy for advanced esophageal cancer on health-related quality of life in KEYNOTE-181. *J Clin Oncol* (2022) 40:382–91. doi: 10.1200/JCO.21.00601

71. Shen L, Kato K, Kim S-B, Ajani JA, Zhao K, He Z, et al. Tislelizumab versus chemotherapy as second-line treatment for advanced or metastatic esophageal squamous cell carcinoma (RATIONALE-302): A randomized phase III study. *J Clin Oncol* (2022) 40:3065–76. doi: 10.1200/JCO.21.01926

72. Van Cutsem E, Kato K, Ajani JA, Shen L, Xia T, Ding N, et al. Tislelizumab versus chemotherapy as second-line treatment for advanced or metastatic

esophageal squamous cell carcinoma (ESCC, RATIONALE 302): Impact on health-related quality of life (HRQoL). *JCO* (2022) 40:268–8. doi: 10.1200/JCO.2022.40.4_suppl.268

73. Xu J, Li Y, Fan Q, Shu Y, Yang L, Cui T, et al. Clinical and biomarker analyses of sintilimab versus chemotherapy as second-line therapy for advanced or metastatic esophageal squamous cell carcinoma: a randomized, open-label phase 2 study (ORIENT-2). *Nat Commun* (2022) 13:857. doi: 10.1038/s41467-022-28408-3

74. Huang J, Xu J, Chen Y, Zhuang W, Zhang Y, Chen Z, et al. Camrelizumab versus investigator's choice of chemotherapy as second-line therapy for advanced or metastatic oesophageal squamous cell carcinoma (ESCORT): A multicentre, randomised, open-label, phase 3 study. *Lancet Oncol* (2020) 21:832–42. doi: 10.1016/S1470-2045(20)30110-8

75. Kato K, Cho BC, Takahashi M, Okada M, Lin C-Y, Chin K, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): A multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* (2019) 20:1506–17. doi: 10.1016/S1470-2045(19)30626-6

76. Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): An international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* (2014) 383:31–9. doi: 10.1016/S0140-6736(13)61719-5

77. Moehler M, Dvorkin M, Boku N, Özgüroğlu M, Ryu M-H, Muntean AS, et al. Phase III trial of avelumab maintenance after first-line induction chemotherapy versus continuation of chemotherapy in patients with gastric cancers: Results from JAVELIN gastric 100. *J OF Clin Oncol* (2021) 39:966–77. doi: 10.1200/JCO.20.00892

78. Chung HC, Arkenau H-T, Lee J, Rha SY, Oh D-Y, Wyrwicz L, et al. Avelumab (anti-PD-L1) as first-line switch-maintenance or second-line therapy in patients with advanced gastric or gastroesophageal junction cancer: phase 1b results from the JAVELIN solid tumor trial. *J Immunother Cancer* (2019) 7:30. doi: 10.1186/s40425-019-0508-1

79. Shitara K, Özgüroğlu M, Bang Y-J, Di Bartolomeo M, Mandalà M, Ryu M-H, et al. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. *Lancet* (2018) 392:123–33. doi: 10.1016/S0140-6736(18)31257-1

80. Fuchs CS, Özgüroğlu M, Bang Y-J, Di Bartolomeo M, Mandalà M, Ryu M, et al. Pembrolizumab versus paclitaxel for previously treated patients with PD-L1–positive advanced gastric or gastroesophageal junction cancer (GC): Update from the phase III KEYNOTE-061 trial. *JCO* (2020) 38:4503–3. doi: 10.1200/JCO.2020.38.15_suppl.4503

81. Fuchs CS, Ozguroglu M, Bang Y-J, Di Bartolomeo M, Mandala M, Ryu M, et al. The association of molecular biomarkers with efficacy of pembrolizumab versus paclitaxel in patients with gastric cancer (GC) from KEYNOTE-061. *J Clin Oncol* (2020) 8:A159. doi: 10.1200/JCO.2020.38.15_suppl.4512

82. Shitara K, Ozguroglu M, Bang Y-J, Di Bartolomeo M, Mandala M, Ryu M, et al. The association of tissue tumor mutational burden (tTMB) using the foundation medicine genomic platform with efficacy of pembrolizumab versus paclitaxel in patients (pts) with gastric cancer (GC) from KEYNOTE-061. *J Clin Oncol* (2020) 38:4537. doi: 10.1200/JCO.2020.38.15_suppl.4537

83. Foote MB, Maron SB, Cercek A, Argilés G, Rousseau B, Diaz LA. TMB cut-offs fail to predict benefit of PD-1 blockade in gastroesophageal adenocarcinoma in KEYNOTE-061. *Ann Oncol* (2021) 32:1188–9. doi: 10.1016/j.annonc.2021.06.006

84. Van Cutsem E, Amonkar M, Fuchs CS, Alsina M, Özgüroğlu M, Bang Y-J, et al. Health-related quality of life in advanced gastric/gastroesophageal junction cancer with second-line pembrolizumab in KEYNOTE-061. *Gastric Cancer* (2021) 24:1330–40. doi: 10.1007/s10120-021-01200-w

85. Fuchs CS, Ozguroglu M, Bang Y-J, Di Bartolomeo M, Mandala M, Ryu M-H, et al. Pembrolizumab versus paclitaxel for previously treated PD-L1-positive advanced gastric or gastroesophageal junction cancer: 2-year update of the randomized phase 3 KEYNOTE-061 trial. *GASTRIC Cancer* (2022) 25:197–206. doi: 10.1007/s10120-021-01227-z

86. Chung HC, Kang Y-K, Chen Z, Bai Y, Ishak WZW, Shim BY, et al. Pembrolizumab versus paclitaxel for previously treated advanced gastric or gastroesophageal junction cancer (KEYNOTE-063): A randomized, open-label, phase 3 trial in Asian patients. *CANCER* (2022) 128:995–1003. doi: 10.1002/cncr.34019

87. Coccolini F, Nardi M, Montori G, Ceresoli M, Celotti A, Cascinu S, et al. Neoadjuvant chemotherapy in advanced gastric and esophago-gastric cancer. *Meta-analysis randomized trials. Int J Surg* (2018) 51:120–7. doi: 10.1016/j.ijsu.2018.01.008

88. Wang R, Song S, Harada K, Ghazanfari Amlashi F, Badgwell B, Pizzi MP, et al. Multiplex profiling of peritoneal metastases from gastric adenocarcinoma identified novel targets and molecular subtypes that predict treatment response. *Gut* (2020) 69:18–31. doi: 10.1136/gutjnl-2018-318070

89. Al-Batran S-E, Hofheinz RD, Pauligk C, Kopp H-G, Haag GM, Luley KB, et al. Histopathological regression after neoadjuvant docetaxel, oxaliplatin, fluorouracil, and leucovorin versus epirubicin, cisplatin, and fluorouracil or capecitabine in patients with resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4-AIO): Results from the phase 2 part of a multicentre, open-label, randomised phase 2/3 trial. *Lancet Oncol* (2016) 17:1697–708. doi: 10.1016/S1470-2045(16)30531-9

90. Alderson D, Cunningham D, Nankivell M, Blazeby JM, Griffin SM, Crellin A, et al. Neoadjuvant cisplatin and fluorouracil versus epirubicin, cisplatin, and capecitabine followed by resection in patients with oesophageal adenocarcinoma (UK MRC OE05): an open-label, randomised phase 3 trial. *Lancet Oncol* (2017) 18:1249–60. doi: 10.1016/S1470-2045(17)30447-3

91. Research C for DE and *FDA Approves nivolumab for resected esophageal or GEJ cancer* (2021). FDA. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-nivolumab-resected-esophageal-or-gej-cancer (Accessed October 13, 2022).

92. Kelly RJ, Ajani JA, Kuzdzal J, Zander T, Van Cutsem E, Piessen G, et al. Adjuvant nivolumab in resected esophageal or gastroesophageal junction cancer. *New Engl J OF Med* (2021) 384:1191–203. doi: 10.1056/NEJMoa2032125

93. Popper U, Rumpold H. Update ESMO: gastric and esophageal cancer. *memo* (2021) 14:180–3. doi: 10.1007/s12254-021-00694-5

94. Jiang H, Yu X, Li N, Kong M, Ma Z, Zhou D, et al. Efficacy and safety of neoadjuvant sintilimab, oxaliplatin and capecitabine in patients with locally advanced, resectable gastric or gastroesophageal junction adenocarcinoma: early results of a phase 2 study. *J Immunother Cancer* (2022) 10:e003635. doi: 10.1136/jitc-2021-003635

95. Bang Y-J, Van Cutsem E, Fuchs CS, Ohtsu A, Tabernero J, Ilson DH, et al. KEYNOTE-585: Phase III study of perioperative chemotherapy with or without pembrolizumab for gastric cancer. *Future Oncol* (2019) 15:943–52. doi: 10.2217/fon-2018-0581

96. Lei M, Siemers NO, Pandya D, Chang H, Sanchez T, Harbison C, et al. Analyses of PD-L1 and inflammatory gene expression association with efficacy of nivolumab ± ipilimumab in gastric Cancer/Gastroesophageal junction cancer. *Clin Cancer Res* (2021) 27:3926–35. doi: 10.1158/1078-0432.CCR-20-2790

97. Kubota Y, Kawazoe A, Sasaki A, Mishima S, Sawada K, Nakamura Y, et al. The impact of molecular subtype on efficacy of chemotherapy and checkpoint inhibition in advanced gastric cancer. *Clin Cancer Res* (2020) 26:3784–90. doi: 10.1158/1078-0432.CCR-20-0075

98. Kim ST, Cristescu R, Bass AJ, Kim K-M, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* (2018) 24:1449–58. doi: 10.1038/s41591-018-0101-z

99. Huang J, Xu B, Mo H, Zhang W, Chen X, Wu D, et al. Safety, activity, and biomarkers of SHR-1210, an anti-PD-1 antibody, for patients with advanced esophageal carcinoma. *Clin Cancer Res* (2018) 24:1296–304. doi: 10.1158/1078-0432.CCR-17-2439

100. Ota Y, Takahari D, Suzuki T, Osumi H, Nakayama I, Oki A, et al. Changes in the neutrophil-to-lymphocyte ratio during nivolumab monotherapy are associated with gastric cancer survival. *Cancer Chemother Pharmacol* (2020) 85:265–72. doi: 10.1007/s00280-019-04023-w

101. Sunakawa Y, Matoba R, Inoue E, Sakamoto Y, Kawabata R, Ishiguro A, et al. Genomic pathway of gut microbiome to predict efficacy of nivolumab in advanced gastric cancer: DELIVER trial (JACCRO GC-08). *161 [Abstract]. J Clin Oncol* (2021) 39:161–1. doi: 10.1200/JCO.2021.39.3_suppl.161

102. Quéméneur L, Beloeil L, Michallet M-C, Angelov G, Tomkowiak M, Revillard J-P, et al. Restriction of *De novo* nucleotide biosynthesis interferes with clonal expansion and differentiation into effector and memory CD8 T cells. *J Immunol* (2004) 173:4945–52. doi: 10.4049/jimmunol.173.8.4945

103. Galluzzi L, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol* (2020) 17:725–41. doi: 10.1038/s41571-020-0413-z

104. Shah MA, Bennouna J, Doi T, Shen L, Kato K, Adenis A, et al. KEYNOTE-975 study design: a phase III study of definitive chemoradiotherapy plus pembrolizumab in patients with esophageal carcinoma. *Future Oncol* (2021) 17:1143–53. doi: 10.2217/fon-2020-0969

105. Janjigian YY, Maron SB, Chatila WK, Millang B, Chavan SS, Alterman C, et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. *Lancet Oncol* (2020) 21:821–31. doi: 10.1016/S1470-2045(20)30169-8

106. Herbst RS, Arkenau H-T, Santana-Davila R, Calvo E, Paz-Ares L, Cassier PA, et al. Ramucirumab plus pembrolizumab in patients with previously treated advanced non-small-cell lung cancer, gastro-oesophageal cancer, or urothelial carcinomas (JVDF): a multicohort, non-randomised, open-label, phase 1a/b trial. *Lancet Oncol* (2019) 20:1109–23. doi: 10.1016/S1470-2045(19)30458-9

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Patients with positive HER-2 amplification advanced gastroesophageal junction cancer achieved complete response with combined chemotherapy of AK104/cadonilimab (PD-1/CTLA-4 bispecific): A case report

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Background: Human epidermal growth factor receptor 2 (HER2) is the most prominent therapeutic target for advanced gastric (G)/GEJ cancer. However, targeted therapy did not significantly improve survival. Currently, there are no regimens for the treatment of HER-2 amplification that exclude targeted agents.

Case presentation: A 42-year-old man was diagnosed with adenocarcinoma of GEJ (stage IV) with liver metastasis and lung metastasis. The patient was enrolled in a trial that excluded patients with known HER2-positivity: AK104, a PD-1/CTLA-4 bispecific antibody, combined with chemotherapy (mXELOX) as first-line therapy for advanced gastric G/GEJ cancer (NCT03852251). After six cycles of AK104 combined with chemotherapy therapy, immune-related pulmonary toxicity was observed. We rechallenged AK104 after hormone therapy, and no further pulmonary toxicity was observed. Immune-related hepatitis occurred in the patient during immunotherapy combined with single-drug capecitabine therapy. After combining steroid therapy with mycophenolate mofetil, the patient's immune hepatitis improved. Nevertheless, the patient was excluded from the clinical study due to the long-term absence of medication. Antitumor therapy was also discontinued in view of the patient's adverse immune response. The patient did not receive subsequent immune antitumor therapy, and immune-related hepatitis still occurred intermittently, but the disease evaluation was maintained at PR. A complete response was confirmed by PET/CT and the biopsy specimen from gastroscopy on 2020-06-10. Next generation sequencing of biopsy tissue was used to guide subsequent therapy at a recent follow-up visit. The results indicated that ERBB2 mutations occurred at copy number 58.4934 (HER-2), TMB = 3.1, MSS. IHC: EBV (–), PD-L1 CPS = 3, HER-2 (3+).

Conclusion: Patients with HER-2-positive advanced GEJ cancer received PD-1/CTLA-4 bispecific immunotherapy combined with chemotherapy and achieved complete remission. It offers a novel, highly specific, and highly potent therapeutic option for HER-2-positive patients. Its use should be considered as a new treatment when trastuzumab is not viable. Currently, we are working to overcome this resistance.

KEYWORDS

PD-1/CTLA-4 bispecific, AK104/cadonilimab, HER-2 positive, complete response, advanced gastroesophageal junction cancer

Introduction

HER-2 is the most prominent therapeutic target in advanced gastric (G) or gastroesophageal junction (GEJ) cancer (1). Since 2010, combination therapy with the anti-HER2 antibody trastuzumab and chemotherapy has become the standard first-line treatment for patients with HER-2-positive G/GEJ cancer (2). The development of a novel bispecific antibody that simultaneously binds to two distinct HER-2 epitopes (KN026) and the use of antibody–drug conjugates (ADC, such as T-DM1 and DS8201 and RC48) having a bystander effect are providing new tools to fight heterogeneity in HER-2 positive advanced cancer (3–5). Several studies have confirmed that anti-HER-2 effects involve antibody-dependent cell-mediated cytotoxicity by immune mechanisms superior to intracellular signaling (6). Immunotherapy plays an increasingly important role in the field of anti-tumor drugs and has achieved considerable clinical success. In the process of HER-2 negative advanced gastric cancer therapy, immune checkpoint inhibitors (natriculumab/sintilimab) combined with chemotherapy compared to pure chemotherapy for advanced G/GEJ First-line treatment of cancer has achieved overall survival (OS) and progression-free survival (PFS) benefits (7, 8). As indicated by the recent positive results of the KEYNOTE-811 trial, the immune effects of anti-HER-2 therapy can be better understood, and the effectiveness of the combination of immunotherapy and anti-HER2 therapy can be elucidated (9). This combination of immunologic targeting and chemotherapy has been recommended by the FDA. Currently, there are no regimens for the treatment of HER-2 amplification positivity that exclude targeted agents. We report a case of immune checkpoint inhibition combined with chemotherapy for the treatment of patients.

Case report

A 42-year-old man has no clear incentive to present an eating obstruction in July 2020. Symptoms worsen when hard and dry foods are consumed, accompanied by paroxysms of dull pain in the upper left abdomen, no chest tightness or pain, no nausea and vomiting, no hematemesis and melena, no fever and chills, and other discomfort. No history of autoimmune disease, no pneumonia, interstitial lung disease, no chronic obstructive pulmonary disease (COPD), denial of hepatitis B virus (HBV) or hepatitis C virus (HCV), human immunodeficiency virus (HIV) carrier, no recent vaccinations. He visited a local hospital on 13 August 2020. Gastroscopy revealed the lower esophagus, cardia and cardia by lumen narrowing, allowing endoscopy to pass through. There is a huge ulcer in the cardia. The nodules at the bottom are uneven and covered with dirt moss (Figures 1A, B). Biopsy pathology: poorly differentiated adenocarcinoma (Figure 1C). The patient came to our hospital for further diagnosis and treatment 17 August 2020. Contrast-enhanced Computed tomography (CT) of the cervicothoracic abdomen and pelvis demonstrated: cardiac cancer involving the esophagus and lesser curvature of stomach, with multiple lymph node metastasis; superior lobe metastasis of the left lung; hepatic metastasis (Figures 1D–F). Eastern Oncology Collaborative Group (EOCG): 1, the patients had poor economic foundation, but as the breadwinner of the family, the patients and their families had a strong desire for therapy.

After communication with the patient and comprehensive consideration, the patient requested to be enrolled in the "open-label study of AK104 (PD-1/CTLA-4 bispecific antibody)" (10). Patients with unknown HER-2 status or negative results could be included in the group. He did not undergo HER-2 and PD-L1

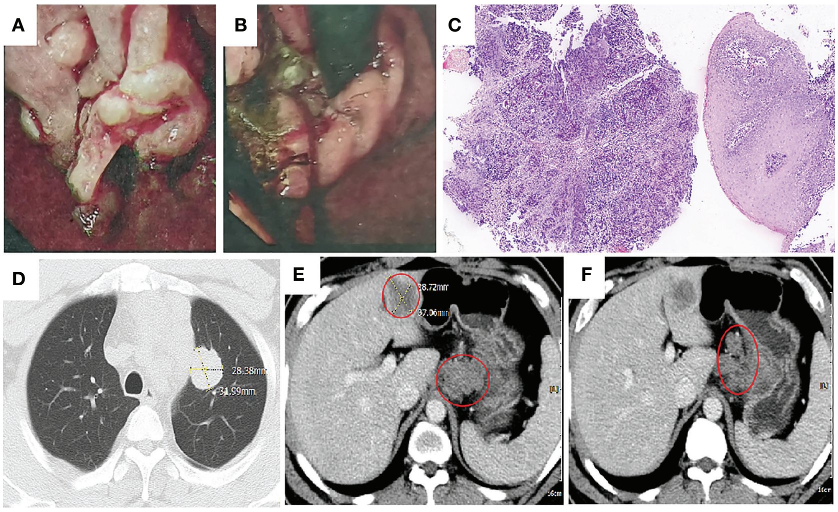

FIGURE 1
Imaging taken at baseline before initiation of treatment. **(A, B)** Gastroscopy illustrated lower esophagus, cardia and cardia by lumen narrowing. There is a huge ulcer in the cardia. The nodules at the bottom are uneven and covered with dirt moss. **(C)** Hemotoxylin and eosin (H&E): cardia with poorly differentiated adenocarcinoma (H&E, ×100 original magnification). **(D–F)** Computed tomography (CT) taken at the primary GEJ cancer and liver metastasis, lung metastasis and multiple lymph node metastasis.

tests at enrollment. Six cycles of AK104 + mXELOX/q14d (AK104 6 mg/kg d1+ oxaliplatin 85 mg/m^2 d1 + capecitabine 1,000 mg/m^2 d1–10/Q14d) were initiated on 27 August 2020. A partial response (PR) was assessed by CT after three and six cycles of treatment (primary foci and hepatic and lung metastatic lesions were markedly decreased). After the sixth treatment cycle, the patient showed symptoms of fatigue, wheezing after activity, palpitation, cough, phlegm, dry mouth, and loss of appetite. On 15 November 2020, general bacterial sputum culture and identification were performed. No bacteria associated with inflammation were identified. Detection of 13 respiratory pathogens: hemophilus influenzae positive. PCT: 0.10 ng/ml. Chest CT: multiple floc and patchy high-density shadows in both lungs, appearance of interstitial pneumonia (Figure 2A). He had not caught a cold recently and had no symptoms of fever. In addition, symptoms and additional examinations were combined to rule out the virus/bacterial pneumonia, considering the possibility of immune pneumonia. Antitumor therapy was interrupted, methylprednisolone sodium succinate (MPSS) 80 mg iv drip for 5 days, oral prednisone acetate tablets (taper off), and the patient's symptoms were markedly improved. A CT scan performed on 28 December 2020 showed that the pneumonia was better than before, and the lung metastatic lesions continued PR (Figure 2B).

A cycle of oxaliplatin 85 mg/m^2 d1 + capecitabine 1,000 mg/m^2 d1–10/Q14d 1 cycle was initiated on 14 January 2021. AK104 6 mg/kg d1 + capecitabine 1,000 mg/m^2 d1–10/Q14d regimen maintenance treatment commenced on 4 February 2021. During the CT evaluation, his condition was sustained at PR on 2 September 2021 monitoring of liver function: ALT 173.7 U/L and AST 148.4 U/L (Figure 2C). We delivered liver preservation therapy and, on 3 September 2021, retest liver function: ALT 189.0 U/L and AST 114.8 U/L. At this time, oxaliplatin had been discontinued for 7 months, so it was considered that liver damage was likely to be related to immunity. We gave MPSS 1 mg/kg combined with liver protection and gallbladder therapy to improve the liver function test on 14 September 2021: ALT 69.4 U/L and AST 26.5 U/L. Then the patient was treated at home with oral prednisone, and liver function returned to normal after regular review. 5 October 2021: ALT 650.4 U/L, AST 499.6 U/L, TBil 35 umol/L, DBil 23.1 umol/L, I-Bil 11.9 umol/L. Incorporating the patient's symptoms and hematologic findings, we diagnosed grade 3 immune-mediated hepatitis. MPSS 2 mg/kg combined with liver protection and gallbladder treatment was used to improve immune hepatitis. 14 October 2021: ALT 153.6 U/L, AST 35.2 U/L. 18 October 2021: ALT 171.3 U/L, AST 41.2 U/L. Considering corticosteroid resistance in patients, we treated them with the incorporation of mycophenolate mofetil. 25 October 2021: ALT 84.7 U/L↑, AST 18.2 U/L, TBil 17.3 umol/L↑, DBil 7.3 umol/L. The patient is getting better right now. Nevertheless, the patient was excluded from the clinical study due to the long-term absence of medication. Antitumor therapy was also discontinued in view of the adverse immune response of the patient. The patient did not receive subsequent immune antitumor therapy, and immune-related hepatitis still occurred intermittently, but the disease evaluation was maintained at PR. CR was confirmed by FDG-PET and the biopsy specimen from gastroscopy on 10 June 2020 (Figures 3A–I). Next-generation sequencing (NGS)-Geneseeq

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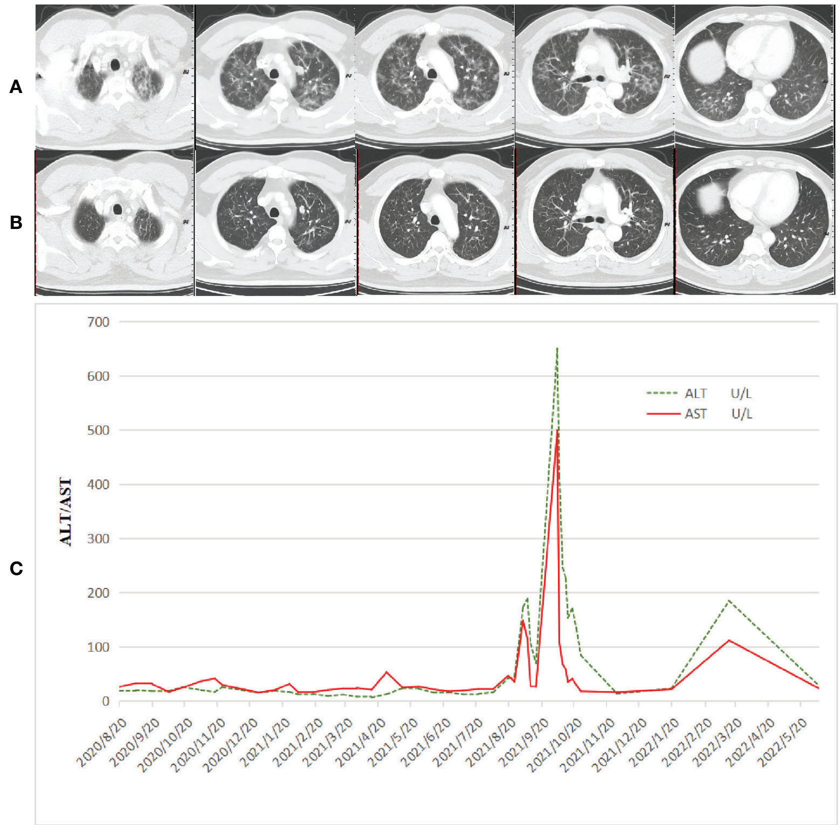


FIGURE 2
Adverse reactions that occurred during treatment. **(A)** CT taken at multiple floc and patchy high-density shadows in both lungs, appearance of interstitial pneumonia. **(B)** CT taken at the pneumonia was better than before, and the lung metastatic lesions continued PR. **(C)** Immune-related hepatitis occurred in the patient during immunotherapy combined with single-drug capecitabine therapy. After combined steroid therapy with mycophenolate mofetil, the patient's immune hepatitis improved. The patient did not receive subsequent immune antitumor therapy, and immune-related hepatitis still occurred intermittently.

PRIME (425-Cancer Gene Panel) of first biopsy tissue to guide subsequent therapy at a recent follow-up visit. The results indicated that TP53, JAK3, JARID2, CDKN2C, GREM1, EMSY, ERBB2 mutations; copy number 58.4934 (ERBB2), 15.158 (CCNE1); structural variation (ERBB2, CDK12); tumor mutational burden (TMB) = 3.1, microsatellite stability (MSS) (Figure 4A, Supplementary Figure S1). Immunohistochemistry (IHC): EBV (−), PD-L1 CPS = 3, HER-2 (3+) (Figures 4B–D).

Discussion

Gastric cancer is a heterogeneous disease, and HER-2-positive patients have heterogeneous responses to current standard therapies. One of the key reasons for this is insufficient attention to the underlying molecular mechanisms that lead to differences in cancer aggressiveness and treatment outcomes (11). Several studies have confirmed that anti-HER-2 effects involve antibody-dependent cell-mediated cytotoxicity by immune mechanisms superior to intracellular signaling (6). In two cancer models in immunocompetent mice, recruitment or downregulation of macrophages and NK cells (the primary effector cells of Ab-dependent cellular cytotoxicity) blocked trastuzumab's effect on tumor control. Ab-dependent cellular cytotoxicity (ADCP) and Ab-dependent cellular phagocytosis (ADCC) were validated as novel mechanisms of action of trastuzumab. It is proposed that activation of macrophages and NK cells can strengthen the anti-cancer efficacy of trastuzumab and other Ab immunotherapies by enhancing ADCP and ADCC, demonstrating that targeted effects are secondary to immune effects (12, 13). Interim data for the phase III KEYNOTE-811 trial (NCT03615326) have been published (9). The objective response rate (a secondary end point) in the first 264 patient incidents was 74.4% in the pembrolizumab group and 51.9% in the placebo group (P = 0.00006), and complete responses were more frequent (11.3% versus 3.1%). The result of the trial is still unknown, but the combination of animal experiments and the current results suggests that the immune effects of anti-HER-2 therapy can be better understood.

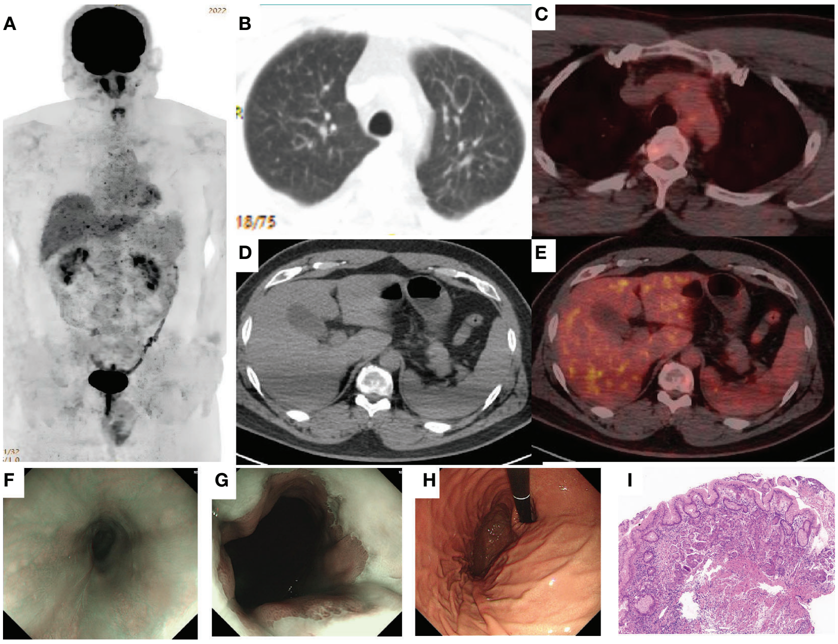
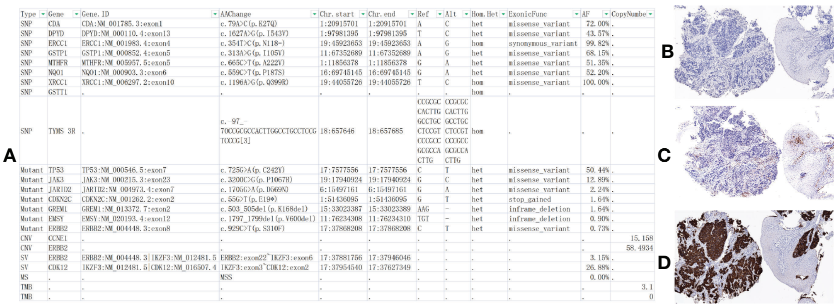


FIGURE 3
Complete response (CR) was confirmed by PET/CT and the biopsy specimen from gastroscopy. **(A–E)** 18F-2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) imaging shows the best overall response of CR to treatment with AK104. FDG-avidity was abolished in the gastroesophageal junction, liver, lung, and multiple lymph nodes. **(F–H)** Gastroscopy showed that the dentate line was seen 40 cm away from the incisor, and the mucosa was slightly rough without stenosis, which was biopsied at 12 o'clock. **(I)**. H&E: There was infiltration of inflammatory cells in the superficial layer of the cardia mucosa, while the glands in the deep layer were normal and no cancer cells were found (H&E, ×100 original magnification).

For this patient, participating in AK104 combined with chemotherapy is both an opportunity and a challenge. If the HER-2 positive status was known in advance, this patient would not have been able to participate in this trial, and the current standard anti-HER-2 treatment would have been applied, and he might not have achieved CR. By a stroke of luck, the shackles of guidelines can be broken, and the innovative application of excluding anti-HER-2 therapy can achieve this amazing clinical effect (Figure 5). The rapid development of antineoplastic drugs has greatly altered the way in which cancer is treated. At present, there are more and more ways to treat tumors, and many of them are too complicated. The therapeutic effect is not significantly improved,



Type	Gene	Gene. ID	AAChange	Chr. start	Chr. end	Ref	Alt	Hom. Het	ExonicFunc	AF	CopyNumbe
SNP	CDA	CDA:NM_001785.3:exon1	c.79A>C(p.K27Q)	1:20915701	1:20915701	A	C	het	missense_variant	72.00%	.
SNP	DPYD	DPYD:NM_000110.4:exon13	c.1627A>G(p.I543V)	1:97981395	1:97981395	T	C	het	missense_variant	43.57%	.
SNP	ERCC1	ERCC1:NM_001983.4:exon4	c.354T>C(p.N118=)	19:45923653	19:45923653	A	G	hom	synonymous_variant	99.82%	.
SNP	GSTP1	GSTP1:NM_000852.4:exon5	c.313A>G(p.I105V)	11:67352689	11:67352689	A	G	het	missense_variant	68.15%	.
SNP	MTHFR	MTHFR:NM_005957.5:exon5	c.665C>T(p.A222V)	1:11856378	1:11856378	G	A	het	missense_variant	51.35%	.
SNP	NQO1	NQO1:NM_000903.3:exon6	c.559C>T(p.P187S)	16:69745145	16:69745145	G	A	het	missense_variant	52.20%	.
SNP	XRCC1	XRCC1:NM_006297.2:exon10	c.1196A>G(p.Q399R)	19:44055726	19:44055726	T	C	hom	missense_variant	100.00%	.
SNP	GSTT1	.	.	.	.	.	.	hom	.	.	.
SNP	TYMS 3R	.	c.-97_-70CCGCGCCACTTGGCCTGCCTCCGTCCCG[3]	18:657646	18:657685	CCGCGCCACTTGGCCTGCCTCCGTCCCGCCGCGCCACTTG	CCGCGCCACTTGGCCTGCCTCCGTCCCGCCGCGCCACTTG	hom	.	.	.
Mutant	TP53	TP53:NM_000546.5:exon7	c.725G>A(p.C242Y)	17:7577556	17:7577556	C	T	het	missense_variant	50.44%	.
Mutant	JAK3	JAK3:NM_000215.3:exon23	c.3200C>G(p.P1067R)	19:17940924	19:17940924	G	C	het	missense_variant	12.89%	.
Mutant	JARID2	JARID2:NM_004973.4:exon7	c.1705G>A(p.D569N)	6:15497161	6:15497161	G	A	het	missense_variant	2.24%	.
Mutant	CDKN2C	CDKN2C:NM_001262.2:exon2	c.55G>T(p.E19*)	1:51436095	1:51436095	G	T	het	stop_gained	1.64%	.
Mutant	GREM1	GREM1:NM_013372.7:exon2	c.503_505del(p.K168del)	15:33023387	15:33023389	AAG	-	het	inframe_deletion	1.64%	.
Mutant	EMSY	EMSY:NM_020193.4:exon12	c.1797_1799del(p.V600del)	11:76234308	11:76234310	TGT	-	het	inframe_deletion	0.90%	.
Mutant	ERBB2	ERBB2:NM_004448.3:exon8	c.929C>T(p.S310F)	17:37868208	17:37868208	C	T	het	missense_variant	0.73%	.
CNV	CCNE1	.	.	.	.	.	.	.	.	.	15.158
CNV	ERBB2	.	.	.	.	.	.	.	.	.	58.4934
SV	ERBB2	ERBB2:NM_004448.3|IKZF3:NM_012481.5	ERBB2:exon22~IKZF3:exon6	17:37881756	17:37946046	.	.	.	.	3.15%	.
SV	CDK12	IKZF3:NM_012481.5|CDK12:NM_016507.4	IKZF3:exon3~CDK12:exon2	17:37954540	17:37627349	.	.	.	.	26.88%	.
MS	.	.	MSS	.	.	.	.	.	.	0.00%	.
TMB	.	.	.	.	.	.	.	.	.	.	3.1
TMB	.	.	.	.	.	.	.	.	.	.	0

FIGURE 4
At the latest follow-up, next-generation sequencing (NGS) and immunohistochemistry (IHC) were performed on the patients' first biopsy tissue. **(A)** NGS-Geneseeq PRIME (425-Cancer Gene Panel) of biopsy tissue to guide subsequent therapy at a recent follow-up visit. The results indicated that TP53, JAK3, JARID2, CDKN2C, GREM1, EMSY, ERBB2 mutations; copy number 58.4934 (HER-2), 15.158 (CCNE1); structural variation (SV) ERBB2, CDK12; tumor mutational burden (TMB) = 3.1, microsatellite stability (MSS). **(B–D)** IHC: EBV (–), PD-L1 Combined Positive Score, CPS = 3 (PD-L1,22C3), HER-2 (3+) (×100 original magnification). *At position 55, base G changes to T, causing a codon that should have been translated to E(glutamic acid) to become a stop codon, resulting in translation termination.

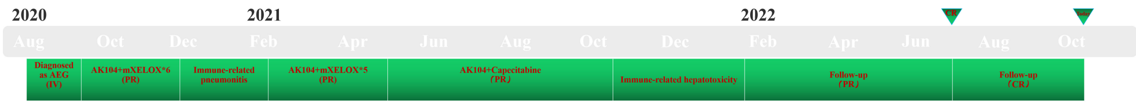


FIGURE 5
Timelines of events.

but adverse reactions are considerably increased. The aim of our therapy is to cure the disease rather than complicate its treatment. Cross-border competition–competitors are not from the same industry. Just as in the days of the horse-drawn carriage, people were looking for a faster horse, but even a faster horse could not beat the later invention of the automobile. Understanding something from other cognitive dimensions often opens the problem-solving landscape. For HER-2 positive patients, PD-1/CTLA-4 bispecific therapy has a good effect on MSI-H/dMMR population like PD-1/PD-L1 (14), and then achieve curve overtaking and lane change acceleration, bringing new first-line treatment options for more patients with positive HER-2 amplification.

Conclusions

In short, patients with HER-2-positive advanced GEJ cancer received PD-1/CTLA-4 bispecific immunotherapy combined with chemotherapy and achieved complete remission. The simplest and most effective treatment is the best regimen. It provides a framework for future clinical and translational research of [TP53, JAK3, JARID2, CDKN2C, GREM1, EMSY, ERBB2 mutations; copy number 58.4934 (ERBB2), 15.158 (CCNE1); structural variation (ERBB2, CDK12); IHC: HER-2 (3+), EBV (–), TMB-L (3.1), MSS] subtype gastric cancer. This case illustrates the clinical benefits of this regimen, which may become a first-line therapy option for HER-2-positive patients, but further clinical trials are needed to confirm this.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of the Shandong Cancer Hospital and Institute, Shandong First Medical University, and Shandong Academy of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JP, QZ, and YL analyzed and interpreted the patient data regarding the disease and the diagnosis. ZP performed the histological examination and diagnosis. JP, ZC, and BL dealt with the therapeutic management of the patient. All authors read and approved the final manuscript.

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Conflict of interest

Author YL was employed by Nanjing Geneseeq Technology Inc.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1049518/full#supplementary-material

References

1. Oh DY, Bang YJ. HER2-targeted therapies - a role beyond breast cancer. *Nat Rev Clin Oncol* (2020) 17(1):33–48. doi: 10.1038/s41571-019-0268-3

2. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial [published correction appears in lancet. *Lancet* (2010) 376(9742):687–97. doi: 10.1016/S0140-6736(10)61121-X

3. Zhang J, Ji D, Cai L, Yao H, Yan M, Wang X, et al. First-in-human HER2-targeted bispecific antibody KN026 for the treatment of patients with HER2-positive metastatic breast cancer: Results from a phase I study. *Clin Cancer Res* (2022) 28(4):618–28. doi: 10.1158/1078-0432.CCR-21-2827

4. Högner A, Moehler M. Immunotherapy in gastric cancer. *Curr Oncol* (2022) 29(3):1559–74. doi: 10.3390/curroncol29030131

5. Peng Z, Liu T, Wei J, Wang A, He Y, Yang L, et al. Efficacy and safety of a novel anti-HER2 therapeutic antibody RC48 in patients with HER2-overexpressing, locally advanced or metastatic gastric or gastroesophageal junction cancer: A single-arm phase II study. *Cancer Commun (Lond)* (2021) 41 (11):1173–82. doi: 10.1002/cac2.12214

6. Augustin JE, Soussan P, Bass AJ. Targeting the complexity of ERBB2 biology in gastroesophageal carcinoma. *Ann Oncol* (2022) 33(11):1134–48. doi: 10.1016/j.annonc.2022.08.001

7. Janjigian YY, Shitara K, Moehler M, Garrido M, Salman P, Shen L, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): A randomised, open-label, phase 3 trial. *Lancet* (2021) 398 (10294):27–40. doi: 10.1016/S0140-6736(21)00797-2

8. Xu J, Jiang H, Pan Y, Gu K, Cang S, Han L, et al. Sintilimab plus chemotherapy (chemo) versus chemo as first-line treatment for advanced gastric or gastroesophageal junction (G/GEJ) adenocarcinoma (ORIENT-16).2021 ESMO. *LBA53*. doi: 10.1016/j.annonc.2021.08.2133

9. Janjigian YY, Kawazoe A, Yañez P, Li N, Lonardi S, Kolesnik O, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. *Nature* (2021) 600(7890):727–30. doi: 10.1038/s41586-021-04161-3

10. Ji J, Shen L, Gao X, Ji K, Chen Y, Xu N, et al. A phase Ib/II, multicenter, open-label study of AK104, a PD-1/CTLA-4 bispecifc antibody, combined with chemotherapy (chemo) as frst-line therapy for advanced gastric (G) or gastroesophageal junction (GEJ) cancer [abstract no. 308]. *J Clin Oncol* (2022) 40(4 Suppl.):308. doi: 10.1200/JCO.2022.40.4_suppl.308

11. Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* (2015) 21(5):449–56. doi: 10.1038/nm.3850

12. Jaime-Ramirez AC, Mundy-Bosse BL, Kondadasula S. Il-12 enhances the antitumor actions of trastuzumab *via* NK cell IFN-γ production. *J Immunol* (2011) 186(6):3401–9. doi: 10.4049/jimmunol.1000328

13. Shi Y, Fan X, Deng H, Brezski RJ, Rycyzyn M, Jordan RE, et al. Trastuzumab triggers phagocytic killing of high HER2 cancer cells *in vitro* and *in vivo* by interaction with fcγ receptors on macrophages. *J Immunol* (2015) 194(9):4379–86. doi: 10.4049/jimmunol.1402891

14. Chao J, Fuchs CS, Shitara K, Tabernero J, Muro K, Van Cutsem E, et al. Assessment of pembrolizumab therapy for the treatment of microsatellite instability-high gastric or gastroesophageal junction cancer among patients in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 clinical trials. *JAMA Oncol* (2021) 7(6):895–902. doi: 10.1001/jamaoncol.2021.0275

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Maintenance therapy of low-dose nivolumab, S-1, and leucovorin in metastatic pancreatic adenocarcinoma with a germline mutation of *MSH6*: A case report

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Immune checkpoint inhibitors (ICIs) provide substantial benefits to a small subset of patients with advanced cancer with mismatch repair deficiency (MMRD) or microsatellite instability (MSI), including patients with pancreatic ductal adenocarcinoma (PDAC). However, the long duration of ICI treatment presents a considerable financial burden. We present the case of a 63-year-old woman with metastatic PDAC refractory to conventional chemotherapy. Genetic analyses identified an *MSH6* germline mutation and a high tumor mutation burden (TMB). Complete response (CR) was achieved after a short course of low-dose nivolumab (20 mg once every 2 weeks) with chemotherapy. CR was maintained for over 1 year with low-dose nivolumab and de-escalated chemotherapy without any immune-related adverse events. This case supports the further exploration of low-dose, affordable ICI-containing regimens in patients with advanced MSI-high/TMB-high cancer.

KEYWORDS

pancreatic ductal adenocarcinoma, nivolumab, maintenance therapy, mismatch repair, case report

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy with poor prognosis, and there has been little progress in the development of novel therapeutics for its treatment. Standard systemic therapy, comprising gemcitabine-based or 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX)-like regimens, is the recommended first-line chemotherapy for metastatic PDAC with good performance status (PS). These regimens have been the standard for more than 10 years (1). Beyond progression under frontline gemcitabine-based therapy, a combination of nanoliposomal irinotecan, fluorouracil, and leucovorin (NaFL), which was tested in the NAPOLI-1 trial, has demonstrated marginal efficacy in terms of response rate (RR) and survival (1). According to data from randomized trials and real-world data, the clinical benefits decrease with successive lines of chemotherapy. Moreover, given its considerable toxicity, it may not be justified to administer further multiagent chemotherapy to patients with deteriorating PS beyond first-line chemotherapy.

In the past 10 years, immunotherapies based on immune checkpoint blockade with manageable toxicities have revolutionized the landscape of anticancer treatment, particularly for refractory solid tumors other than PDAC. Single-agent or combination immune checkpoint inhibitors (ICIs) have exhibited poor RR and survival in advanced PDAC, even when used in conjunction with chemotherapy (2). Nevertheless, tumors associated with DNA mismatch repair deficiency (MMRD) and characterized by microsatellite instability (MSI) and high tumor mutation burden (TMB) represent a small subset (~1%) of PDAC with high RR and long duration of response (DOR) to ICIs (3, 4). Although they provide considerable benefits for a subgroup of patients, the optimal dosing, timing, and combination of ICIs are unknown, and their financial burden is high.

Herein, we report a case of metastatic *MSH6*-mutated PDAC refractory to standard frontline palliative chemotherapy regimens indicating a complete and durable response achieved by low-dose nivolumab plus chemotherapy.

Case presentation

A 63-year-old woman initially presented with intermittent periumbilical pain for half a year. She had undergone resection and adjuvant chemotherapy for stage I ovarian micropapillary serous carcinoma at the age of 52. In addition, she had a thoracic spinal epidural schwannoma that had been resected at the age of 61. Her sister had metachronous endometrial cancer and breast cancer in her 60s, and her father had lung cancer. Abdominal magnetic resonance imaging revealed an infiltrative hypoenhancing tumor measuring 2.2 cm in diameter at the pancreatic head. She underwent the Whipple procedure in September 2019, and the pathology report indicated pT2N0 stage IB poorly differentiated PDAC. Because of the cancer history of the patient and her family, genetic tests were recommended. Germline testing of a blood sample revealed a heterozygous mutation of the *MSH6* gene [c.3018C>G (p.Tyr1006Ter)]. The tumor tissue panel revealed the same *MSH6* mutation, heterozygous deletion of the *MLH1* gene, and additional genetic alterations (Supplementary Table 1). The tumor was MSI-high with a TMB of 52.8 mutations per megabase. Immunohistochemistry (IHC) revealed complete loss of MSH6 expression but a weak and heterogeneous expression of MLH1 in the neoplastic ducts. The expression of MSH2 and PMS2 was preserved (Supplementary Figure 1).

Subsequently, six monthly cycles of adjuvant gemcitabine and tegafur/gimeracil/oteracil (S-1) were administered. Recurrence with peritoneal metastases was noted soon after completion of adjuvant chemotherapy with doubling of the cancer antigen 19-9 (CA 19-9) level. One cycle of gemcitabine with nab-paclitaxel was administered. However, chemotherapy was temporarily withheld for the treatment of cryptococcal pneumonia. The level of CA 19-9 rapidly increased during the 4-month chemotherapy-free period. After the successful treatment of the infection, palliative chemotherapy was changed to NaFL.

Following eight cycles of NaFL, peritoneal metastases progressed with new liver metastases (Figures 1A, B). Based on the results of genetic tests, standard-dose anti-programmed cell death 1 (anti-PD-1) therapy was recommended, but this treatment was not affordable for the patient. With the approval of the patient, a biweekly low dose of nivolumab (0.3 mg/kg, 20 mg) combined with cisplatin (40 mg/m^2), gemcitabine (500 mg/m^2), S-1 (20 mg bid), and leucovorin (15 mg bid) was started in February 2021. After six cycles of nivolumab with chemotherapy, in April 2021, computed tomography revealed marked tumor reduction. Because of cisplatin-associated renal dysfunction and extreme tumor reduction, nivolumab, S-1, and leucovorin have been administered without gemcitabine and cisplatin since July 2021. With ongoing low-dose nivolumab plus S-1 and leucovorin for more than 1 year, CR has been maintained (Figures 1C, D), and she has remained asymptomatic with gradually recovered renal function. The treatment course is summarized in Figure 2.

Discussion

Maintenance therapy in advanced malignancies is understudied in cancer types with poor RR and short progression-free survival (PFS) with chemotherapy, such as PDAC. In the largest prospective study on PDAC, the PANOPTIMOX-PRODIGE 35 trial, the comparable median PFS and overall survival (OS) between a maintenance LV5FU2 regimen following disease control with eight cycles of FOLFIRINOX and 12 cycles of FOLFIRINOX in the first-line setting were demonstrated (5). However, patients may not recover from the toxicity of chemotherapy, such as the neurotoxicity of oxaliplatin; toxicity may even progress further as a result of restarting the same regimen (5). Sunitinib, a multitargeted receptor tyrosine kinase inhibitor, may be an acceptable alternative, although inadequate because of the limited benefit to PFS reported in the PACT-12 trial (6).

By contrast, meaningfully prolonged PFS has been achieved with maintenance olaparib, a poly(ADP–ribose) polymerase inhibitor, in metastatic PDAC with germline *BRCA1* or *BRCA2* mutations (7). However, the financial burden of olaparib may preclude the recommendation of olaparib in

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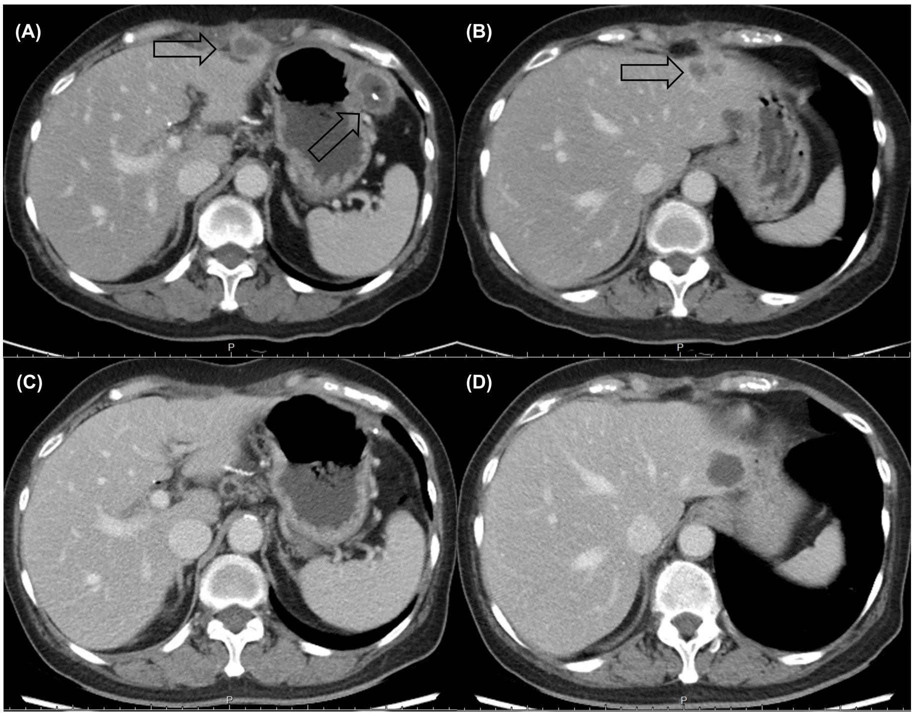

FIGURE 1
Computed tomography revealed multiple rim-enhancing **(A)** peritoneal metastases and **(B)** liver metastases before low-dose nivolumab. A durable complete response (**C**, **D**) was maintained after more than 1 year of nivolumab-based treatment.

daily practice among the small group of patients because no OS benefit was reported in the POLO trial (7).

The safety and efficacy of pembrolizumab, an anti-PD-1 antibody, has been well documented in patients with MMRD (4). In the pancreatic cancer subgroup of the KEYNOTE-158 study utilizing pembrolizumab at 200 mg once every 3 weeks, the median DOR was 13.4 (8.1 to 16.0+) months (4). However, the financial burden of prolonged use is even greater than that of olaparib, and this may limit access to full-dose anti-PD-1 therapy in low- and middle-income populations. However, in the phase I trial of nivolumab monotherapy, the plateau and dynamic levels of PD-1 occupancy on the circulating $CD3^+$ lymphocytes were similar among dose levels ranging from 0.3 to 10 mg/kg (8). The *in vitro* nivolumab concentration of 0.04 μg/mL could occupy >70% PD-1 on T cells, and pharmacodynamic tests indicated sufficient and durable PD-1 blockade at a low serum level (8, 9). Furthermore, the RR was not correlated with the dose of nivolumab; this provides ethical and scientific support for the application of low-dose nivolumab. For tumor-infiltrating lymphocytes (TILs), considerably more CD8+ TILs were observed in patients with MMRD PDAC (all with *MSH6* loss) compared to those without (median, 626 *vs.* 124 cells/mm^2) (10). Therefore, a high number of neoantigen-specific TILs in patients with MMRD PDAC is expected and may largely compensate for the potentially inferior efficacy of nivolumab at even low doses.

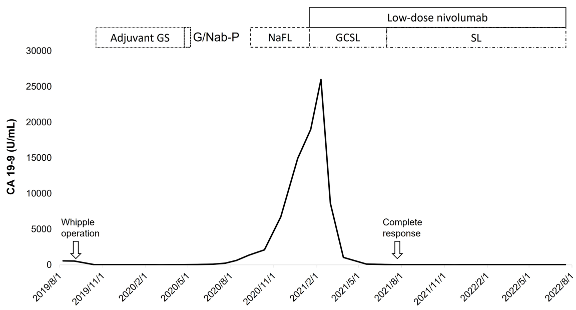

FIGURE 2
With a short duration of low-dose nivolumab plus gemcitabine (G), cisplatin (C), S-1 (S), and leucovorin (L), the level of CA 19-9 decreased rapidly with a complete response of tumors, which was durable under the maintenance therapy with low-dose nivolumab plus de-escalated chemotherapy (S-1 and leucovorin). (F, fluorouracil; Na, nanoliposomal irinotecan; Nab-P, nab-paclitaxel).

Regarding our case, the history of multiple malignancies in her family and the genetic analyses of blood and tumor tissue were consistent with the presence of MMRD. IHC confirmed the loss of MSH6 expression. The heterogeneous and weak expression of MLH1 may probably reflect the heterogeneity of the promoter hypermethylation and also partially contribute to the high TMB in tumor cells. Short disease-free survival after adjuvant chemotherapy and rapid progression under third-line NaFL indicated limited treatment options and poor survival from further palliative chemotherapy if she had PDAC that was MMR-proficient or without actionable genetic alterations, such as *KRAS* G12C or *BRCA* mutations. Although the 2.1-month median time (range: 1.3–10.6) to response reported in the KEYNOTE-158 study was similar to that of fourth-line gemcitabine, cisplatin, S-1, and leucovorin in our case (4), the possibility of cytoreduction or enhancement of the antitumor immune responses from chemotherapy cannot be excluded. Because of the heavily pretreated status, considerable tumor burden, and uncertain efficacy of the low-dose nivolumab in our case, the administration of chemoimmunotherapy was reasonable to maximize the chance of disease control.

A previous pilot study, exploring cisplatin plus S-1 in pancreatic cancer patients who had failed postoperative gemcitabine, demonstrated RR of 29.4%, stable disease of 11.8%, and median OS of 10 months (11). Regarding the disease-free survival of more than 6 months with adjuvant gemcitabine plus S-1 in this patient and the activity of cisplatin plus S-1, the application of gemcitabine, cisplatin, low-dose S-1, and leucovorin was a reasonable and feasible chemotherapy backbone for the heavily pretreated patient. However, the timing of anti-PD-1 therapy in patients with MMRD PDAC remains undetermined. In the KEYNOTE-177 study on metastatic colorectal cancer with MMRD, the non-significant difference reported in median OS between the first-line pembrolizumab and chemotherapy was probably due to the crossover to PD-1 pathway blockade in 60% of the chemotherapy arm; this also reflects the uncertainty regarding treatment timing (12).

Conclusion

Optimal patient selection is crucial for a favorable outcome even in cancer types with poor prognoses. Our case supports the further exploration of low-dose nivolumab in patients with MSI-high/TMB-high PDAC. This treatment has the advantages of relative safety and affordability.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/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

Study design: SH-P, S-HY Data collection and analysis: SHP, B-BC, T-CK, J-CL Manuscript preparation: SH-P, S-HY.

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.

Ono Pharmaceutical Co., Ltd supported nivolumab for an ongoing investigator-initiated clinical trial in pancreatic cancer at National Taiwan University Hospital. But this case report is not part of the clinical trial and the patient reported here did not participate in the nivolumab trial. This case report did not receive any funding from Ono Pharmaceutical Co., Ltd.

Publisher’s note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1077840/full#supplementary-material

References

1. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. *Lancet* (2020) 395:2008–20. doi: 10.1016/S0140-6736(20)30974-0

2. Bockorny B, Grossman JE, Hidalgo M. Facts and hopes in immunotherapy of pancreatic cancer. *Clin Cancer Res* (2022) 28:4606–4617. doi: 10.1158/1078-0432.CCR-21-3452

3. Grant RC, Denroche R, Jang GH, Nowak KM, Zhang A, Borgida A, et al. Clinical and genomic characterisation of mismatch repair deficient pancreatic adenocarcinoma. *Gut* (2021) 70:1894–903. doi: 10.1136/gutjnl-2020-320730

4. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite Instability/Mismatch repair-deficient cancer: Results from the phase II KEYNOTE-158 study. *J Clin Oncol* (2020) 38:1–10. doi: 10.1200/JCO.19.02105

5. Dahan L, Williet N, Le Malicot K, Phelip JM, Desrame J, Bouché O, et al. PRODIGE 35 Investigators/Collaborators. randomized phase II trial evaluating two sequential treatments in first line of metastatic pancreatic cancer: Results of the PANOPTIMOX-PRODIGE 35 trial. *J Clin Oncol* (2021) 39:3242–50. doi: 10.1200/JCO.20.03329

6. Reni M, Cereda S, Milella M, Novarino A, Passardi A, Mambrini A, et al. Maintenance sunitinib or observation in metastatic pancreatic adenocarcinoma: a phase II randomised trial. *Eur J Cancer* (2013) 49:3609–15. doi: 10.1016/j.ejca.2013.06.041

7. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N Engl J Med* (2019) 381:317–27. doi: 10.1056/NEJMoa1903387

8. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* (2010) 28:3167–75. doi: 10.1200/JCO.2009.26.7609

9. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* (2012) 366:2443–54. doi: 10.1056/NEJMoa1200690

10. Fraune C, Burandt E, Simon R, Hube-Magg C, Makrypidi-Fraune G, Kluth M, et al. MMR deficiency is homogeneous in pancreatic carcinoma and associated with high density of Cd8-positive lymphocytes. *Ann Surg Oncol* (2020) 27:3997–4006. doi: 10.1245/s10434-020-08209-y

11. Togawa A, Yoshitomi H, Ito H, Kimura F, Shimizu H, Ohtsuka M, et al. Treatment with an oral fluoropyrimidine, s-1, plus cisplatin in patients who failed postoperative gemcitabine treatment for pancreatic cancer: a pilot study. *Int J Clin Oncol* (2007) 12:268–73. doi: 10.1007/s10147-007-0674-x

12. Diaz LAJr, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. KEYNOTE-177 investigators. pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. *Lancet Oncol* (2022) 23:659–70. doi: 10.1016/S1470-2045(22)00197-8

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Interim result of phase II, prospective, single-arm trial of long-course chemoradiotherapy combined with concurrent tislelizumab in locally advanced rectal cancer

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Background: Neoadjuvant chemoradiotherapy is the standard treatment for locally advanced rectal cancer, with modest benefits on tumor regression and survival. Since chemoradiotherapy combined with immune checkpoint inhibitors has been reported to have synergic effects. This study aims to explore the safety and efficacy of long-course chemoradiotherapy combined with concurrent tislelizumab as a neoadjuvant treatment regimen for patients with locally advanced rectal cancer.

Methods: This manuscript reported the interim result of a prospective, multicenter, single-arm, phase II trial. Patients with mid-to-low locally advanced rectal cancer with clinical stages of cT3-4a N0M0 or cT1-4a N1-2M0 were included. The patients received long-course radiotherapy (50 Gy/25 f, 2 Gy/f, 5 days/week) and three 21-day cycles of capecitabine (1000 mg/m2, bid, day1-14) plus concurrent three 21-day cycles of tislelizumab (200 mg, day8), followed by a radical surgery 6-8 weeks after radiotherapy. The primary endpoint was the pathological complete response rate. (Clinical trial number: NCT04911517)

Results: A total of 26 patients completed the treatment protocol between April 2021 and June 2022. All patients completed chemoradiotherapy, 24 patients received three cycles of tislelizumab, and 2 patients received two cycles. The pathological complete remission (ypT0N0) was achieved in 50% (13/26) of the patients with all proficient mismatch repair tumors. The immune-related adverse event occurred in 19.2% (5/26) of patients. Patients with no CEA elevation or age less than 50 were more likely to benefit from this treatment regimen.

Conclusion: Long-course chemoradiotherapy combined with concurrent tislelizumab in patients with locally advanced low rectal cancer had favorable safety and efficacy, and does not increase the complication rate of surgery. Further study is needed to confirm these results.

KEYWORDS

rectal cancer, chemoradiotherapy, immune checkpoint inhibitors, neoadjuvant therapy, combination therapy

Introduction

Worldwide, colorectal cancer (CRC) is the third most common malignancy (1). Rectal cancer accounts for more than 1/3 of CRC patients. For those with mid-to-low locally advanced rectal cancer (LARC), long-course chemoradiotherapy (CRT) followed by total mesorectal excision (TME) is the standard treatment (2, 3). Generally, the pathological complete response (pCR) rate in conventional CRT was only 10%-20% (4–6). To obtain better oncological outcomes and preservation of organ function, treatment combinations in neoadjuvant therapy have been explored to achieve a higher rate of tumor downstaging.

Immune checkpoint inhibitors (ICIs) have been proven effective in many solid tumors (7–9). In deficient mismatch repair (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer, the ICIs appear favorable clinical benefits (10), while in proficient mismatch repair (pMMR) or microsatellite stable (MSS) subsets, the slight efficacies of ICIs have been reported (11–13). Thus, a combination of CRT and ICIs has been expected to treat such refractory tumors. Preclinical studies have shown a synergistic antitumor effect of this treatment regimen. Radiotherapy promotes the presentation of tumor-derived antigens, upregulates the PD-L1 expression, increases the CD3/CD8 T-cell infiltration, and activates the innate immune pathway (14, 15). These tumor microenvironment remolding effects may enhance the anti-tumor efficacies of ICIs.

A few studies have explored the ICIs combined with CRT in neoadjuvant therapy for LARC. A promising pCR rates of 25%-48.1% were reported with only mild toxicities (16–20). The VOLTAGE-A study added 5 cycles of nivolumab after long-course chemoradiotherapy. A 30% and 60% pCR rates were observed in MSS and MSI-H patients respectively (18). The optimal timing of ICIs use in neoadjuvant therapy is inconclusive. Several studies have shown that ICIs appear to have better synergy with radiotherapy when administered concurrently (21, 22). And the PACIFIC trial demonstrated that the durvalumab given within 14 days after radiation may prolong the overall survival (23). Thus, the PACIFIC-2 aimed to evaluate the benefit of concurrent durvalumab with chemoradiation (NCT03519971). Given these results, we designed this phase II, multicenter, prospective, single-arm trial to evaluate the efficacy and safety of LR-CRT combined with concurrent tislelizumab in patients with LARC (24). In this manuscript, we will report the interim result of this study.

Materials and methods

This NCRT-PD1-LARC was a prospective, multicenter, single-arm, phase II trial (Clinical trial number: NCT04911517). The study design was described previously (24). To allow patient enrollment in accordance with clinical practice, we undertook a protocol amendment to include patients with mid-to-low locally advanced rectal cancer (0-10cm above anal verge) with cT3-4aN0M0 or cT1-4a N1-2M0 pre-staged by MRI. The major exclusion criteria were congenital or acquired immune deficiency and present or previous active malignancies (except the diagnosis of rectal cancer this time). The protocol and amendments were approved by the ethics committee of Beijing Friendship Hospital, Capital Medical University on March 30th, 2021, and February 25th, 2022, respectively. The informed consent of study participation was signed before treatment.

Therapeutic schedule

Eligible patients received long course radiotherapy (50 Gy/25 f, 2 Gy/f, 5 days/week) in the first five weeks and three 21-day cycles of capecitabine (1000 mg/m2, bid, po, day1-14) plus tislelizumab (200 mg, iv.gtt, day8) in the first nine weeks. All patients receive the total mesorectal excision surgery 6-8 weeks after completion of the radiotherapy. Adjuvant therapy regimens after surgery are recommended for chemotherapy according to NCCN guidelines.

Patients are required to complete a baseline assessment prior to treatment, including a complete medical history and physical examination, chest CT, abdominal and pelvic CT, rectal MRI, and colonoscopy. These examinations need to be evaluated again before surgery, and the clinical efficacy is evaluated according to the criteria of the Response Evaluation Criteria In Solid Tumors (RECIST) ver.1.1. Adverse events monitoring is followed up at least every 3 weeks during neoadjuvant therapy. The adverse event was managed according to the consensus recommendations from the Society for Immunotherapy of Cancer (SITC) toxicity management working group.

Postoperative follow-up is performed every 3 months for 1 year and every 6 months thereafter until 5 years after surgery or to death. The complication classification refers to the Clavien-Dindo classification [9].

Outcomes

The primary outcome was the pathologic complete response (pCR) rate, defined as the proportion of patients with pCR (ypT0N0). The secondary outcomes were as follows (1): The tumor regression was evaluated according to the criteria of the American Joint Committee on Cancer (AJCC) 8th edition. Tumor regression grade (TRG) 0 indicates no residual tumor cells; TRG 1 indicates single or small groups of cells, TRG 2 indicates residual cancer with a desmoplastic response, and TRG 3 indicates minimal evidence of tumor response (2). objective response rate (ORR) is the result of complete response plus partial response rate (3). neoadjuvant rectal (NAR) score was calculated from clinical T stage, pathological T and N stages. A higher score represents a poorer prognosis (4). R0 resection rate was defined as the percentage of the negative margin microscopically (5). Anal preservation rate was defined as the percentage of the patients who received the anal-preserving surgery (6). 3-year local recurrence rate was defined as the percentage of patients who had local recurrence within 3 years after TME surgery (7). 3-year disease-free survival rate is defined as the percentage of patients without recurrence, metastasis, or death within 3 years (8). 3-year overall survival rate was defined as the percentage of patients alive at the 3-year follow-up (9). Safety analysis includes adverse events and postoperative complications. Adverse events were assessed using Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0, and postoperative complications were assessed using e Clavien–Dindo classification ver. 2.0.

Statistical analysis

The pCR rate in patients with NCRT was reported to be 15% according to previous studies. We assumed the pCR rate in this trial could increase to 40%. With a one-sided alpha of 5%, power of 80%, and a 10% dropout, 50 patients were needed in this single arm.

Statistical analyses were in progress using the SPSS software (version 22.0). Continuous variables will be presented as means ± standard deviation. Categorical variables will be presented as numbers and percentages. The efficacy and safety analyses were performed in patients treated with at least one dose of tislelizumab and who received radical surgery to obtain the pathological results. Comparisons were performed using Fisher's exact test or the $\chi 2$ test. P values <0.05 were considered statistically significant.

Results

Patient characteristics and compliance

At the time of the interim analysis, 38 patients were enrolled in this ongoing study from April 2021 to June 2022. Among them, 26 patients have received neoadjuvant therapy and completed treatment protocol. All patients received the full course of radiotherapy (50Gy) and chemotherapy without dose modification (100%, 26/26). And 24 patients received 3 cycles of tislelizumab (92.3%, 24/26), 2 patients received 2 cycles (first and third cycles) due to adverse events (grade 3 immune checkpoint inhibitor-associated colitis and grade 1 hyperthyroidism). Patient characteristics were shown in Table 1.

TABLE 1 Patient characteristics.

Age, years, means (standard)	60.5 (11.8)
Sex, n (%)	
Male	14 (53.8)
Female	12 (46.2)
ECOG performance status, n (%)	
0	16 (61.5)
1	10 (38.5)
Clinical T category, n (%)	
cT2	4 (15.4)
cT3	19 (73.1)
cT4	3 (11.5)
Clinical N category, n (%)	
cN0	12 (46.2)
cN1	9 (34.6)
cN2	5 (19.2)
EMVI, n (%)	
Negative	9 (34.6)
Positive	17 (65.4)
MRF, n (%)	
Negative	22 (84.6)
Positive	4 (15.4)
Distance from primary tumor to anal verge	
Means (standard)	4.9 (2.6)
<5cm, n (%)	12 (46.2)
5-10cm, n (%)	14 (53.8)
Length of tumor lesion, cm, means (standard)	3.7 (1.7)
CEA evaluated, n (%)	9 (34.6)
Time from the end of CRT to radical surgery, weeks, means (weeks)	8.0 (1.7)
Surgery	
Anal-preserving surgery, n (%)	23 (88.5)
Not anal-preserving surgery, n (%)	3 (11.5)

Surgery

The interval between the completion of radiotherapy and surgery was 8.0 ± 1.7 weeks. A total of 27 patients underwent TME surgery with R0 resection. The anal preservation was 88.5% (23/26). The blood loss was 74.1 ± 41.7 ml. The length of surgery was 222.0 ± 50.6 min. None of the patients had intraoperative complications. Six patients (23.1%) had postoperative complications, including rectovaginal fistula in one patient (grade III), anastomosis leak in one patient (grade II), ileus in two patients (grade II), and deep vein thrombosis in one patient (grade II). The length of the patient's hospital stay was 12.4 ± 2.9 days. No treatment-related death occurred.

Efficacy

The interval between the end of radiotherapy and preoperative MRI evaluation was 6.0 ± 1.9 weeks. The efficacy evaluation was shown in Table 2. Of the 26 patients, 46.2% (12/26) achieved a complete response, 26.9% (7/26) achieved a partial response, and 26.9% (7/26) achieved stable disease. No patients present with progressive disease. The objective response rate was 73.1% (19/26). All the patients were pMMR subsets, 50% (13/26) patients achieved pCR(ypT0N0), 53.8% (14/26) achieved TRG 0, 26.9% (7/26) patients achieved TRG 1, and 19.2% (5/26) achieved TRG 2. The positive lymph nodes (pN+) were found in 4 patients, of which 2 patients had metastatic lymph nodes and 2 patients had tumor deposits. The NAR scores were 7.2 ± 10.4.

TABLE 2 Efficacy evaluation.

RECIST evaluation, n (%)	
CR	12 (46.2)
PR	7 (26.9)
SD	7 (26.9)
ORR	19 (73.1)
T category, n (%)	
ypT0	14 (53.8)
ypT1	3 (11.5)
ypT2	2 (7.7)
ypT3	7 (26.9)
N category, n (%)	
ypN0	22 (84.6)
ypN1	3 (11.5)
ypN2	1 (3.8)
TRG, n (%)	
0	14 (53.8)
1	7 (26.9)
2	5 (19.2)
pCR, n (%)	13 (50.0)

Safety

The adverse events that emerged during the neoadjuvant therapy were summarized in Table 3. Most treatment-related adverse events were grade 1-2, with only one grade 3 adverse event occurring. The most common treatment-related AEs were fatigue (53.8%), pruritus (42.3%), and radiation enteritis (38.5%). Immune-related adverse events (irAE) occurred in five (19.2%) patients, including one patient with grade 3 immune checkpoint inhibitor-associated colitis, one patient with grade 1 hyperthyroidism, one patient with grade 1 hypothyroidism, one patient with grade 1 hypopigmentation, and one patient with grade 1 bullous pemphigoid. No grade 4 or 5 adverse event occurred in this study.

Predictive factors analysis for treatment response

The clinical features were examined to analyze the predictive factors for pCR and the results were shown in Table 4. The univariate

TABLE 3 Adverse events.

	Patients (n=26)	
Treatment-related AEs, n (%)	Grade I-II	Grade III
Fatigue	14 (53.8)	0
Pruritus	11 (42.3)	0
Radiation Proctitis	10 (38.5)	0
Nausea	8 (30.8)	0
Leukopenia	8 (30.8)	0
Rash	7 (26.9)	0
Diarrhea	7 (26.9)	0
Anemia	6 (23.1)	0
Abdominal pain	5 (19.2)	0
Neutropenia	4 (15.4)	0
Arthralgia	2 (7.7)	0
Alanine transaminase increased	2 (7.7)	0
Chest pain	1 (3.8)	0
Hyperthyroidism	1 (3.8)	0
Hypothyroidism	1 (3.8)	0
Skin depigmentation	1 (3.8)	0
Bullous pemphigoid	1 (3.8)	0
Immune checkpoint inhibitor-associated colitis	0	1 (3.8)
Immune-related AEs, n (%)		
Immune checkpoint inhibitor-associated colitis	0	1 (3.8)
Hyperthyroidism	1 (3.8)	0
Hypothyroidism	1 (3.8)	0
Skin depigmentation	1 (3.8)	0
Bullous pemphigoid	1 (3.8)	0

TABLE 4 Clinical features of patients with response to the treatment.

	pCR (n=13)	Non-pCR (n=13)	p
Age, years, n (%)			0.030*
<50	4 (30.8)	0 (0)	
≥50	9 (69.2)	13 (100)	
Sex, n (%)			0.431
Male	6 (46.2)	8 (61.5)	
Female	7 (53.9)	5 (38.5)	
CEA level, ng/ml, n (%)			0.004**
<5	12 (92.3)	5 (38.5)	
≥5	1 (7.7)	8 (61.5)	
Differentiation grade			0.095
1	3 (23.1)	0 (0)	
2	9 (69.2)	13 (100)	
3	1 (7.7)	0 (0)	
Clinical T classification, n (%)			0.619
1-2	3 (23.1)	2 (15.4)	
3-4	10 (76.9)	11 (84.6)	
Clinical N classification, n (%)			1
Negative	6 (46.2)	6 (46.2)	
Positive	7 (53.9)	7 (53.9)	
Distance from AV (cm), n (%)			0.431
<5	7 (53.9)	5 (35.5)	
5-10	6 (46.2)	8 (61.5)	
EMVI, n (%)			0.680
Negative	5 (38.5)	4 (30.8)	
Positive	8 (61.5)	9 (69.2)	
MRF, n (%)			0.277
Negative	10 (76.92)	12 (92.3)	
Positive	3 (23.08)	1 (7.7)	
Radiotherapy-surgery interval, weeks, n (%)			0.216
≥7	7 (53.9)	10 (76.9)	
<7	6 (46.2)	3 (23.1)	

*p<0.05, **p<0.01.

analysis suggested that age <50 years, without pre-treatment carcinoembryonic antigen (CEA) elevation, may be beneficial from the treatment regimen. The pCR rate was 100% (4/4) in young onset rectal cancer patients (age<50) and 40.9% (9/22) in other patients (p=0.03). And the pCR rate was only 11.1% (1/9) in patients with elevated CEA and 70.6% (12/17) in patients without CEA elevation (p=0.004). No significant differences were found in other clinical factors.

Discussion

While the ICIs have shown promise in dMMR/MSI-H rectal cancers, they are generally ineffective in pMMR/MSS rectal cancers (11). However, CRT combined with ICIs is considered to have a good synergistic effect. A more immunologically active microenvironment was found after CRT: an increase in CD8+ T-cell infiltration and upregulated PD-L1 expression (14, 15). In this rationale, an addition of ICIs may enhance the anti-tumor effect. The clinical efficacy of chemoradiotherapy combined with immunotherapy has been proven effective in many tumors (25–31), particularly in non-small cell lung cancer, this regimen has rarely been reported as neoadjuvant therapy in rectal cancer. To our knowledge, our study is the first to propose a neoadjuvant therapy of a concurrent long-course CRT and ICIs combination and achieved a high pCR rate of 50% in pMMR LARC patients with no serious adverse events occurring. The pCR rate reached 50%, much higher than the 10%-20% of traditional neoadjuvant therapies (4–6) and also higher than the 25%-46.2% of other studies using ICI combined with CRT (16–20).

This study reported a fairly good tumor regression efficacy. The CR and ORR reached 46.2% and 73.1%, respectively. The improvement of CR rate will be of great significance to the organ preservation of LARC patients after radiotherapy and chemotherapy through “Watch and Waite” policy or selective local excision. In the Maas study, 192 patients treated with traditional chemoradiotherapy, 21 patients (10.9%) achieved clinical complete regression and underwent organ preservation through “Watch and Waite” policy (32). In the ACCORD12/PRODIGE 2 study, 201 LARC patients were evaluated for clinical tumor response after neoadjuvant therapy, and ths score was: complete response: 8%; partial response: 68%; stable: 21%; progression: 3%. The CR rate of CAPOX+radiotherapy group was higher than that of capecitabine+radiotherapy group (9.3% vs 6.7%) (33). Our study reported a similar ORR rate, but a significantly higher CR rate (46.2%). Therefore, it is promising to further study and explore organ preservation after chemoradiotherapy combined with immunotherapy.

Various combination regimens of CRT and ICIs have been reported. In the VOLTAGE-A study, 5 cycles of nivolumab followed by CRT resulted in a 30% pCR rate in pMMR rectal cancer patients (18). It is suggested that the use of ICIs in advance in the course of radiotherapy and chemotherapy may achieve a better synergistic effect. The dose scheduling with concurrent but not sequential therapy was also proved to be effective in tumor regression in preclinical studies (22). The neoadjuvant therapy of adding ICIs to the regimen of short-course radiotherapy combined with CAPOX or FOLFOX also achieved favorable results, WUGO-001 and AVERECTAL studies reported the pCR rate of 48.1% and 37.5% respectively (17, 34). However, the NRG-GI002 study reported a similar pCR rate comparing the concurrent long-course CRT plus pembrolizumab and long-course CRT alone after FOLFOX induction (31.9% versus 29.4%) (16). This suggests that chemotherapy may be more effective as a consolidation regimen rather than an induction regimen.

It is critical to screen the beneficiaries of this neoadjuvant strategy. The VOLTAGE-A study showed that the elevated expression of PD-L1 and CD8/eTreg ratio before treatment were more likely to benefit from the immunotherapy. Among patients with PD-L1 (TPS) ≥ 1%, 75% of patients achieved pCR, while in the PD-L1 (TPS) <1% group, only 17% of patients achieved pCR (18). By analyzing the clinical features, we found CEA was a negative predictor of tumor response. The pCR rate of 11.1% was achieved in patients with CEA elevating compared with 70.6% in those without CEA elevating. This was consistent with previous studies that pre-treatment CEA was inversely correlated with pCR (35, 36). Another predictive factor that we identified was age less than 50 years. These young-onset rectal patients have a promising response to the neoadjuvant treatment with a 100% (4/4) pCR rate. Certain pathological characteristics were reported in colorectal patients less than 50 years, including poor tumor differentiation and low tumor-infiltrating lymphocytes, which were considered to have poor anti-tumor immune response (37). However, this condition may be reversed under the regimen of chemoradiotherapy combined with immunotherapy.

This manuscript reported the interim result of this study. The limitations include the small sample size, single-arm design, and no long-term survival data. Despite this, the result of the high pCR rate was encouraging. We will continue to complete study enrollment and follow-up. Biomarkers will also be analyzed using pre and post-treatment tumor samples. Further large randomized controlled phase III study is worth to

In conclusion, long-course chemoradiotherapy combined with tislelizumab followed by TME surgery showed a favorable pCR rate and well-tolerated toxicities in pMMR rectal cancer patients. Patients with no CEA elevation or young-onset rectal cancer are more likely to benefit from this treatment regimen. Further large-scale randomized controlled studies are required to confirm this result.

Data availability statement

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

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

HY and ZZ designed this study; JG, ZY, XZ, ZB, WD, QW, JH, AL, GL, YS, DK enrolled and managed patients, and collected the data. JZ reviewed the MRI. GC and RX were responsible for pathological assessment. YL provided the administrative support. JG, XZ, ZY drafted the manuscript and all authors reviewed. HY and ZZ had full access to all the data in the study and had final responsibility for the decision to submit for publication. The final version was approved to be submitted by all authors. HY and ZZ are guarantors of the work.

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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. Sauer R, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* (2004) 351(17):1731–40. doi: 10.1056/NEJMoa040694

3. Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery–the clue to pelvic recurrence? *Br J Surg* (1982) 69(10):613–6. doi: 10.1002/bjs.1800691019

4. Smith CA, Kachnic LA. Evolving treatment paradigm in the treatment of locally advanced rectal cancer. *J Natl Compr Canc Netw* (2018) 16(7):909–15. doi: 10.6004/jnccn.2018.7032

5. Bahadoer RR, Dijkstra EA, van Etten B, Marijnen CAM, Putter H, Kranenbarg EM, et al. Short-course radiotherapy followed by chemotherapy before total mesorectal excision (Tme) versus preoperative chemoradiotherapy, tme, and optional adjuvant chemotherapy in locally advanced rectal cancer (Rapido): A randomised, open-label, phase 3 trial. *Lancet Oncol* (2021) 22(1):29–42. doi: 10.1016/s1470-2045(20)30555-6

6. Jin J, Tang Y, Hu C, Jiang LM, Jiang J, Li N, et al. Multicenter, Randomized, phase Iii trial of short-term radiotherapy plus chemotherapy versus long-term chemoradiotherapy in locally advanced rectal cancer (Stellar). *J Clin Oncol* (2022) 40(15):1681–92. doi: 10.1200/jco.21.01667

7. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* (2010) 363(8):711–23. doi: 10.1056/NEJMoa1003466

8. Wang J, Lu S, Yu X, Hu Y, Sun Y, Wang Z, et al. Tislelizumab plus chemotherapy vs chemotherapy alone as first-line treatment for advanced squamous non-Small-Cell lung cancer: A phase 3 randomized clinical trial. *JAMA Oncol* (2021) 7(5):709–17. doi: 10.1001/jamaoncol.2021.0366

9. Shen L, Kato K, Kim SB, Ajani JA, Zhao K, He Z, et al. Tislelizumab versus chemotherapy as second-line treatment for advanced or metastatic esophageal squamous cell carcinoma (Rationale-302): A randomized phase iii study. *J Clin Oncol* (2022) 40 (26):3065–76. doi: 10.1200/jco.21.01926

10. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, et al. Pd-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. *N Engl J Med* (2022) 386(25):2363–76. doi: 10.1056/NEJMoa2201445

11. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in mmr-proficient and mmr-deficient early-stage colon cancers. *Nat Med* (2020) 26(4):566–76. doi: 10.1038/s41591-020-0805-8

12. Zhang X, Yang Z, An Y, Liu Y, Wei Q, Xu F, et al. Clinical benefits of pd-1/Pd-L1 inhibitors in patients with metastatic colorectal cancer: A systematic review and meta-analysis. *World J Surg Oncol* (2022) 20(1):93. doi: 10.1186/s12957-022-02549-7

13. Yang Z, Wu G, Zhang X, Gao J, Meng C, Liu Y, et al. Current progress and future perspectives of neoadjuvant anti-Pd-1/Pd-L1 therapy for colorectal cancer. *Front Immunol* (2022) 13:1001444. doi: 10.3389/fimmu.2022.1001444

14. Demaria S, Golden EB, Formenti SC. Role of local radiation therapy in cancer immunotherapy. *JAMA Oncol* (2015) 1(9):1325–32. doi: 10.1001/jamaoncol.2015.2756

15. 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

16. Rahma OE, Yothers G, Hong TS, Russell MM, You YN, Parker W, et al. Use of total neoadjuvant therapy for locally advanced rectal cancer: Initial results from the pembrolizumab arm of a phase 2 randomized clinical trial. *JAMA Oncol* (2021) 7 (8):1225–30. doi: 10.1001/jamaoncol.2021.1683

17. Lin Z, Cai M, Zhang P, Li G, Liu T, Li X, et al. Phase Ii, single-arm trial of preoperative short-course radiotherapy followed by chemotherapy and camrelizumab in locally advanced rectal cancer. *J Immunotherapy Cancer* (2021) 9(11). doi: 10.1136/jitc-2021-003554

18. Bando H, Tsukada Y, Inamori K, Togashi Y, Koyama S, Kotani D, et al. Preoperative chemoradiotherapy plus nivolumab before surgery in patients with microsatellite stable and microsatellite instability-high locally advanced rectal cancer. *Clin Cancer Res* (2022) 28(6):1136–46. doi: 10.1158/1078-0432.Ccr-21-3213

19. Shamseddine A, Zeidan YH, El Husseini Z, Kreidieh M, Al Darazi M, Turfa R, et al. Efficacy and safety-in analysis of short-course radiation followed by mfolfox-6 plus avelumab for locally advanced rectal adenocarcinoma. *Radiat Oncol* (2020) 15(1):233. doi: 10.1186/s13014-020-01673-6

20. Salvatore L, Bensi M, Corallo S, Bergamo F, Pellegrini I, Rasola C, et al. Phase Ii study of preoperative (Preop) chemoradiotherapy (Ctrt) plus avelumab (Ave) in patients (Pts) with locally advanced rectal cancer (Larc): The avana study. *J Clin Oncol* (2021) 39 (15_suppl):3511–11. doi: 10.1200/JCO.2021.39.15_suppl.3511

21. Buchwald ZS, Wynne J, Nasti TH, Zhu S, Mourad WF, Yan W, et al. Radiation, immune checkpoint blockade and the abscopal effect: A critical review on timing, dose and fractionation. *Front Oncol* (2018) 8:612. doi: 10.3389/fonc.2018.00612

22. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, McKenna C, Jones S, Cheadle EJ, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent pd-L1 blockade. *Cancer Res* (2014) 74(19):5458–68. doi: 10.1158/0008-5472.CAN-14-1258

23. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage iii non-Small-Cell lung cancer. *N Engl J Med* (2017) 377 (20):1919–29. doi: 10.1056/NEJMoa1709937

24. Yang Z, Zhang X, Zhang J, Gao J, Bai Z, Deng W, et al. Rationale and design of a prospective, multicenter, phase ii clinical trial of safety and efficacy evaluation of long course neoadjuvant chemoradiotherapy plus tislelizumab followed by total mesorectal excision for locally advanced rectal cancer (Ncrt-Pd1-Larc trial). *BMC Cancer* (2022) 22 (1):462. doi: 10.1186/s12885-022-09554-9

25. Wu J, Deng R, Ni T, Zhong Q, Tang F, Li Y, et al. Efficacy and safety of Radiotherapy/Chemoradiotherapy combined with immune checkpoint inhibitors for locally advanced stages of esophageal cancer: A systematic review and meta-analysis. *Front Oncol* (2022) 12:887525. doi: 10.3389/fonc.2022.887525

26. Patel P, Alrifai D, McDonald F, Forster M. Beyond chemoradiotherapy: Improving treatment outcomes for patients with stage iii unresectable non-Small-Cell lung cancer through immuno-oncology and durvalumab (Imfinzi, astrazeneca uk limited). *Br J Cancer* (2020) 123(Suppl 1):18–27. doi: 10.1038/s41416-020-01071-5

27. Balasubramanian A, Onggo J, Gunjur A, John T, Parakh S. Immune checkpoint inhibition with chemoradiotherapy in stage iii non-Small-Cell lung cancer: A systematic review and meta-analysis of safety results. *Clin Lung Cancer* (2021) 22(2):74–82. doi: 10.1016/j.cllc.2020.10.023

28. Huang J, Xu J, Chen Y, Zhuang W, Zhang Y, Chen Z, et al. Camrelizumab versus investigator's choice of chemotherapy as second-line therapy for advanced or metastatic oesophageal squamous cell carcinoma (Escort): A multicentre, randomised, open-label, phase 3 study. *Lancet Oncol* (2020) 21(6):832–42. doi: 10.1016/s1470-2045 (20)30110-8

29. McBride S, Sherman E, Tsai CJ, Baxi S, Aghalar J, Eng J, et al. Randomized phase ii trial of nivolumab with stereotactic body radiotherapy versus nivolumab alone in metastatic head and neck squamous cell carcinoma. *J Clin Oncol* (2021) 39(1):30–7. doi: 10.1200/jco.20.00290

30. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The checkmate 143

 10.3389/fonc.2023.1057947

phase 3 randomized clinical trial. *JAMA Oncol* (2020) 6(7):1003–10. doi: 10.1001/jamaoncol.2020.1024

31. Schoenfeld JD, Giobbie-Hurder A, Ranasinghe S, Kao KZ, Lako A, Tsuji J, et al. Durvalumab plus tremelimumab alone or in combination with low-dose or hypofractionated radiotherapy in metastatic non-Small-Cell lung cancer refractory to previous Pd(L)-1 therapy: An open-label, multicentre, randomised, phase 2 trial. *Lancet Oncol* (2022) 23(2):279–91. doi: 10.1016/s1470-2045(21)00658-6

32. Maas M, Beets-Tan RG, Lambregts DM, Lammering G, Nelemans PJ, Engelen SM, et al. Wait-and-See Policy for Clinical Complete Responders after Chemoradiation for Rectal Cancer. *J Clin Oncol* (2011) 29(35):4633–40. doi: 10.1200/jco.2011.37.7176

33. Gérard JP, Chamorey E, Gourgou-Bourgade S, Benezery K, de Laroche G, Mahé MA, et al. Clinical complete response (Ccr) after neoadjuvant chemoradiotherapy and conservative treatment in rectal cancer. findings from the accord 12/Prodige 2 randomized trial. *Radiother Oncol* (2015) 115(2):246–52. doi: 10.1016/j.radonc.2015.04.003

34. Shamseddine A, Zeidan Y, Bouferraa Y, Turfa R, Kattan J, Mukherji D, et al. So-30 efficacy and safety of neoadjuvant short-course radiation followed by Mfolfox-6 plus avelumab for locally-advanced rectal adenocarcinoma: Averectal study. *Ann Oncol* (2021) 32(Suppl 3):215. doi: 10.1016/j.annonc.2021.05.054

35. Lee JH, Hyun JH, Kim DY, Yoo BC, Park JW, Kim SY, et al. The role of fibrinogen as a predictor in preoperative chemoradiation for rectal cancer. *Ann Surg Oncol* (2015) 22 (1):209–15. doi: 10.1245/s10434-014-3962-5

36. Das P, Skibber JM, Rodriguez-Bigas MA, Feig BW, Chang GJ, Wolff RA, et al. Predictors of tumor response and downstaging in patients who receive preoperative chemoradiation for rectal cancer. *Cancer* (2007) 109(9):1750–5. doi: 10.1002/cncr.22625

37. Ugai T, Väyrynen JP, Lau MC, Borowsky J, Akimoto N, Väyrynen SA, et al. Immune cell profiles in the tumor microenvironment of early-onset, intermediate-onset, and later-onset colorectal cancer. *Cancer Immunol Immunother* (2022) 71(4):933–42. doi: 10.1007/s00262-021-03056-6

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A retrospective case-series of influence of chronic hepatitis B on synchronous liver metastasis of colorectal cancer

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Main point: Our retrospective analysis of a large number of cases found in patients with primary colorectal cancer (CRC) carrying positive HBsAg inhibited the occurrence of synchronous liver metastases (SLM). However, liver cirrhosis caused by non-HBV factors promoted the occurrence of SLM.

Objectives: This study aimed to investigate the effect of HBV on the occurrence of synchronous liver metastases (SLM) of colorectal cancer (CRC).

Methods: Univariate and multivariate analyses were used to analyze the influence of clinical parameters on the occurrence of SLM.

Results: A total of 6, 020 patients with primary CRC were included in our study, of which 449 patients carrying HBsAg(+) accounted for 7.46%. 44 cases of SLM occurred in the HBsAg(+) group, accounting for 9.80%, which was much lower than 13.6% (758/5571) in the HBsAg(-) group (X=5.214, P=0.022). Among CRC patients with HBsAg(-), the incidence of SLM was 24.9% and 14.9% in the group with high APRI and FIB-4 levels, respectively, which were significantly higher than that in the compared groups (12.3% and 12.5%, all P<0.05). Compared with the control group, female patients, late-onset patients, and HBV-infective patients had lower risks of SLM (HR=0.737, 95%CI: 0.614-0.883, P<0.001; HR=0.752, 95% CI: 0.603-0.943, P=0.013; HR=0.682, 95%CI: 0.473-0.961, P=0.034).

Conclusions: The carriage of HBsAg(+) status inhibited the occurrence of SLM from CRC. HBV-causing liver cirrhosis did not further influence the occurrence of SLM, whereas non-HBV-factor cirrhosis promoted the occurrence of SLM. Nevertheless, this still required prospective data validation.

KEYWORDS

chronic hepatitis B, synchronous liver metastasis, colorectal cancer, HBV, liver cirrhosis

Introduction

Colorectal cancer (CRC) was one of the most common malignancies of the gastrointestinal tract. CRC ranked the third in incidence and was the second leading cause of cancer-related deaths worldwide (1). CRC was also one of the most prevalent cancers in China, where the mortality rate of CRC was about 13.13/100, 000, accounting for 7.8% of the total number of deaths among patients with malignant tumors (2). Recurrence and distant metastasis were the two main factors affecting the survival of CRC (3). The most common target organ of distant hematogenous metastases of CRC was liver (4). Colonic venous blood converged into the hepatic portal vein through the superior and inferior mesenteric veins, respectively. It was the anatomical structure and portal circulation pathway that made the liver the preferred site for distant metastases. About 15-25% of patients suffered from synchronous liver metastasis (SLM), while another 15-25% developed metachronous liver metastases postoperatively (5). Ultimately, approximately 50% of patients developed liver metastases at some point throughout the course of their disease (6). Patients with untreated liver metastases had a median survival of only 6.9 months (7). Although complete surgical resection was provided, the median survival period was less than 35 months (7). Obviously, CRC liver metastasis was a thorny problem in clinical diagnosis and treatment.

According to WHO, 1.1 million people were newly infected with chronic hepatitis B virus (HBV) in 2017 (7). As of 2016, there were 267 million chronic hepatitis B (CHB) infections worldwide and 1.4 million deaths from viral hepatitis, 96% of which were caused by hepatitis B and C viruses (7, 8). China conducted the first national hepatitis seroepidemiological survey in 1992, according to which approximately 120 million people in China carried HBsAg (+), and nearly 300, 000 died from HBV infection each year (9). CRC patients with comorbid chronic HBV infection were also more common in clinical practice. However, whether CHB promoted or suppressed synchronous liver metastasis was controversial. Some concluded that the incidence of liver metastases was reduced in CRC patients with concomitant CHB infection (10). Obviously, CHB infection had a suppressive effect on liver metastases, but the sample size included was small. Although others thought that CRC with concomitant CHB infection promoted liver metastases (11, 12), the inclusion criteria were controversial.

Thus, in our study, a retrospective analysis of a large sample was conducted to explore the effect of HBV on SLM of CRC, aiming to clarify a clear connection between HBV and SLM in CRC, to provide a basis for further clinical and basic research on CRC liver metastasis.

Patients and methods

Clinical information

A total of 6, 020 consecutive patients with CRC who were admitted to Shanghai Changzheng Hospital from July 2010 to June 2021 were selected. The study was approved by the ethics committee of Shanghai Changzheng hospital. The clinical data collected included age, gender, tumor metastasis, HBV carrier status, blood type, CEA, CA199, AFP, primary tumor location, primary tumor diameter, tissue type, degree of differentiation, and depth of tumor invasion. Locations of the primary tumor were divided into left colon, right colon, and rectum. Diameter of the primary tumor was divided into ≤3 cm and >3 cm according to the size. Tissue types were divided into adenocarcinoma and other types (carcinoid, signet ring cell carcinoma, mucinous adenocarcinoma, etc.). Degrees of differentiation were divided into undifferentiated-poor differentiation and medium-well differentiation. Depths of invasion were divided into T_{1-2} group and T_{3-4} according to the TNM staging standard formulated by AJCC. Each patient selectively underwent X-ray, abdominal B-ultrasound, chest CT, abdomen CT, abdominal MRI, or PET-CT according to the diagnosis and treatment needs.

Inclusion and exclusion criteria

Inclusion criteria

(1) Colonoscopy biopsy or surgical pathology was performed (2). Specific HBV carrier statuses were recorded, such as HBsAg, HBsAg, HBsAg, HBeAb, and HBcAb (3). The diagnosis of distant metastases was issued with clear imaging data support, such as B-ultrasound, CT, MRI, or PET-CT.

Exclusion criteria

(1) Benign colorectal diseases: colorectal polyps, familial polyposis, ulcerative colitis, Crohn's disease. (2) Other diseases of the colorectum: neuroendocrine tumors, lymphoma, intestinal tuberculosis, typhoid fever, intestinal amebiasis, Intestinal schistosomiasis, etc. (3) Serious lack of clinical data: such as age, gender, primary tumor location, SLM information, etc. (4) Combined with other archenteric malignant tumors. (5) Patients who had undergone surgery or radiotherapy and chemotherapy at the time of admission. (6) Patients who had lung metastasis and concomitant metastases of other organs, such as liver metastasis.

Diagnostic criteria

1. CRC: all patients included in the study had a definite diagnosis of CRC. Patients who underwent surgery had a complete postoperative pathology report. Patients with advanced stage or metastases who did not undergo surgery were diagnosed by colonoscopy biopsy. 2. Liver cirrhosis: Aspartate aminotransferase-to-platelet ratio index (APRI) and Fibrosis 4 Score (FIB-4) were used as an indirect indicator for the diagnosis of liver cirrhosis with the cut-off values of 0.5 and 1.45, respectively (13, 14). APRI lower than 0.5 was generally considered to exclude liver cirrhosis, and FIB-4 lower than 1.45 was generally considered to exclude liver cirrhosis (14). 3. Definition of SLM of CRC: according to international consensus (15) and the "Guidelines for the diagnosis and comprehensive treatment of liver metastases of CRC in China (2020)" (16), synchronous liver metastasis referred to liver

metastases found before or at the time of diagnosis of CRC. 4. Imaging diagnosis of SLM: at least 2 or more imaging physicians with associate high title issued the corresponding diagnostic reports. The confirmation of intraoperative liver metastases should be determined by at least 2 experienced surgeons.

Statistical analysis

SPSS 20.0 statistical software was used for statistical analysis. The numerical variables were converted into categorical variables, which were uniformly tested by the chi-square test. Univariate analysis was performed on the factors that might affect SLM, and multivariate Logistic regression analysis was performed on the statistically significant indexes. P<0.05 was statistically significant.

Results

Clinical characteristics

As shown in Table 1, a total of 6, 020 patients with primary CRC were enrolled in this study, 3810 males and 2210 females, with an age range of 14-105 years and a median age of 63.0 years. Among them, there were 449 CRC patients with HBsAg(+), accounting for 7.46%. There were 802 patients with synchronous liver metastasis in all cases, among which 44 patients with HBsAg(+) complicated with synchronous liver metastasis, accounting for 9.80% in the HBsAg (+) group; while 758 patients with HBsAg (–), accounting for 13.6% in the HBsAg (–) group. Compared with the HBsAg (–) group, the proportion of SLM was lower in the HBsAg(+) group. There was a statistical difference between the two groups (P<0.05), which suggested that HBV might inhibit the occurrence of SLM in CRC.

In order to know the published data on the effect of HBV on CRC liver metastasis in the past 20 years, we searched CRC patients in PubMed, Web of Science, and Embase with the keywords HBsAg, HBV, CRC, colon cancer, rectal cancer, and liver metastasis. We searched 13 retrospective analyses, of which 10 articles were published by Chinese scholars, 2 by Italian scholars, and 1 by Japanese scholars. Among them, 4 suggested that HBV promoted CRC liver metastasis, and 9 suggested that HBV inhibited CRC liver metastasis (Table 2). In studies with over 3, 000 CRC patients enrolled, HBV was believed to promote the occurrence of liver metastases. However, the definitions of liver metastases above were controversial and failed to distinguish SLM from metachronous liver metastases (Table 2). Even for SLM, the established criteria were inconsistent.

In addition, we also found that the status of HBsAg in CRC patients was also related to age and AFP. Early-onset CRC patients (age <50 years old) accounted for 27.2% (122/449) in HBsAg(+) group, which was more than 15.0% (834/5571) in HBsAg (–) group. Among the patients with HBsAg(+), elevated AFP levels accounted

TABLE 1 Clinical parameters and characteristics.

Clinical parameters	Enrolled cases N=6020	HBsAg(+) N=449	HBsAg (–) N=5571	χ^2	P value
Gender				3.674	0.055
male	3810	303(67.5%)	3507(63.0%)		
female	2210	146(32.5%)	2064(37.0%)		
Age (years)				46.304	**0.000**
<50	956	122(27.2%)	834(15.0%)		
≥50	5064	327(72.8%)	4737(85.0%)		
Blood type				6.273	0.180
O	1918	159(35.4%)	1759(31.6%)		
A	1919	140(31.2%)	1779(31.9%)		
B	1526	99(22.0%)	1427(25.6%)		
AB	561	47(10.5%)	514 (9.2%)		
missing data	96	4 (0.9%)	92 (1.7%)		
CEA				0.936	0.632
normal	3365	261(58.1%)	3104(55.7%)		
high	2596	184(41.0%)	2412(43.3%)		
missing data	59	4 (0.9%)	55 (1.0%)		

(Continued)

TABLE 1 Continued

Clinical parameters	Enrolled cases N=6020	HBsAg(+) N=449	HBsAg (–) N=5571	χ^2	P value
CA199				2.655	0.265
normal	4813	353(78.6%)	4460(80.1%)		
high	1103	84(18.7%)	1019(18.3%)		
missing data	104	12 (2.7%)	92 (1.7%)		
AFP				10.519	**0.012**
normal	5860	427(95.1%)	5433(97.5%)		
high	13	4 (0.9%)	9 (0.2%)		
missing data	147	18 (4.0%)	129 (2.3%)		
Tumor location				0.773	0.679
right colon	1380	102(22.7%)	1278(22.9%)		
left colon	1520	121(26.9%)	1399(25.1%)		
rectum	3120	226(50.3%)	2894(51.9%)		
Tumor size (cm)				0.942	0.642
≤3	1756	139(31.0%)	1617(29.0%)		
>3	4239	308(68.6%)	3931(70.6%)		
missing data	25	2 (0.4%)	23 (0.4%)		
Pathological type				0.243	0.622
adenocarcinoma	5221	386(86.0%)	4835(86.8%)		
#others	799	63(14.0%)	736(13.2%)		
Differentiation				2.320	0.313
G1-G2	342	31 (6.9%)	311 (5.6%)		
G3-G4	5389	401(89.3%)	4988(89.5%)		
missing data	289	17 (3.8%)	272 (4.9%)		
Invasion depth				1.686	0.430
T1-T2	1715	120(26.7%)	1595(28.6%)		
T3-T4	3929	305(67.9%)	3624(65.1%)		
missing data	376	24 (5.3%)	352 (6.3%)		
SLM				5.214	**0.022**
yes	802	44 (9.8%)	758(13.6%)		
no	5218	405(90.2%)	4813(86.4%)		

other types: carcinoid, signet ring cell carcinoma, mucinous adenocarcinoma, etc. P<0.05 was statistically significant. P-values less than 0.5 are marked in bold.

for 0.9% (4/449), higher than 0.2% (9/5571) in the HBsAg(-) group. We assumed that this was probably because the infection of HBV could cause damage to hepatic cells, leading to the elevation of AFP, which seemed not to contradict the conclusion that HBsAg(+) inhibited SLM in CRC patients. However, HBsAg status was not related to gender, blood type, CEA, CA199, tumor location, tumor size, tissue type, degree of differentiation, and depth of invasion (P>0.05) (Table 1).

TABLE 2 Effects of HBV on CRC liver metastases published during 1999-2022.

Years	Nation	Cases	HBsAg+ratio	Rate of CRLM: HBsAg(+) vs HBsAg(-)	Inhibit or promote	Journal
2019	China (17)	7187	5.12%	13.40% vs. 8.54%	+	Annals of Oncology
2018	China (12)	4033	6.1%	15.57% vs. 8.60%	+	Clinical infectious diseases
2022	China (18)	3914	13.19%	16.95% vs. 13.06%	+	Scientific Report
2022	China (11)	3132	13.2%	16.5% vs. 12.7%	+	Cancer Management and Research
2014	China (19)	1413	–	9.4% vs. 23.9%	–	Hepatogastroenterology
2011	China (10)	1298	2.9%	14.2% vs. 28.2%	–	World journal of gastroenterology
2020	China (20)	884	33.60%	1.68% vs. 5.28%	–	International Journal of Colorectal Disease
2005	Italy (21)	630	9.21%	17.2% vs. 33.1%	–	Minerva chirurgica
2001	China (22)	512	14.45%	13.51% vs. 27.17%	–	American journal of surgery
2013	Italy (23)	488	6.35%	3.2% vs. 9.4%	–	Annali italiani di chirurgia
1999	Japan (24)	438	8.45%	8.11% vs. 21.20%	–	American journal of surgery
2012	China (25)	354	19.77%	2.86% vs. 16.9%	–	Hepatogastroenterology
2018	China (26)	289	12.1%	18.42% vs. 81.58%	–	Journal of Cancer

+: Promote; -: Inhibit. CRLM: CRC liver metastasis.

APRI, FIB-4 promoted SLM in non-HBsAg (+) group

We further explored the effects of e-antigen, liver cirrhosis indicators, and virus carrier status on the occurrence of simultaneous liver metastases. In 449 HBsAg(+) patients, the effects of e-antigen, liver cirrhosis index, and virus carrier status on the occurrence of SLM were analyzed. Different from a previous report (12), we did not find that e-antigen, liver cirrhosis indicators (APRI and FIB-4), and virus replication status [HBsAg/HBeAg/HBcAb(+) and HBsAg/HBeAb/HBcAb(+)] had any effect on the occurrence of simultaneous liver metastases in HBsAg(+) CRC patients ($P>0.05$) (Table 3). Interestingly, in the non-HBsAg+ group, the incidence of SLM in the high APRI and FIB-4 groups was 24.9% and 14.9%, respectively, which was significantly higher than that in the low APRI and FIB-4 groups (12.3% and 12.5%, $P < 0.05$) (Table 4), suggesting that cirrhosis or liver fibrosis may promote the occurrence of SLM in non-HBV-infected CRC.

TABLE 3 Effect of e-antigen, liver cirrhosis, and virus carrier status on CRLM in HBsAg(+) group.

Group	SLM, N (%)	No SLM, N (%)	P value
HBeAg			1.000
+	5(10.9%)	41(89.1%)	
-	39(9.7%)	364(90.3%)	
APRI			0.111
APRI high level	4(5.0%)	76(95.0%)	
APRI low level	40(10.8%)	329(89.2%)	
FIB-4			0.963
FIB-4 high level	22(9.7%)	204(90.3%)	
FIB-4 low level	22(9.9%)	201(90.1%)	
Virus carrier status			0.499
HBsAg/HBeAg/HBcAb(+)	5(11.6%)	38(88.4%)	
HBsAg/HBeAb/HBcAb(+)	21(8.4%)	228(91.6%)	
Unknown	18(11.5%)	139(88.5%)	

CRLM: CRC liver metastasis. P<0.05 was statistically significant.

TABLE 4 Effect of liver cirrhosis index on CRLM in non-HBsAg+ group.

Group	SLM, N (%)	No SLM, N (%)	P value
APRI			<0.001
APRI high level	148(24.9%)	446(75.1%)	
APRI low level	610(12.3%)	4367(87.7%)	
FIB-4			0.004
FIB-4 high level	427(14.9%)	2441(85.1%)	
FIB-4 low level	331(12.2%)	2372(87.8%)	

CRLM: CRC liver metastasis. P<0.05 was statistically significant.

Univariate and multivariate analysis on SLM in CRC

Univariate analysis showed that gender, age, CEA, CA199, tumor location, tumor size, tissue type, degree of differentiation, depth of infiltration, and HBsAg status were factors influencing the occurrence of CRC SLM (P<0.05). Further, we found that gender, age, CEA, CA199, tumor size, tissue type, degree of differentiation, depth of invasion and HBsAg status were independent factors affecting the occurrence of SLM in CRC (P<0.05) (Supplementary Table 1).

Excluding groups with incomplete data on clinical parameters (CEA, CA199, tumor size, tissue type, degree of differentiation, and depth of infiltration), gender, age, and HBsAg status were independent factors influencing the occurrence of SLM (P< 0.05), while tumor location was not an independent factor (P>0.05). Compared with the control group, female patients had a lower risk of developing CRC synchronous liver metastasis (HR=0.737, 95%CI: 0.614—0.883, P<0.001). Similar results have been observed in late-onset CRC patients (HR=0.752, 95%CI:0.603—0.943, P=0.013) and CRC patients with HBsAg(+) (HR=0.682, 95% CI:0.473—0.961, P=00.034) (Supplementary Table 5).

Effect of HBV on SLM in the early-onset CRC group

The above results suggested that the proportion of HBsAg(+) in early-onset CRC patients was higher, suggesting that early-onset CRC might be a suppressive factor for SLM.

Therefore, in order to further explore whether the low incidence of SLM in early-onset CRC was related to HBV infection, we investigated the effect of HBsAg status on SLM. As seen in Supplementary Table 2, in the early-onset CRC group, HBsAg status was not associated with the occurrence of SLM (P=0.108). Apparently, the occurrence of SLM in early-onset CRC was more closely related to exposure factors, dietary habits, body immune status, gene expression, and mutation correlation.

Similarly, Supplementary Table 3 showed that in the early-onset CRC with HBsAg+ group, e-antigen, liver cirrhosis indicators, and virus carrier status were not associated with the occurrence of SLM.

Effect of HBV on SLM in colon cancer

Although we found that after dividing the CRC into the left half, right half, and rectum according to the tumor location, the tumor part was not an independent factor affecting the occurrence of synchronous liver metastasis. However, after dividing CRC into colon and rectum, the rate of concurrent liver metastases from colon cancer was 15.2% (442/2458), which was higher than that in rectal cancer (10.11%, 360/3562), also being an independent factor influencing the occurrence of SLM (P<0.5), consistent with the data reported in the literature (16). Therefore, we further explored the effect of HBV on synchronous liver metastasis in colon cancer. In Supplementary Tables 4, 5, we found that HBsAg status, e-antigen, APRI, and FIB-4 were unrelated to the occurrence of SLM (P>0.05). We speculated that the higher incidence of SLM in the colon might be more attributed to anatomical superior and inferior mesenteric venous reflux to the portal system, while the rectal portion returned to the inferior vena cava (body circulation).

Effect of HBV on synchronous extrahepatic (lung) metastases

The effect of HBV on extrahepatic metastasis, especially lung metastasis, remained unclear. Our study found that the rates of synchronous lung metastases in HBsAg(+) and HBsAg(-) were 1.7% and 1.53%, respectively. There was no statistical difference between them (P=0.576) (Table 5). This suggested that the occurrence of synchronous lung metastases was not related to the status of HBV infection, but more probably associated with systemic blood circulation and lung microenvironment.

Discussion

Recurrence and metastasis were the leading causes of death in CRC patients (27). The liver was the most common metastatic organ of CRC (28). Resection of liver metastases was the preferred method for the treatment of CRC with liver metastases (29). However, approximately 75% of patients relapsed within 2 years (30). Due to a large number of HBV infective patients and CRC patients worldwide, so what was the relationship between HBV

TABLE 5 Influence of HBsAg status on lung metastasis.

Parameters	Synchronous lung metastases, N (%)	No synchronous lung metastases, N (%)	Total	χ^2	P value
HBsAg				0.313	0.576
+	6(1.7%)	443(98.3%)	449		
-	94(1.53%)	5477(98.47%)	5571		
Total	100(1.7%)	5920(98.3%)	6020		

P<0.05 was statistically significant.

infection and CRLM? Before discussing the relationship between HBV and CRLM, we first defined the definition of synchronous CRLM. The Expert Group on the Treatment of Liver Metastases discussed this issue and reached a consensus (31) that SLM were referred to as simultaneously discovered liver metastases detected at the time of primary CRC tumor diagnosis. Although the classification of SLM had reached an international consensus, actually the standards in researches were not uniform. Some argued that SLM were liver metastases found at the time of CRC diagnosis or within 6 months after radical resection of the primary CRC (15). Nevertheless, if liver metastases happened within 6 months after surgery, it meant that metastases had already occurred before surgery. In the early stages of metastasis, minimal residual diseases were undetectable. Because CT only could distinguish lesions larger than 0.5 cm, while B-ultrasound only larger than 1 cm (32). In addition, the reports on the incidence of synchronous and metachronous liver metastases were controversial due to the limited sample size (21, 23). Here, we selected SLM according to the international consensus (31) that liver metastases found before or at the time of diagnosis of CRC, which could allow us to judge the occurrence of SLM more accurately.

The controversy was still ongoing regarding the impact of HBV infection on the risk of CRC liver metastases. Most studies thought that HBV infection inhibited the occurrence of CRC liver metastases (26, 33); at the same time, other few studies held an opposite view (11, 17). A retrospective study by Huo et al. (12) collected 4,033 CRC patients to conclude that concomitant chronic HBV infection significantly increased the risk of CRC liver metastases with a higher hazard risk (2.317), compared with CRC patients not infected with HBV. However, the mechanism of HBV infection promoting CRC liver metastasis was unclear. Chemokines in tumor microenvironment promoted malignant tumor metastasis through multiple mechanisms (34). CRC cells recruited specific subsets of myeloid cells to facilitate cancer cell growth in the liver through the chemokine CCL2 (35). They were combined with monocyte chemoattractant protein (MCP-1) to form MCP-1/CCR2, which promoted the growth of CRC in the animal. Once HBV infection occurred, the expression level of MCP-1 was up-regulated (36). This might hint that HBV infection facilitated liver metastasis of CRC. Also, the expressions of chemokines CCL20, CXCL6, and CXCL9/10/11 increased in HBV-infected patients, which were all related to the occurrence of CRC (36–38).

We found that HBV may inhibit SLM in CRC. We enrolled 6, 020 cases, of which 802 patients developed SLM. There were 44 cases with simultaneous liver metastasis in the HBV infective group. Compared with the HBsAg (–) group, the proportion of SLM in the HBsAg(+) group was lower (9.80% vs. 13.6%). Utsunomiya et al. (24) found that liver metastases were rare in HBV or HCV-infected CRC patients. Song et al. (22) reported that HBV-infected patients had fewer CRC liver metastases and more prolonged survival than non-HBV-infective patients. Another research showed that HBV infection and liver cirrhosis could reduce the incidence of liver metastases in CRC patients, but did not affect their survival rate. Wang et al. (25) and Qiu et al. (10) also came to a similar conclusion that HBV inhibited the SLM of CRC.

The mechanism by which HBV infection inhibited CRC liver metastasis was also still unclear. Some studies held that HBV enhanced the host's cellular and humoral immune function after HBV entered the body. HBV replication not only enhanced the killing of cytotoxic T lymphocytes (CTL) and Kupffer cells to wipe out cancer cells, but activated cytokines such as TNF-α and INF-γ to boost the antitumor effects (10, 39). HBV infection promoted the production of cytokines such as INF-γ and IL-6 by activating Kupffer cells, CTLs, and monocytes, while INF-γ inhibited the formation of neovascularization in cancer metastases. IL-6 indirectly increased liver ECM, thereby inhibiting CRC cell metastasis or making it difficult for CRC cells to transfer to the liver for growth and proliferation (40). During the progression from CHB to cirrhosis, Kupffer cell activation led to tissue damage and even liver fibrosis, and inhibited CRC liver metastasis. Other studies reported that microRNAs silenced target genes through mRNA degradation or translation inhibition to inhibit the occurrence of liver metastasis, such as miRNA-145, Let-7, etc (41, 42). Also, tumor liver metastases were intrinsic to tumor cells and influenced by the local metastatic tumor microenvironment (43). The imbalance between matrix metalloproteinases (MMPs) and their inhibitors contributed to CRC progression and invasion (44). MMP inhibitors used to treat CRC in animal models suggested that increased expression of MMPs inhibited the colonization of chronic hepatitis-infected tumor cells and hindered colon cancer liver metastasis (45). Another possible explanation we thought was that CRC secreted CEA that could specifically bind to the CEA receptor on liver Kupffer cells so that Kupffer cells produced IL-α, IL-1β, IL-6, and TNF-α, inducing liver Sinusoidal endothelial cells to express intercellular adhesion molecules. Next, metastatic cancer cells adhered to the liver sinusoidal endothelial cells, so as not to enter the liver.

Liver cirrhosis was a common clinical chronic progressive liver disease, diffuse liver damage formed by long-term or repeated action of one or more causes (46–49). In China, most of them were post-hepatitis cirrhosis, while a few were alcoholic cirrhosis and schistosomiasis (12, 50, 51). What was the relationship between post-hepatitis cirrhosis and CRC liver metastasis? Huo et al. (12) used APRI as an evaluation index for the severity of liver cirrhosis and found that in CRC patients with positive HBsAg, patients with high APRI (>0.5) had a lower probability of developing SLM than patients with low APRI (≤0.5). Liver metastases from CRC were rarely shown in patients with liver cirrhosis, a retrospective study in the United States showed (52). However, a study in Taiwan put forward the opposite view, arguing that the risk of liver metastases in CRC patients with liver cirrhosis was underestimated, presenting that the risk of liver metastases in CRC patients with liver cirrhosis was higher (53). Nevertheless, in 449 cases of HBsAg(+) CRC patients in our study, we did not find that e-antigen and liver cirrhosis indicators (APRI, FIB-4) had any effect on the occurrence of SLM. This suggested that HBV-induced liver cirrhosis did not further affect the occurrence of SLM. There might be the following reasons we thought for the above results: 1. A better indicator of HBV replication was the level of DNA replication. 2. APRI and FIB-4 could not accurately reflect the actual degree of liver cirrhosis or liver fibrosis. 3. The information on whether patients took hepatoprotective or antiviral drugs or not was missing. But interestingly, we found that in HBsAg (–) CRC patients, the incidences of SLM in the high APRI and FIB-4 groups were 24.9% and 14.9%, respectively, which were significantly higher than those in the low APRI and FIB-4 groups (12.3% and 12.5%), suggesting that non-HBV factors in liver cirrhosis promoted the occurrence of SLM from CRC. The possible underlying mechanism was the effect of mechanical factors, such as mesenteric circulation and hepatic capillaries, which promoted liver metastasis (54). Patients with liver cirrhosis had intestinal epithelial barrier dysfunction compared with healthy subjects (55–57). In addition, vascular remodeling and tortuosity led to direct shunting of portal and arterial blood supply to the hepatic outflow tract, and eventual vessel tortuosity and slow blood flow further facilitated cancer cell seeding (57, 58). The new finding opened up new ideas for us to further study the pathogenetic mechanism of synchronous liver metastasis of CRC, but it still needed prospective data verification.

This study had the following deficiencies and limitations: 1. Status of HBV carriers. It would be more convincing to clarify the role of HBV-DNA status in tumor pathogenesis. 2. HBV treatment and outcome. Antiviral therapy duration, regimen, and outcomes also affected final clinical outcomes. Besides, many retrospective studies have not been able to investigate whether the tumor occurred or HBV infection first. 3. Different definitions of liver metastases and defects in detection methods. Different definitions of liver metastases in CRC would inevitably lead to bias in the analysis of results. Also, the resolutions and models of imaging equipment in different hospitals or different periods of the same hospital were quite different, resulting in diagnostic defects and final research bias.

In conclusion, despite the controversies shown in the review of literature, the retrospective analysis of a large number of cases in our study found that in patients with primary CRC, carrying positive HBsAg might inhibit the occurrence of SLM. As for early-onset CRC patients, it seemed that HBsAg status was not associated with the occurrence of SLM. The rate of concurrent liver metastases from colon cancer was higher than that in rectal cancer. However, HBsAg status seemed unrelated to the occurrence of SLM in colon cancer. Besides, HBV-induced liver cirrhosis appeared not to further affect the occurrence of SLM while liver cirrhosis caused by non-HBV factors promoted the occurrence of SLM. Meanwhile, it seemed that HBsAg status had no effects on the incidences of lung metastasis in CRC. These findings still required prospective data validation.

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 human participants were reviewed and approved by the ethics committee of Shanghai Changzheng hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LZ, PG, and YL contributed equally to this work and share first authorship. All authors contributed to the article and approved the submitted version.

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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

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References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin* (2020) 70(3):145–64. doi: 10.3322/caac.21601

2. Liu S, Zheng R, Zhang M, Zhang S, Sun X, Chen W. Incidence and mortality of colorectal cancer in China, 2011. *Chin J Cancer Res* (2015) 27(1):22–8. doi: 10.3978/j.issn.1000-9604.2015.02.01

3. Carpizo DR, D'Angelica M. Liver resection for metastatic colorectal cancer in the presence of extrahepatic disease. *Lancet Oncol* (2009) 10(8):801–9. doi: 10.1016/S1470-2045(09)70081-6

4. 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

5. Qin S, Liu GJ, Huang M, Huang J, Luo Y, Wen Y, et al. The local efficacy and influencing factors of ultrasound-guided percutaneous microwave ablation in colorectal liver metastases: A review of a 4-year experience at a single center. *Int J Hyperthermia* (2019) 36(1):36–43. doi: 10.1080/02656736.2018.1528511

6. Carpizo DR, Are C, Jarnagin W, Dematteo R, Fong Y, Gonen M, et al. Liver resection for metastatic colorectal cancer in patients with concurrent extrahepatic disease: Results in 127 patients treated at a single center. *Ann Surg Oncol* (2009) 16 (8):2138–46. doi: 10.1245/s10434-009-0521-6

7. Stewart CL, Warner S, Ito K, Raoof M, Wu GX, Kessler J, et al. Cytoreduction for colorectal metastases: Liver, lung, peritoneum, lymph nodes, bone, brain. when does it palliate, prolong survival, and potentially cure? *Curr Probl Surg* (2018) 55(9):330–79. doi: 10.1067/j.cpsurg.2018.08.004

8. Margonis GA, Sergentanis TN, Ntanasis-Stathopoulos I, Andreatos N, Tzanninis IG, Sasaki K, et al. Impact of surgical margin width on recurrence and overall survival following R0 hepatic resection of colorectal metastases: A systematic review and meta-analysis. *Ann Surg* (2018) 267(6):1047–55. doi: 10.1097/SLA.0000000000002552

9. Weng JJ, Wei JZ, Li M, Lu JL, Qin YD, Jiang H, et al. Effects of hepatitis b virus infection and antiviral therapy on the clinical prognosis of nasopharyngeal carcinoma. *Cancer Med* (2020) 9(2):541–51. doi: 10.1002/cam4.2715

10. Qiu HB, Zhang LY, Zeng ZL, Wang ZQ, Luo HY, Keshari RP, et al. Hbv infection decreases risk of liver metastasis in patients with colorectal cancer: A cohort study. *World J Gastroenterol* (2011) 17(6):804–8. doi: 10.3748/wjg.v17.i6.804

11. Zhou J, Guo X, Huang P, Tan S, Lin R, Zhan H, et al. Hbv infection status indicates different risks of synchronous and metachronous liver metastasis in colorectal cancer: A retrospective study of 3132 patients with a 5-year follow-up. *Cancer Manag Res* (2022) 14:1581–94. doi: 10.2147/CMAR.S350276

12. Huo T, Cao J, Tian Y, Shi X, Wu L, Zhang M, et al. Effect of concomitant positive hepatitis b surface antigen on the risk of liver metastasis: A retrospective clinical study of 4033 consecutive cases of newly diagnosed colorectal cancer. *Clin Infect Dis* (2018) 66(12):1948–52. doi: 10.1093/cid/cix1118

13. Takase B, Goto T, Hamabe A, Uehata A, Kuroda K, Satomura K, et al. Flow-mediated dilation in brachial artery in the second half of pregnancy and prediction of pre-eclampsia. *J Hum Hypertens* (2003) 17(10):697–704. doi: 10.1038/sj.jhh.1001599

14. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. Fib-4: An inexpensive and accurate marker of fibrosis in hcv infection. *Comparison Liver Biopsy Fibrotest. Hepatol* (2007) 46(1):32–6. doi: 10.1002/hep.21669

15. Siriwardena AK, Mason JM, Mullamitha S, Hancock HC, Jegatheeswaran S. Management of colorectal cancer presenting with synchronous liver metastases. *Nat Rev Clin Oncol* (2014) 11(8):446–59. doi: 10.1038/nrclinonc.2014.90

16. Chinese College of S, Section of Gastrointestinal Surgery BoSCMA, Section of Colorectal Surgery BoSCMA, Section of Colorectal Oncology OBCMA, Colorectal Cancer Professional Committee CA-CA and Colorectal Cancer Professional Committee CMDA, et al. [China guideline for diagnosis and comprehensive treatment of colorectal liver metastases (Version 2020)]. *Zhonghua Wei Chang Wai Ke Za Zhi* (2021) 24(1):1–13. doi: 10.3760/cma.j.cn.441530-20201225-00680

17. Zhao L, Song L, Cao J, Yang Y. Active chronic hepatitis b increases the risk of liver metastasis of colorectal cancer: A retrospective clinical study of 7187 consecutive cases of newly diagnosed colorectal cancer. *Ann Oncol* (2019) 30(Supplement 5):v212. doi: 10.1093/annonc/mdz246.039

18. Zhou J, Huang P, Guo X, Tan S, Lin R, Zhan H, et al. Hbv infection statuses indicate different risks of synchronous and metastasis liver metastasis in colorectal cancer. (2021). doi: 10.21203/rs.3.rs-285576/v1. Scientific Reports; Preprint (Version 1) Available at Research Square.

19. Qian HG, Hao CY. Hepatitis b virus infection is an independent factor influencing the occurrence of liver metastasis in colorectal cancer: A retrospective analysis of 1413 cases. *Hepatogastroenterology* (2014) 61(135):1908–14.

20. Jiaming Z, Pinzhu H, Xiaoyan G, Shuyun T, Rongwan L, Huanmiao Z, et al. Hbv infection may reduce the risk of metachronous liver metastasis in postoperative pathological stage 2 colorectal cancer. *Int J Colorectal Dis* (2020) 35(12):2205–17. doi: 10.1007/s00384-020-03712-w

21. Iascone C, Ruperto M, Barillari P. [Colorectal carcinoma metastasis in livers infected with hepatitis b or c virus]. *Minerva Chir* (2005) 60(2):77–81.

22. Song E, Chen J, Ou Q, Su F. Rare occurrence of metastatic colorectal cancers in livers with replicative hepatitis b infection. *Am J Surg* (2001) 181(6):529–33. doi: 10.1016/s0002-9610(01)00634-1

23. Li Destri G, Castaing M, Ferlito F, Minutolo V, Di Cataldo A, Puleo S. Rare hepatic metastases of colorectal cancer in livers with symptomatic hbv and hcv hepatitis. *Ann Ital Chir* (2013) 84(3):323–7.

24. Utsunomiya T, Saitsu H, Saku M, Yoshida K, Matsumata T, Shimada M, et al. Rare occurrence of colorectal cancer metastasis in livers infected with hepatitis b or c virus. *Am J Surg* (1999) 177(4):279–81. doi: 10.1016/s0002-9610(99)00045-8

25. Wang FS, Shao ZG, Zhang JL, Liu YF. Colorectal liver metastases rarely occur in patients with chronic hepatitis virus infection. *Hepatogastroenterology* (2012) 59 (117):1390–2. doi: 10.5754/hge11747

26. Zhao Y, Lin J, Peng J, Deng Y, Zhao R, Sui Q, et al. Hepatitis b virus infection predicts better survival in patients with colorectal liver-only metastases undergoing liver resection. *J Cancer* (2018) 9(9):1560–7. doi: 10.7150/jca.24544

27. Steeg PS. Targeting metastasis. *Nat Rev Cancer* (2016) 16(4):201–18. doi: 10.1038/nrc.2016.25

28. Chen HN, Shu Y, Liao F, Liao X, Zhang H, Qin Y, et al. Genomic evolution and diverse models of systemic metastases in colorectal cancer. *Gut* (2022) 71(2):322–32. doi: 10.1136/gutjnl-2020-323703

29. Hadden WJ, de Reuver PR, Brown K, Mittal A, Samra JS, Hugh TJ. Resection of colorectal liver metastases and extra-hepatic disease: A systematic review and proportional meta-analysis of survival outcomes. *HPB (Oxford)* (2016) 18(3):209–20. doi: 10.1016/j.hpb.2015.12.004

30. Jones RP, Jackson R, Dunne DF, Malik HZ, Fenwick SW, Poston GJ, et al. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. *Br J Surg* (2012) 99(4):477–86. doi: 10.1002/bjs.8667

31. Adam R, de Gramont A, Figueras J, Kokudo N, Kunstlinger F, Loyer E, et al. Managing synchronous liver metastases from colorectal cancer: A multidisciplinary international consensus. *Cancer Treat Rev* (2015) 41(9):729–41. doi: 10.1016/j.ctrv.2015.06.006

32. Wang JP. [Chinese standard for the diagnosis and treatment of colorectal cancer (2010)]. *Zhonghua Wei Chang Wai Ke Za Zhi* (2011) 14(1):1–4.

33. Au KP, Chok KSH, Chan ACY, Dai WC, Cheung TT, Lo CM. Impact of hepatitis b carrier status on the outcomes of surgical treatment of colorectal liver metastases. *World J Surg* (2018) 42(8):2642–50. doi: 10.1007/s00268-018-4483-3

34. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol* (2017) 17(9):559–72. doi: 10.1038/nri.2017.49

35. Zhao L, Lim SY, Gordon-Weeks AN, Tapmeier TT, Im JH, Cao Y, et al. Recruitment of a myeloid cell subset (Cd11b/Gr1 mid) *Via* Ccl2/Ccr2 promotes the development of colorectal cancer liver metastasis. *Hepatology* (2013) 57(2):829–39. doi: 10.1002/hep.26094

36. Iwata T, Tanaka K, Inoue Y, Toiyama Y, Hiro J, Fujikawa H, et al. Macrophage inflammatory protein-3 alpha (Mip-3a) is a novel serum prognostic marker in patients with colorectal cancer. *J Surg Oncol* (2013) 107(2):160–6. doi: 10.1002/jso.23247

37. Halama N, Zoernig I, Berthel A, Kahlert C, Klupp F, Suarez-Carmona M, et al. Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-Ccr5 therapy in cancer patients. *Cancer Cell* (2016) 29(4):587–601. doi: 10.1016/j.ccell.2016.03.005

38. Toiyama Y, Fujikawa H, Kawamura M, Matsushita K, Saigusa S, Tanaka K, et al. Evaluation of Cxcl10 as a novel serum marker for predicting liver metastasis and prognosis in colorectal cancer. *Int J Oncol* (2012) 40(2):560–6. doi: 10.3892/ijo.2011.1247

39. Lara-Pezzi E, Majano PL, Gomez-Gonzalo M, Garcia-Monzon C, Moreno-Otero R, Levrero M, et al. The hepatitis b virus X protein up-regulates tumor necrosis factor alpha gene expression in hepatocytes. *Hepatology* (1998) 28(4):1013–21. doi: 10.1002/hep.510280416

40. Naito M, Hasegawa G, Ebe Y, Yamamoto T. Differentiation and function of kupffer cells. *Med Electron Microsc* (2004) 37(1):16–28. doi: 10.1007/s00795-003-0228-x

41. Akao Y, Nakagawa Y, Naoe T. Let-7 microrna functions as a potential growth suppressor in human colon cancer cells. *Biol Pharm Bull* (2006) 29(5):903–6. doi: 10.1248/bpb.29.903

42. Sachdeva M, Mo YY. Microrna-145 suppresses cell invasion and metastasis by directly targeting mucin 1. *Cancer Res* (2010) 70(1):378–87. doi: 10.1158/0008-5472.CAN-09-2021

43. Mueller MM, Fusenig NE. Friends or foes - bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* (2004) 4(11):839–49. doi: 10.1038/nrc1477

44. Islekel H, Oktay G, Terzi C, Canda AE, Fuzun M, Kupelioglu A. Matrix metalloproteinase-9,-3 and tissue inhibitor of matrix metalloproteinase-1 in colorectal cancer: Relationship to clinicopathological variables. *Cell Biochem Funct* (2007) 25(4):433–41. doi: 10.1002/cbf.1325

45. Shalinsky DR, Brekken J, Zou H, McDermott CD, Forsyth P, Edwards D, et al. Broad antitumor and antiangiogenic activities of Ag3340, a potent and selective mmp inhibitor undergoing advanced oncology clinical trials. *Ann N Y Acad Sci* (1999) 878:236–70. doi: 10.1111/j.1749-6632.1999.tb07689.x

46. Bernsmeier C, van der Merwe S, Perianin A. Innate immune cells in cirrhosis. *J Hepatol* (2020) 73(1):186–201. doi: 10.1016/j.jhep.2020.03.027

47. Zermatten MG, Fraga M, Moradpour D, Bertaggia Calderara D, Aliotta A, Stirnimann G, et al. Hemostatic alterations in patients with cirrhosis: From primary hemostasis to fibrinolysis. *Hepatology* (2020) 71(6):2135–48. doi: 10.1002/hep.31201

48. Loomba R, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. *Gut* (2020) 69(7):1343–52. doi: 10.1136/gutjnl-2018-317593

49. O'Leary JG, Greenberg CS, Patton HM, Caldwell SH. Aga clinical practice update: Coagulation in cirrhosis. *Gastroenterology* (2019) 157(1):34–43 e1. doi: 10.1053/j.gastro.2019.03.070

50. Xiao J, Wang F, Wong NK, He J, Zhang R, Sun R, et al. Global liver disease burdens and research trends: Analysis from a Chinese perspective. *J Hepatol* (2019) 71 (1):212–21. doi: 10.1016/j.jhep.2019.03.004

51. Zhang L, Schuppan D. Traditional Chinese medicine (Tcm) for fibrotic liver disease: Hope and hype. *J Hepatol* (2014) 61(1):166–8. doi: 10.1016/j.jhep.2014.03.009

52. Gervaz P, Pak-art R, Nivatvongs S, Wolff BG, Larson D, Ringel S. Colorectal adenocarcinoma in cirrhotic patients. *J Am Coll Surg* (2003) 196(6):874–9. doi: 10.1016/S1072-7515(03)00117-0

53. Chiou WY, Chang CM, Tseng KC, Hung SK, Lin HY, Chen YC, et al. Effect of liver cirrhosis on metastasis in colorectal cancer patients: A nationwide population-based cohort study. *Jpn J Clin Oncol* (2015) 45(2):160–8. doi: 10.1093/jjco/hyu178

54. Lalor PF, Lai WK, Curbishley SM, Shetty S, Adams DH. Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions in vivo. *World J Gastroenterol* (2006) 12(34):5429–39. doi: 10.3748/wjg.v12.i34.5429

55. Benjamin J, Singla V, Arora I, Sood S, Joshi YK. Intestinal permeability and complications in liver cirrhosis: A prospective cohort study. *Hepatol Res* (2013) 43 (2):200–7. doi: 10.1111/j.1872-034X.2012.01054.x

56. Zuckerman MJ, Menzies IS, Ho H, Gregory GG, Casner NA, Crane RS, et al. Assessment of intestinal permeability and absorption in cirrhotic patients with ascites using combined sugar probes. *Dig Dis Sci* (2004) 49(4):621–6. doi: 10.1023/b:ddas.0000026307.56909.21

57. Pijls KE, Jonkers DM, Elamin EE, Masclee AA, Koek GH. Intestinal epithelial barrier function in liver cirrhosis: An extensive review of the literature. *Liver Int* (2013) 33(10):1457–69. doi: 10.1111/liv.12271

58. Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet* (2008) 371(9615):838–51. doi: 10.1016/S0140-6736(08)60383-9

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Comparison of neoadjuvant immunotherapy versus routine neoadjuvant therapy for patients with locally advanced esophageal cancer: A systematic review and meta-analysis

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Background: The neoadjuvant use of immune checkpoint inhibitor combined with chemotherapy (nICT) or chemoradiotherapy (nICRT) in locally advanced esophageal cancer (EC) is currently an area of active ongoing research. Therefore, we carried out a comprehensive meta-analysis to compare the efficacy and safety of the new strategy with routine neoadjuvant strategy, which included neoadjuvant chemotherapy (nCT) and neoadjuvant chemoradiotherapy (nCRT).

Patients and methods: MEDLINE (via PubMed), Embase (via OVID), ISI Web of Science database and Cochrane Library were included. And, all of them were searched for eligible studies between January, 2000 and February, 2023. The pathological complete response (pCR) and major pathological response (MPR) were primary outcome of our study. The second outcome of interest was R0 resection rate. Odds ratio (OR) and associated 95% CI were used as the effect indicators comparing the safety and efficiency of the neoadjuvant immunotherapy with the routine neoadjuvant therapy. Fixed-effect model (Inverse Variance) or random-effect model (Mantel-Haenszel method) was performed depending on the statistically heterogeneity.

Results: There were eight trials with 652 patients were included in our meta-analysis. The estimated pCR rate was higher in the neoadjuvant immunotherapy group (OR =1.86; 95% CI, 1.25–2.75; I^2 = 32.8%, P=0.166). The different results were found in the esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) subgroups, the estimated OR was 2.35 (95%CI, 1.00–2.72; I^2 = 30.9%, P=0.215) in the EAC subgroup, and 2.35 (95% CI, 1.20–4.54; I^2 = 45.3%, P=0.161) in the ESCC subgroup, respectively. The neoadjuvant immunotherapy also showed the advantage in the MPR rates (OR =2.66; 95% CI, 1.69–4.19; I^2 = 24.3%, P=0.252). There was no obvious difference between the neoadjuvant immunotherapy and routine neoadjuvant therapy with respect to surgical resection rate, R0 resection rate, surgical delay rate; while more treatment-related adverse events were observed for the neoadjuvant immunotherapy for pneumonitis/pneumonia (OR=3.46, 95% CI, 1.31–9.16; I^2 = 67.3%, *P*=0.005) and thyroid dysfunction (OR=4.69, 95% CI, 1.53–14.36; I^2 = 56.5%, *P*=0.032).

Conclusion: The pooled correlations indicated that the neoadjuvant immunotherapy (both nICT and nICRT) could significantly increase the rates of pCR and MPR, compared with routine neoadjuvant therapy (both nCT and nCRT) in the treatment of locally advanced EC. The neoadjuvant immunotherapy and routine neoadjuvant therapy were with acceptable toxicity. However, randomized studies with larger groups of patients need to performed to confirm these results.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42020155802.

KEYWORDS

esophageal cancer, neoadjuvant, immune checkpoint inhibitor, chemotherapy, chemoradiotherapy, pathological complete response, meta-analysis

Introduction

Esophageal cancer is one of the deadliest cancers. As the eighth most commonly diagnosed cancer worldwide, there were 544,000 cancer-related deaths of EC in 2020, ranked sixth of cancer-related mortality (1). According to the latest data of China National Cancer Center, esophageal cancer ranked the sixth and the mortality ranked the fourth. EC includes two main histological subtypes, EAC and ESCC. The ESCC accounts for about 90% of esophageal cancer patients. As an aggressive cancer, the five-year survival rate of ESCC was just 35–45%, and the EAC was even lower.

Surgery remains the mainstay for ESCC or EAC, but surgery alone did not show satisfactory clinical data. Some studies showed that neoadjuvant therapy was the most effective strategy in improving survival of resectable esophageal cancer (2, 3). At present, the neoadjuvant therapy is widely applied to improve long-term survival rate in clinical trials. There were two randomized controlled trials (RCTs) demonstrated the neoadjuvant CRT (nCRT) was an effective and safe therapy strategy for locally advanced EC, NEOCRTEC5010 (nCRT for ESCC) and CROSS (nCRT for EC) (4, 5). In addition, the neoadjuvant chemotherapy (nCT) was another standard treatment for locally advanced ESCC patients, especially in Japan (6). However, the 5-year overall survival rate of nCRT or nCT was only 47%, and 3-year disease free survival was about 49%.

Immune checkpoint inhibitors (ICIs) combined with chemotherapy, as first line, obviously improved survival data of patients with advanced/metastatic esophageal cancer (7–11). The efficacy of neoadjuvant ICIs combined with nCT has been previously reported in esophageal cancer (12, 13). Recent meta-analyses have demonstrated the neoadjuvant ICIs combined with nCT or nCRT had promising clinical result and acceptable safety outcomes for patients with locally advanced EC (14–17). Nevertheless, there was no any meta-analyses comparing neoadjuvant ICIs combined with nCT or nCRT with routine neoadjuvant therapy, which included nCRT and nCT.

We summarized the recent studies and carried out this systematic review and meta-analysis to compare the efficacy and safety of the neoadjuvant immunotherapy with the routine neoadjuvant therapy followed by esophagectomy for patients with locally advanced EC.

Methods

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) and Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guidelines (18, 19) (checklists presented in the Supplement). This systematic review and meta-analysis were registered at International Prospective Register of Systematic Reviews (CRD42020155802).

Search strategy and study selection

We identified eligible studies comparing the neoadjuvant immunotherapy with routine neoadjuvant therapy in the treatment of locally advanced EC in the MEDLINE (via PubMed), Embase (via OVID), ISI Web of Science database and Cochrane Library, between January, 2000 and February, 2023. The language was limited to English. The following search terms or keywords were used: esophageal cancer (MeSH) OR esophageal squamous cell carcinoma OR esophageal adenocarcinoma AND neoadjuvant OR preoperative AND programmed cell death 1 (PD-1) OR programmed cell death ligand 1 (PD-L1) OR immunotherapy (Supplement Table S2). The last search was conducted on February 6, 2023. All titles and abstracts were screened and reviewed carefully.

Two authors (H.D.X. and K.L.) independently retrieved the available literature to identify the eligible studies. The studies were chosen on the basis of the following criteria: (a) studies only including patients with esophagus cancer or esophagogastric cancer; (b) the primary efficacy outcomes were pathological complete response rate; complete (R0) tumor resection rate; adverse events of neoadjuvant treat; (c) Randomized Controlled Trials (RCTs) or Retrospective experiments comparing neoadjuvant ICIs combined with nCT or nCRT for treating EC and (d) The experimental design met the requirements and included patients with ESCC and EAC. Exclusion criteria were as the following criteria: (a) studies reporting incomplete or inconsistent outcomes; and (b) duplicate studies, studies reporting animal experiments, case reports, cohort studies, and review articles.

Data collection and quality assessment

Data extraction was respectively and carefully performed by two reviewers (H.D.X. and K.L.). The following information was collected: first author, year of publication, region, characteristics of the study population (number, sex and age), TNM stage, treatment therapy, adverse events of neoadjuvant therapies, postoperative complications, and pathological response. If the HR and its 95% CI were not directly provided in the original articles, the extracted survival information and the published risk table were used to reconstruct the survival curve for each included study using the method of David (20). The extraction of information was repeated if there were apparent discrepancies. Reviewers would contact the corresponding authors of the studies to access relevant data to analysis, when no sufficient data in publications were extracted. The methodological quality was assessed by reviewers (H.D.X. and K.L.) using the Newcastle-Ottawa Scale (NOS). Moderate quality was defined as 4-6 scores, and 7-9 scores was high quality. An additional adjudicator (L.W.) would be invited into the discussion to resolve the discrepancies between the reviewers. To ensure that patients were not counted several times, we selected data with the largest number of participants if a medical database was used by multiple studies in adjacent time periods and the number of patients were similar.

Outcome measures

The neoadjuvant immunotherapy comprised neoadjuvant immune checkpoint inhibitor in combination with chemotherapy (nICT) and neoadjuvant immune checkpoint inhibitor in combination with chemoradiotherapy (nICRT). The routine neoadjuvant therapy included neoadjuvant chemotherapy (nCT) and neoadjuvant chemoradiotherapy (nCRT).

The pathological TNM stage was staged according to the 8th edition American Joint Committee on Cancer/Union for International Cancer Control staging system (21). We used Response Evaluation Criteria In Solid Tumours guideline version 1.13 system to classify regressive changes after neoadjuvant treatment based on histopathological results to reveal prognostic information (22). The treatment related adverse events (TRAEs) were assessed by Common Terminology Criteria for Adverse Events, version 4.0 (23).

Pathologic complete response (pCR) was defined as no evidence of residual tumor cells of the complete resected tumor specimen of neoadjuvant therapy and resection. The major pathological response (MPR) was defined as less than 10% of residual tumor cells. In the present study, the pCR and MPR rates were considered to be the primary outcomes. R0 resection was defined as a microscopically margin-negative resection without microscopic tumor on the primary tumor bed. The R0 surgical resection rate was set as the secondary outcome for comparing neoadjuvant immunotherapy plus chemotherapy with chemotherapy alone for patients.

Statistical analysis

The primary outcome of interest was pathologic response (pCR and MPR). The second outcome of interest was R0 resection rate. Odds ratio (OR) and associated 95% CI were used as the effect indicators comparing the safety and efficiency of the neoadjuvant immunotherapy with the routine neoadjuvant therapy. To minimize the influence of recall and selection bias that occur in retrospective studies, we performed stratified analyses to assess the association in all cohort studies. The heterogeneity between studies was evaluated with Q and I^2 statistics (24). The results were calculated using a random-effect model (Mantel-Haenszel method) when statistically heterogeneity (I^2 >50%) between studies were found. If low heterogeneity (I^2 ≤50%) was between studies fixed-effect model (Inverse Variance) was performed.

Sensitivity analysis, subgroup analysis and meta-regression were all performed to explore the sources of heterogeneity. The potential publication bias was further validated by the Egger's and Begg's test (25). All statistical analyses were two sides; and P value less than 0.05 was considered statistically significant. Statistical analysis was performed using the STATA version 15.0 (Stata Corp LP, College Station, Texas, USA).

Results

Characteristics of included studies

After reviewing 557 publications found using the predefined search terms. All investigators finally agreed to include eight eligible studies (26–33) with 652 patients in our meta-analysis (Table 1). The PRISMA flow chart of this meta-analysis was shown in Figure 1. Among them, five studies were conducted on esophageal squamous cell carcinoma (ESCC) (26–28, 31, 32), and the other three addressed esophageal adenocarcinoma cancer (EAC) (29, 30, 33). About the neoadjuvant strategies, there were four studies that studied nICT vs nCT (26, 28, 31, 33), two studies that studied nICT vs nCRT (27, 32), two studies that studied nICRT vs nCRT (29, 30). The

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TABLE 1 Characteristics of included studies for the meta-analyses.

Study	Country	Enrolled patients				Intervention	ICI	Neoadjuvant cycle	NCT or ChiCTR identifier
		Sample size, No.	Male, No. (%)	Clinical stage	Histological type				
Bingjiang Huang et al, 2021 (26)	China	54	51 (94.4%)	cT2-4N1-3M0	ESCC	nICT vs nCT	pembrolizumab	2	ChiCTR2000035079
Zhinuan Hong et al, 2022 (27)	China	87	68 (78.2%)	cT1N1-3M0 or cT2-4aN 0-3M0	ESCC	nICT vs nCRT	sintilimab pembrolizumab toripalimab camrelizumab	2-4	NR
Shaowu Jing et al, 2022 (28)	China	94	63 (67.0%)	cT3-4aN0-2M0	ESCC	nICT vs nCT	sintilimab pembrolizumab toripalimab camrelizumab	1-3	NR
Smita Sihag et al, 2021 (29)	USA	168	146 (86.9%)	NR	EAC	nICRT vs nCRT	durvalumab	2	NCT02962063
Tom van don Ende et al, 2021 (30)	Netherlands	80	71 (88.7%)	NR	EAC	nICRT vs nCRT	atezolizumab	5	NCT03087864
Zhinuan Hong et al, 2021 (31)	China	122	101 (82.8%)	cT1N1-3 M0 or cT2-4aN 0-3M0	ESCC	nICT vs nCT	sintilimab pembrolizumab camrelizumab	2-4	ChiCTR2100045659
Jiahan Cheng et al, 2022 (32)	China	149	123 (82.6%)	cT2-4N1-3M0	ESCC	nICT vs nCRT	sintilimab pembrolizumab camrelizumab toripalimab tislelizumab	2-4	NR
Xuewei Ding et al, 2023 (33)	China	47	NR	NR	EAC	nICT vs nCT	sintilimab	3	NCT04982939

ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma; nICT, neoadjuvant immune checkpoint inhibitor in combination with chemotherapy; nICRT, neoadjuvant immune checkpoint inhibitor in combination with chemoradiotherapy; nCT, neoadjuvant chemotherapy; nCRT, neoadjuvant chemoradiotherapy; NR, not reported.

sample size was ranged from 47 to 168. The Newcastle-Ottawa scores are presented in the Supplement Table S2.

pCR and MPR

Eight studies (26–33) were included in the pCR meta-analysis. Due to the heterogeneity between studies (I^2 = 32.8%, *P*=0.166), the data from the subgroups within a single study was pooled using a fixed-effect model. The estimated pCR rate was higher in the neoadjuvant immunotherapy group, including nICT and nICRT (OR =1.86; 95% CI, 1.25–2.79; Figure 2). As to the difference of the histologic subtypes, the studies were divided into two subgroups (the EAC group and the ESCC group). However, the different results were found in the ESCC and EAC subgroups, the estimated OR was 2.35 (95%CI, 1.20–4.64) in the EAC subgroup, and 1.65 (95% CI, 1.00–2.72) in the ESCC subgroup. The heterogeneity of two subgroups were (I^2 = 45.3%, *P*=0.161) and (I^2 = 30.9%, *P*=0.215), respectively. Interestingly, we found the common result (OR=1.93, 95% CI, 1.08–3.46; I^2 = 57.5%, *P*=0.094) (see Supplementary Material 3: Figure S1), when we deleted all studies included nCRT.

Six studies (27–31, 33) reported on the MPR. When pooling the studies, the pooled MPR was higher in the neoadjuvant immunotherapy group (OR =2.66; 95% CI, 1.69–4.19; Figure 3). Common results were showed in the subgroups, EAC and ESCC. The result was showed in Figure 3.

R0 resection

No difference of R0 resection was founded between two groups (OR=1.79, 95% CI, 0.84–3.84; Figure 4), with moderate heterogeneity (I^2 = 39.9%, *P*=0.156).

Incidence of grade ≥3 TRAEs

Incidence of the overall grade ≥3 TRAEs was significantly higher in patients receiving neoadjuvant immunotherapy compared to patients receiving routine neoadjuvant therapy (neoadjuvant chemotherapy/chemoradiotherapy). Further analyses of individual grade ≥3 TRAEs showed that the neoadjuvant immunotherapy was associated with more pneumonitis/pneumonia (OR=3.46, 95% CI,

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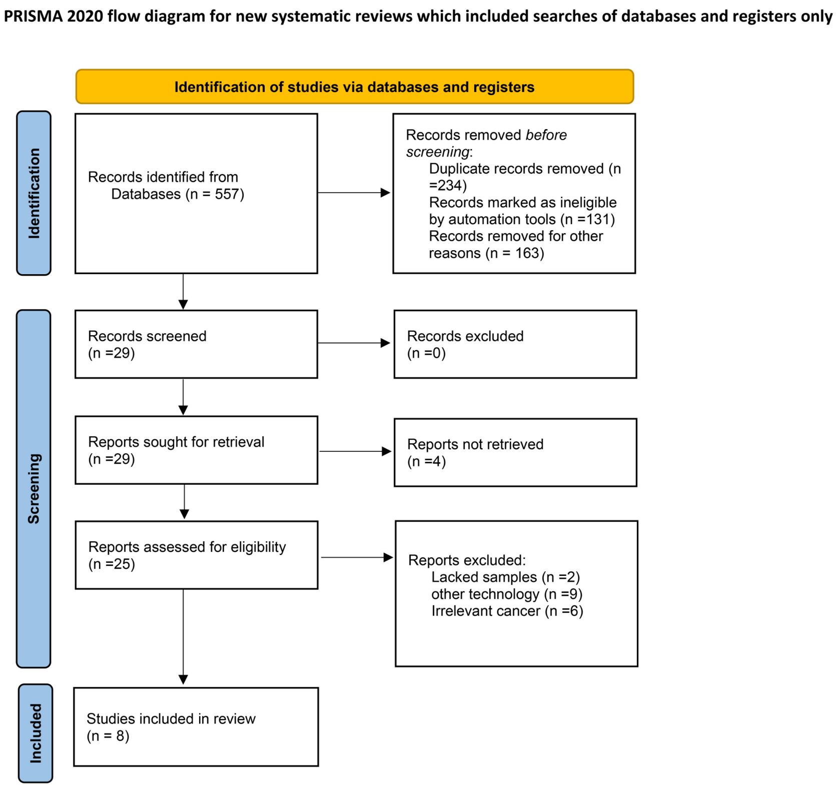

FIGURE 1
Flow diagram of included studies for this meta-analysis.

1.31–9.16; I^2 = 67.3%, *P*=0.005; Figure 5A) and thyroid dysfunction (OR=4.69, 95% CI, 1.53–14.36; I^2 = 56.5%, *P*=0.032; Figure 5B). Other individual grade ≥3 TRAEs including blood system, gastrointestinal system, and hypokalemia were comparable between the neoadjuvant immunotherapy and the routine neoadjuvant therapy (see Supplementary Material 3: Figure S2).

One death was reported in the patients received nICRT, and the death was due to pneumonitis (30).

Surgical safety

Surgical resection rate (OR=0.74, 95% CI, 0.42–1.29; I^2 = 0.0%, *P*=0.478) and surgical delay rate (OR=1.24, 95% CI, 0.79–1.90; I^2 = 22.8%, *P*=0.255) were comparable between the neoadjuvant immunotherapy and the routine neoadjuvant therapy (see Supplementary Material 3: Figure S3). No surgical mortality was reported.

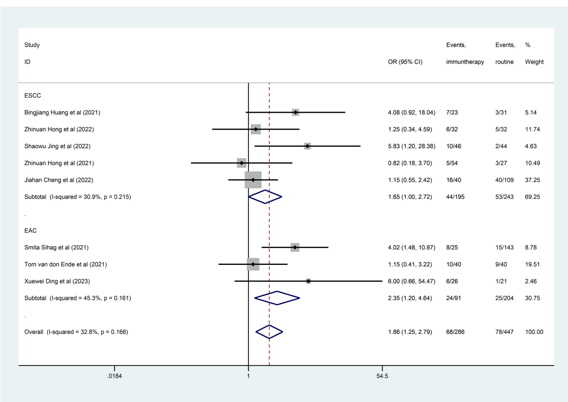

FIGURE 2
Forest plot of pathological complete response (pCR).

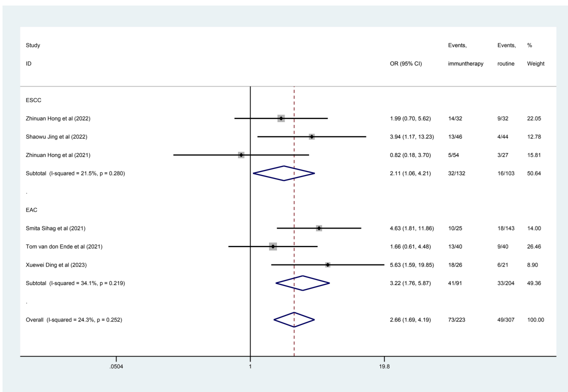

FIGURE 3
Forest plot of major pathological response (MPR).

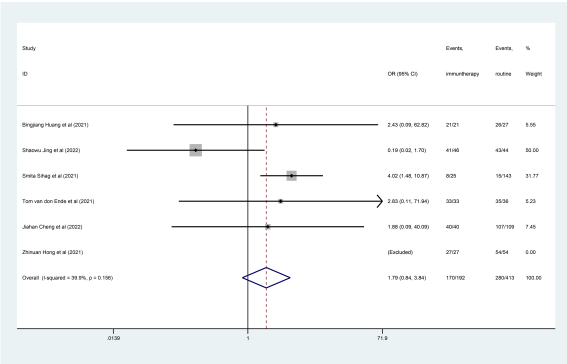

FIGURE 4
Forest plot of R0 resection.

Evaluation of sensitivity and publication bias

We conducted sensitivity analyses to ensure that the combined outcomes were not severely altered by the specific trials, and the overall estimates remained consistent across these analyses.

Egger's test and Begg's test were used to evaluate publication bias. Two regression intercept tests showed that the publication bias was not statistically significant (Supplementary Material 3: Table S3).

Discussion

The neoadjuvant immunotherapy significantly improved pCR rates with tolerable toxicity in EC patients (14–17). However, the best neoadjuvant treatment strategy for EC was still inconclusive. Therefore, we conducted the comprehensive systematic review and meta-analysis to compare the antitumor efficacy and safety of the neoadjuvant immunotherapy with routine neoadjuvant therapy in patients with locally advanced EC. Our meta-analysis showed that the neoadjuvant immunotherapy had better pathologic response than routine neoadjuvant therapy. In addition, no significant differences were found in R0 resection rate.

The nCRT was performed as the standard therapy strategy for locally advanced EC patients, both ESCC and EAC. In the immune era, nCRT was also facing increasingly challenged by the neoadjuvant immunotherapy. The pembrolizumab combined with nCRT was demonstrated to be a safe and effective neoadjuvant treatment strategy for ESCC patients, in PALACE-1 trail. The neoadjuvant therapy did not delay surgery time, and 55.6% of patients received operation achieved pCR (34). Recent Neo-PLANET trail suggested that neoadjuvant camrelizumab plus nCRT exhibited pCR rate was 33.3% and MPR rate was 44.4% in patients with locally advanced EAC patients, with an acceptable safety profile. Although didn't reach final survival outcome, Two-year progression free survival (PFS) and over survival (OS) rates were 66.9% and 76.1%, respectively (13). However, PERFECT trail suggested that the combining nCRT with immunotherapy didn't show satisfactory database in patients with EAC (30). In addition, many trails also evaluated the clinical result of neoadjuvant immunotherapy in locally advanced EC patients, and the security of treatment was also analyzed (12, 35–40). The MPR and pCR for ESCC patients, received surgery, were 52.9%-72.0% and 30.2%-50.0% respectively. Preclinical studies have shown that programmed cell death 1 (PD-1) inhibitor combined with chemotherapy can further enhance the host's immune response and inhibit the immune escape of cancer cells (41). For improving the efficacy, the neoadjuvant immunotherapy was always combined with chemotherapy or chemoradiotherapy (42).

Our study showed that the estimated pCR rates and MPR rates were higher in the neoadjuvant immunotherapy. But we found the pathologic response of the neoadjuvant immunotherapy appeared to be similar to that for nCRT in patients with locally advanced EC. At present, there were only two retrospective studies compared the antitumor efficacy and safety of nCRT with nICT. The study of Jiahan Cheng et al. indicated nICT could result in better outcome and less complications compared with nCRT therapy in locally advanced ESCC patients (32). However, Zhinuan Hong et al.'s study reported the quite opposite result (27). Platinum-based chemotherapy was the most applied neoadjuvant therapy. All included trails are based on the fluoropyrimidine plus platinum

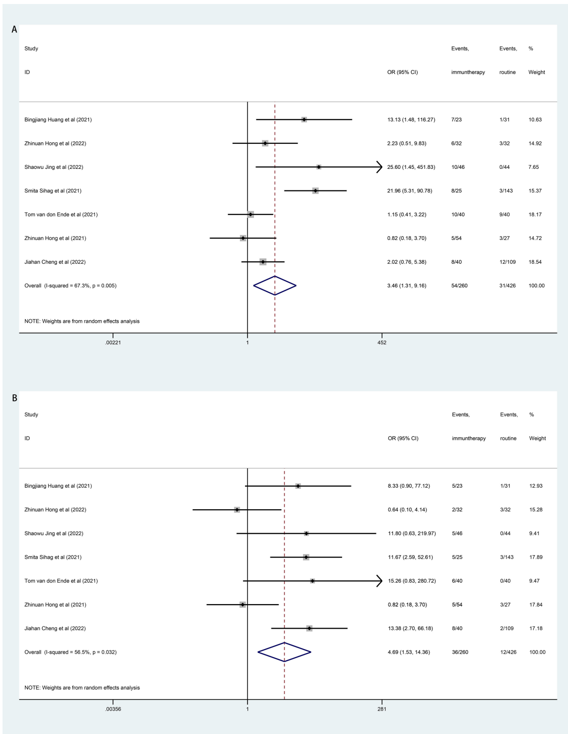

FIGURE 5
Forest plot of treatment related adverse events (TRAEs). **(A)**: Forest Plot of pneumonitis/pneumonia; **(B)**: Forest Plot of thyroid dysfunction.

(FP) or the paclitaxel and carboplatin (PC). A three-arm phase III randomized controlled trial (JCOG1109) is ongoing in Japan (43); its preliminary results showed that the docetaxel, cisplatin plus 5-FU (DCF) would be a better choice. There was no consensus on the best chemotherapy regimen. In addition, the sequence of PD-1/PD-L1 inhibitors and chemotherapy or chemoradiotherapy might impact the pathologic response outcome. Wenqun Xing et al. found that delaying toripalimab to day 3 in nICT achieved a higher pCR rate, compared to on the same day (44). The time for surgical resection is generally 3-6 weeks after the last cycle neoadjuvant therapy. In our meta-analysis, 41.4Gy in was the most frequently used RT schedule in eligible studies of nICRT and nCRT.

There were no biomarkers could predicate clinical outcomes of the neoadjuvant immunotherapy for patients with EC. The most promising tools for predicting the potential for response to the neoadjuvant immunotherapy included PD-L1 expression status, mismatch-repair-deficient/microsatellite instability-high (dMMR/MSI-H), and tumor mutation burden (TMB). A recent meta-analysis suggested that tissue-based PD-L1 expression, more than any variable other than dMMR/MSI-H, identified varying degrees of benefit from ICIs-containing therapy (45). The dMMR/MSI-H also might be a biomarker (46). There was a strong association between TMB and clinical efficacy in advanced EAC patients received first-line pembrolizumab-based therapy, but it did not exclude patients with MSI-H tumors (47). A biomarker could accurately estimate the therapeutic effect of immunotherapy in esophageal cancer was eagerly needed.

Incidence rate of TRAEs was higher in the immunotherapy than routine neoadjuvant therapy. Our meta-analysis also suggested the same result, especially in pneumonitis/pneumonia and thyroid dysfunction. Tom van don Ende et al. reported one death due to pneumonitis (30); and dead cases caused by TRAEs were also reported in the PALACE-1 study (34). Unlike the TRAEs were within 10 days after the end of treatment in routine neoadjuvant therapy, TRAEs of immunotherapy usually occurred three and four weeks after one cycle of immunotherapy (48, 49). In addition, the danger of various TRAEs were totally different. Recent studies revealed that the TRAEs of skin and thyroid even were associated with a better prognosis (50).

Limitations

There were several limitations in our study. Firstly, all included studies were descriptive study and the results have not been evaluated in large-scale controlled trials. Therefore, these findings required further validation by large RCTs. Only the RCTs were the golden standard of comparing the neoadjuvant immunotherapy and the routine neoadjuvant therapy. Secondly, researches for neoadjuvant immunotherapy in EAC remains fairly limited. The few researches were all performed in North America and Europe (29, 30). The diversity between ESCC and EAC might may lead to different responses to the neoadjuvant immunotherapy. Therefore, more clinical trials of neoadjuvant immunotherapy in EAC are needed, especially in East Asia. The main outcome measures are pCR and MPR, both would be typically increased by radiotherapy. A clear comparison between nICT vs nCT and nICRT vs nCRT is not achievable for the smaller sample size of the included studies. Thirdly, all eligible studies concentrated the pathological response rates, but no survival data was reported. The association between pathological response and survival in esophageal cancer deserves further investigation (51). Only the overall survival data was the gold standard to compare the neoadjuvant immunotherapy with routine neoadjuvant therapy. Another main limitation is the heterogeneity of the included studies, which is reflected in the different ICIs.

Conclusions

The current meta-analysis revealed that the neoadjuvant immunotherapy (nICT and nICRT) could significantly increase the rates of pCR and MPR, compared with routine neoadjuvant therapy (nCT and nCRT) in the treatment of locally advanced EC. The neoadjuvant immunotherapy and routine neoadjuvant therapy were with acceptable toxicity. However, randomized studies with larger groups of patients need to performed to confirm these results.

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

FL: conceptualization. HX and KL: methodology. HQ and HX: software. YL: formal analysis. HQ, HX and KL: data curation. YZ: writing original draft preparation. YZ, LW, HQ and FY: writing-review and editing. All authors contributed to the article and approved the submitted version.

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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/fimmu.2023.1108213/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(3):209–49. doi: 10.3322/caac.21660

2. Pasquali S, Yim G, Vohra RS, Mocellin S, Nyanhongo D, Marriott P, et al. Survival after neoadjuvant and adjuvant treatments compared to surgery alone for resectable esophageal carcinoma: A network meta-analysis. *Ann Surg* (2017) 265(3):481–91. doi: 10.1097/sla.0000000000001905

3. Bushan K, Sharma S. Neoadjuvant chemotherapy and surgery versus surgery alone in resectable esophageal cancer. *Indian J Cancer* (2015) 52(3):413–6. doi: 10.4103/0019-509x.176743

4. Yang H, Liu H, Chen Y, Zhu C, Fang W, Yu Z, et al. Neoadjuvant chemoradiotherapy followed by surgery versus surgery alone for locally advanced squamous cell carcinoma of the esophagus (Neocrtec5010): A phase iii multicenter, randomized, open-label clinical trial. *J Clin Oncol Off J Am Soc Clin Oncol* (2018) 36(27):2796–803. doi: 10.1200/jco.2018.79.1483

5. Eyck BM, van Lanschot JJB, Hulshof M, van der Wilk BJ, Shapiro J, van Hagen P, et al. Ten-year outcome of neoadjuvant chemoradiotherapy plus surgery for esophageal cancer: The randomized controlled cross trial. *J Clin Oncol Off J Am Soc Clin Oncol* (2021) 39(18):1995–2004. doi: 10.1200/jco.20.03614

6. Mayanagi S, Irino T, Kawakubo H, Kitagawa Y. Neoadjuvant treatment strategy for locally advanced thoracic esophageal cancer. *Ann gastroenterological Surg* (2019) 3(3):269–75. doi: 10.1002/ags3.12243

7. Sun JM, Shen L, Shah MA, Enzinger P, Adenis A, Doi T, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (Keynote-590): A randomised, placebo-controlled, phase 3 study. *Lancet (London England)* (2021) 398(10302):759–71. doi: 10.1016/s0140-6736(21)01234-4

8. Doki Y, Ajani JA, Kato K, Xu J, Wyrwicz L, Motoyama S, et al. Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *New Engl J Med* (2022) 386(5):449–62. doi: 10.1056/NEJMoa2111380

9. Luo H, Lu J, Bai Y, Mao T, Wang J, Fan Q, et al. Effect of camrelizumab vs placebo added to chemotherapy on survival and progression-free survival in patients with advanced or metastatic esophageal squamous cell carcinoma: The escort-1st randomized clinical trial. *Jama* (2021) 326(10):916–25. doi: 10.1001/jama.2021.12836

10. Lu Z, Wang J, Shu Y, Liu L, Kong L, Yang L, et al. Sintilimab versus placebo in combination with chemotherapy as first line treatment for locally advanced or metastatic oesophageal squamous cell carcinoma (Orient-15): Multicentre, randomised, double blind, phase 3 trial. *BMJ (Clinical Res ed)* (2022) 377:e068714. doi: 10.1136/bmj-2021-068714

11. Wang ZX, Cui C, Yao J, Zhang Y, Li M, Feng J, et al. Toripalimab plus chemotherapy in treatment-naïve, advanced esophageal squamous cell carcinoma (Jupiter-06): A multi-center phase 3 trial. *Cancer Cell* (2022) 40(3):277–88.e3. doi: 10.1016/j.ccell.2022.02.007

12. Yan X, Duan H, Ni Y, Zhou Y, Wang X, Qi H, et al. Tislelizumab combined with chemotherapy as neoadjuvant therapy for surgically resectable esophageal cancer: A prospective, single-arm, phase ii study (Td-nice). *Int J Surg (London England)* (2022) 103:106680. doi: 10.1016/j.ijsu.2022.106680

13. Tang Z, Wang Y, Liu D, Wang X, Xu C, Yu Y, et al. The neo-planet phase ii trial of neoadjuvant camrelizumab plus concurrent chemoradiotherapy in locally advanced adenocarcinoma of stomach or gastroesophageal junction. *Nat Commun* (2022) 13(1):6807. doi: 10.1038/s41467-022-34403-5

14. Ge F, Huo Z, Cai X, Hu Q, Chen W, Lin G, et al. Evaluation of clinical and safety outcomes of neoadjuvant immunotherapy combined with chemotherapy for patients with resectable esophageal cancer: A systematic review and meta-analysis. *JAMA network Open* (2022) 5(11):e2239778. doi: 10.1001/jamanetworkopen.2022.39778

15. Wu J, Deng R, Ni T, Zhong Q, Tang F, Li Y, et al. Efficacy and safety of Radiotherapy/Chemoradiotherapy combined with immune checkpoint inhibitors for locally advanced stages of esophageal cancer: A systematic review and meta-analysis. *Front Oncol* (2022) 12:887525. doi: 10.3389/fonc.2022.887525

16. Wang H, Li S, Liu T, Chen J, Dang J. Neoadjuvant immune checkpoint inhibitor in combination with chemotherapy or chemoradiotherapy in resectable esophageal cancer: A systematic review and meta-analysis. *Front Immunol* (2022) 13:998620. doi: 10.3389/fimmu.2022.998620

17. Wang Z, Shao C, Wang Y, Duan H, Pan M, Zhao J, et al. Efficacy and safety of neoadjuvant immunotherapy in surgically resectable esophageal cancer: A systematic review and meta-analysis. *Int J Surg (London England)* (2022) 104:106767. doi: 10.1016/j.ijsu.2022.106767

18. Ong MF, Soh KL, Saimon R, Wai MW, Mortell M, Soh KG. Fall prevention education to reduce fall risk among community-dwelling older persons: A systematic review. *J Nurs Manage* (2021) 29(8):2674–88. doi: 10.1111/jonm.13434

19. Dickson K, Yeung CA. Prisma 2020 updated guideline. *Br Dental J* (2022) 232(11):760–1. doi: 10.1038/s41415-022-4359-7

20. Miller DJ, Nguyen JT, Bottai M. Emagnification: A tool for estimating effect-size magnification and performing design calculations in epidemiological studies. *Stata J* (2020) 20(3):548–64. doi: 10.1177/1536867x20953567

21. Inada M, Nishimura Y, Ishikawa K, Nakamatsu K, Wada Y, Uehara T, et al. Comparing the 7th and 8th editions of the American joint committee on Cancer/Union for international cancer control tnm staging system for esophageal squamous cell carcinoma treated by definitive radiotherapy. *Esophagus Off J Japan Esophageal Soc* (2019) 16(4):371–6. doi: 10.1007/s10388-019-00675-y

22. 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 (Oxford Engl 1990)* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026

23. Freites-Martinez A, Santana N, Arias-Santiago S, Viera A. Using the common terminology criteria for adverse events (Ctcae - version 5.0) to evaluate the severity of adverse events of anticancer therapies. *Actas dermo-sifiliograficas* (2021) 112(1):90–2. doi: 10.1016/j.ad.2019.05.009

24. Crippa A, Khudyakov P, Wang M, Orsini N, Spiegelman D. A new measure of between-studies heterogeneity in meta-analysis. *Stat Med* (2016) 35(21):3661–75. doi: 10.1002/sim.6980

25. Furuya-Kanamori L, Barendregt JJ, Doi SAR. A new improved graphical and quantitative method for detecting bias in meta-analysis. *Int J Evidence-Based healthcare* (2018) 16(4):195–203. doi: 10.1097/xeb.0000000000000141

26. Huang B, Shi H, Gong X, Yu J, Xiao C, Zhou B, et al. Comparison of efficacy and safety between pembrolizumab combined with chemotherapy and simple chemotherapy in neoadjuvant therapy for esophageal squamous cell carcinoma. *J gastrointestinal Oncol* (2021) 12(5):2013–21. doi: 10.21037/jgo-21-610

27. Hong ZN, Gao L, Weng K, Huang Z, Han W, Kang M. Safety and feasibility of esophagectomy following combined immunotherapy and chemotherapy for locally advanced esophageal squamous cell carcinoma: A propensity score matching analysis. *Front Immunol* (2022) 13:836338. doi: 10.3389/fimmu.2022.836338

28. Jing SW, Zhai C, Zhang W, He M, Liu QY, Yao JF, et al. Comparison of neoadjuvant immunotherapy plus chemotherapy versus chemotherapy alone for patients with locally advanced esophageal squamous cell carcinoma: A propensity score matching. *Front Immunol* (2022) 13:970534. doi: 10.3389/fimmu.2022.970534

29. Sihag S, Ku GY, Tan KS, Nussenzweig S, Wu A, Janjigian YY, et al. Safety and feasibility of esophagectomy following combined immunotherapy and chemoradiotherapy for esophageal cancer. *J Thorac Cardiovasc Surg* (2021) 161(3):836–43.e1. doi: 10.1016/j.jtcvs.2020.11.106

30. van den Ende T, de Clercq NC, van Berge Henegouwen MI, Gisbertz SS, Geijsen ED, Verhoeven RHA, et al. Neoadjuvant chemoradiotherapy combined with atezolizumab for resectable esophageal adenocarcinoma: A single-arm phase ii feasibility trial (Perfect). *Clin Cancer Res an Off J Am Assoc Cancer Res* (2021) 27 (12):3351–9. doi: 10.1158/1078-0432.Ccr-20-4443

31. Hong ZN, Zhang Z, Chen Z, Weng K, Peng K, Lin J, et al. Safety and feasibility of esophagectomy following combined neoadjuvant immunotherapy and chemotherapy for locally advanced esophageal cancer: A propensity score matching. *Esophagus Off J Japan Esophageal Soc* (2022) 19(2):224–32. doi: 10.1007/s10388-021-00899-x

32. Cheng J, Guo M, Yang Y, Liu Y, Hu W, Shang Q, et al. Perioperative outcomes of minimally invasive esophagectomy after neoadjuvant immunotherapy for patients with locally advanced esophageal squamous cell carcinoma. *Front Immunol* (2022) 13:848881. doi: 10.3389/fimmu.2022.848881

33. Ding X, Wang X, Li B, Wang L, Guo H, Shang L, et al. Persist: A multicenter, randomized phase ii trial of perioperative oxaliplatin and s-1 (Sox) with or without sintilimab in resectable locally advanced Gastric/Gastroesophageal junction cancer (Gc/Gejc). *Am Soc Clin Oncol* (2023) 41(4_suppl):364-. doi: 10.1200/JCO.2023.41.4_suppl.364

34. Li C, Zhao S, Zheng Y, Han Y, Chen X, Cheng Z, et al. Preoperative pembrolizumab combined with chemoradiotherapy for oesophageal squamous cell carcinoma (Palace-1). *Eur J Cancer (Oxford Engl 1990)* (2021) 144:232–41. doi: 10.1016/j.ejca.2020.11.039

35. Duan H, Wang T, Luo Z, Wang X, Liu H, Tong L, et al. A multicenter single-arm trial of sintilimab in combination with chemotherapy for neoadjuvant treatment of resectable esophageal cancer (Sin-ice study). *Ann Trans Med* (2021) 9(22):1700. doi: 10.21037/atm-21-6102

36. Zhang Z, Ye J, Li H, Gu D, Du M, Ai D, et al. Neoadjuvant sintilimab and chemotherapy in patients with resectable esophageal squamous cell carcinoma: A prospective, single-arm, phase 2 trial. *Front Immunol* (2022) 13:1031171. doi: 10.3389/fimmu.2022.1031171

37. Lv H, Tian Y, Li J, Huang C, Sun B, Gai C, et al. Neoadjuvant sintilimab plus chemotherapy in resectable locally advanced esophageal squamous cell carcinoma. *Front Oncol* (2022) 12:864533. doi: 10.3389/fonc.2022.864533

38. Shen D, Chen Q, Wu J, Li J, Tao K, Jiang Y. The safety and efficacy of neoadjuvant pd-1 inhibitor with chemotherapy for locally advanced esophageal squamous cell carcinoma. *J gastrointestinal Oncol* (2021) 12(1):1–10. doi: 10.21037/jgo-20-599

39. Shang X, Zhao G, Liang F, Zhang C, Zhang W, Liu L, et al. Safety and effectiveness of pembrolizumab combined with paclitaxel and cisplatin as neoadjuvant therapy followed by surgery for locally advanced resectable (Stage iii) esophageal squamous cell carcinoma: A study protocol for a prospective, single-arm, single-center, open-label, phase-ii trial (Keystone-001). *Ann Trans Med* (2022) 10 (4):229. doi: 10.21037/atm-22-513

40. Liu J, Li J, Lin W, Shao D, Depypere L, Zhang Z, et al. Neoadjuvant camrelizumab plus chemotherapy for resectable, locally advanced esophageal squamous cell carcinoma (Nic-Escc2019): A multicenter, phase 2 study. *Int J Cancer* (2022) 151(1):128–37. doi: 10.1002/ijc.33976

41. Liu J, Chen Z, Li Y, Zhao W, Wu J, Zhang Z. Pd-1/Pd-L1 checkpoint inhibitors in tumor immunotherapy. *Front Pharmacol* (2021) 12:731798. doi: 10.3389/fphar.2021.731798

42. Topalian SL, Taube JM, Pardoll DM. Neoadjuvant checkpoint blockade for cancer immunotherapy. *Sci (New York, NY)* (2020) 367(6477). doi: 10.1126/science.aax0182

43. Nakamura K, Kato K, Igaki H, Ito Y, Mizusawa J, Ando N, et al. Three-arm phase iii trial comparing cisplatin plus 5-fu (Cf) versus docetaxel, cisplatin plus 5-fu (Dcf) versus radiotherapy with cf (Cf-rt) as preoperative therapy for locally advanced esophageal cancer (Jcog1109, next study). *Japanese J Clin Oncol* (2013) 43(7):752–5. doi: 10.1093/jjco/hyt061

44. Xing W, Zhao L, Zheng Y, Liu B, Liu X, Li T, et al. The sequence of chemotherapy and toripalimab might influence the efficacy of neoadjuvant chemoimmunotherapy in locally advanced esophageal squamous cell cancer-a phase ii study. *Front Immunol* (2021) 12:772450. doi: 10.3389/fimmu.2021.772450

45. Yoon HH, Jin Z, Kour O, Kankeu Fonkoua LA, Shitara K, Gibson MK, et al. Association of pd-L1 expression and other variables with benefit from immune checkpoint inhibition in advanced gastroesophageal cancer: Systematic review and meta-analysis of 17 phase 3 randomized clinical trials. *JAMA Oncol* (2022) 8(10):1456–65. doi: 10.1001/jamaoncol.2022.3707

46. Dhakras P, Uboha N, Horner V, Reinig E, Matkowskyj KA. Gastrointestinal cancers: Current biomarkers in esophageal and gastric adenocarcinoma. *Trans Gastroenterol Hepatol* (2020) 5:55. doi: 10.21037/tgh.2020.01.08

47. Lee KW, Van Cutsem E, Bang YJ, Fuchs CS, Kudaba I, Garrido M, et al. Association of tumor mutational burden with efficacy of Pembrolizumab ±Chemotherapy as first-line therapy for gastric cancer in the phase iii keynote-062 study. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2022) 28(16):3489–98. doi: 10.1158/1078-0432.Ccr-22-0121

48. Qin W, Yang L, Fan B, Zou B, Duan Y, Li B, et al. Association between immune-related adverse events and the efficacy of pd-1 inhibitors in advanced esophageal cancer. *Front Immunol* (2022) 13:931429. doi: 10.3389/fimmu.2022.931429

49. Yang Y, Tan L, Hu J, Li Y, Mao Y, Tian Z, et al. Safety and efficacy of neoadjuvant treatment with immune checkpoint inhibitors in esophageal cancer: Real-world multicenter retrospective study in China. *Dis esophagus Off J Int Soc Dis Esophagus* (2022) 35(11). doi: 10.1093/dote/doac031

50. Zhao Q, Zhang J, Xu L, Yang H, Liang N, Zhang L, et al. Safety and efficacy of the rechallenge of immune checkpoint inhibitors after immune-related adverse events in patients with cancer: A systemic review and meta-analysis. *Front Immunol* (2021) 12:730320. doi: 10.3389/fimmu.2021.730320

51. Güç ZG, Turgut B, Avci A, Cengiz F, Eren Kalender M, Alacacioğlu A. Predicting pathological response and overall survival in locally advanced gastric cancer patients undergoing neoadjuvant chemotherapy: The role of Pet/Computed tomography. *Nucl Med Commun* (2022) 43(5):560–7. doi: 10.1097/mnm.0000000000001534

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Assessment of neutrophil subsets and immune checkpoint inhibitor expressions on T lymphocytes in liver transplantation: A preliminary study beyond the neutrophil-lymphocyte ratio

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Background: Advanced stages of cirrhosis are characterized by the occurrence of progressive immune alterations known as CAID (Cirrhosis Associated Immune Dysfunction). In advanced cirrhosis, liver transplantation (LT) remains the only curative treatment. Sepsis, shares many similarities with decompensated cirrhosis in terms of immuno-inflammatory response. In both conditions, the neutrophil-lymphocyte ratio (NLR) is associated with poor outcomes. Based on alterations in sepsis, we hypothesized that we could observe in cirrhotic and LT patients more detailed neutrophil and lymphocyte phenotypes. To this end, along with leukocyte count, we assessed immature neutrophils, LOX-1$^+$ MDSC and PD-1 and TIM-3 lymphocyte expressions in cirrhotic patients before transplantation in association with liver disease severity and during the first month after transplantation.

Methods: We conducted a prospective monocentric study including cirrhotic patients registered on LT waiting-list. Blood samples were collected at enrolment before LT and for 1 month post-LT. In addition to NLR, we assessed by whole blood flow cytometry the absolute count of immature neutrophils and LOX-1$^+$

Abbreviations: ACLF, Acute on Chronic Liver Failure; CAID, Cirrhosis Associated Immune Dysfunction; HV, Healthy Volunteers; Lox1, Lectine-type oxidized LDL receptor 1; LT, Liver Transplantation; MELD, Model of End stage Liver Disease; PD-1, Programmed Death 1; PMN-MDSC, Polymorphonuclear Myeloid Derived Suppressor Cells; TIM3, T cell immunoglobulin and mucin domain 3.

MDSC as well as the expressions of immune checkpoint receptors PD-1 and TIM-3 on T lymphocytes.

Results: We included 15 healthy volunteers (HV) and 28 patients. LT was performed for 13 patients. Pre-LT patients presented with a higher NLR compared to HV and NLR was associated with cirrhosis severity. Increased immature neutrophils and LOX-1$^+$ MDSC counts were observed in the most severe patients. These alterations were mainly associated with acute decompensation of cirrhosis. PD-1 and TIM-3 expressions on T lymphocytes were not different between patients and HV. Post-LT immune alterations were dominated by a transitory but tremendous increase of NLR and immature neutrophils during the first days post-LT. Then, immune checkpoint receptors and LOX-1$^+$ MDSC tended to be overexpressed by the second week after surgery.

Conclusion: The present study showed that NLR, immature neutrophils and LOX-1$^+$ MDSC counts along with T lymphocyte count and checkpoint inhibitor expression were altered in cirrhotic patients before and after LT. These data illustrate the potential interest of immune monitoring of cirrhotic patients in the context of LT in order to better define risk of sepsis. For this purpose, larger cohorts of patients are now necessary in order to move forward a more personalised care of LT patients.

KEYWORDS

transplantation, immunosuppression, cirrhosis, immune checkpoint receptors, PD-1, LOX-1

Introduction

Liver cirrhosis defined by annular fibrosis surrounding regenerating hepatocytes is the terminal evolution of many chronic liver diseases (Anthony et al., 1977). Advanced stages of cirrhosis are characterized by portal hypertension, hepatic insufficiency and by the occurrence of progressive immune alterations known as CAID (Cirrhosis Associated Immune Dysfunction). CAID associates both systemic inflammation and features of immunosuppression as a consequence of alterations of the gut-liver axis inducing intestinal hyper-permeability and dysbiosis (Albillos et al., 2021). This leads to a continuous immune stimulation by microbial antigens and ultimately to immune cell exhaustion (Albillos et al., 2020). As a result, both innate and adaptive immune responses are dysregulated in cirrhotic patients and dramatically worsen with cirrhosis severity such as in the highest severity stage of inflammation represented by ACLF (Acute on Chronic Liver Failure) (Arvaniti et al., 2010). In this context of advanced cirrhosis, liver transplantation (LT) remains the only curative treatment. In addition to CAID, LT amplifies the profound immunosuppressive state of patients due to major surgery, immunosuppressive drugs, and intensive care unit stay). Therefore, infections constitute a major clinical issue in pre- and post-LT patients (Tranah et al., 2022). Before LT, infections in cirrhotic patients are both more frequent and more severe in association with cirrhosis severity and they can delay the access to a graft and increase mortality risk (Finkenstedt et al., 2013). After LT, infections increase morbidity and graft dysfunction (Tranah et al., 2022). Noteworthy, infections represent the major cause of death in the first year following LT in ACLF patients (Sundaram et al., 2020).

Sepsis, a life-threatening organ dysfunction caused by a dysregulated inflammatory host response to infection, shares many similarities with decompensated cirrhosis in terms of immuno-inflammatory response (Singer et al., 2016). It associates overwhelming inflammation and compensatory anti-inflammatory response that may lead to marked immunosuppression. Besides, immune dysfunction in ACLF has been described as a “sepsis-like” immune paralysis (Wasmuth et al., 2005). In sepsis, many immunological parameters have been demonstrated as prognostic marker of higher infectious rick/mortality (Venet and Monneret, 2018). Of them, due to lymphocyte apoptosis and emergency granulopoiesis (Venet et al., 2021) the neutrophil-lymphocyte ratio (NLR) is a widely described prognostic biomarker associated with poor outcomes (Rehman et al., 2020; Abensur Vuillaume et al., 2021; Lorente et al., 2022). Moreover, on neutrophil side, additional dysfunctional subsets have been described: increased immature neutrophils (i.e., CD16^{low}) (Rehman et al., 2020) or occurrence of LOX-1+ myeloid derived suppressive cells (LOX-1$^+$ MDSC) (Coudereau et al., 2022). On lymphocyte side, several reports revealed overexpression of immune checkpoint receptors such as PD-1 (Programmed death-1) and TIM3 (T cell immunoglobulin domain and mucin domain 3) on lymphocyte surface (Guignant et al., 2011; Boomer et al., 2012). Most importantly, in septic patients, all these parameters contribute to immunosuppression and were repeatedly reported to be associated with poor outcomes (mortality, risk of secondary infections, and longer length of ICU stay) (Venet and Monneret, 2018).

So far, although NLR has been studied in cirrhosis (Cai et al., 2017; Bernsmeier et al., 2020; Liu et al., 2021; Magalhães et al., 2021) but never after LT, further phenotyping of additional cell subsets (either neutrophils or lymphocytes) has never been conducted, especially over the pre/post-transplantation period. Explorations in the field may address the unmet clinical need in early recognition of infectious risk in cirrhotic and LT patients. Having similar NLR alterations in cirrhosis and sepsis, we hypothesize that we could observe in cirrhotic patients more detailed neutrophil and lymphocyte phenotype alterations known to be associated with immunosuppression. To this end, along with leukocyte count, we assessed immature neutrophils, LOX-1^{+} MDSC and PD-1 and TIM3 lymphocyte expression in cirrhotic patients before transplantation in association with liver disease severity and during the first month after transplantation. We aimed to better characterize immune alterations in those patients to identify putative biomarkers that may help in defining more individualized medicine.

Materials and methods

Subjects

Patients registered on LT waiting for decompensated cirrhosis or for cirrhosis complicated with hepatocellular carcinoma list at Lyon University Hospital (France) were prospectively enrolled. All patients were eligible to a standard immunosuppressive protocol with administration of simulect (day 0 and day 4), corticoids (at least 7 days), tacrolimus and mycophenolate mofetil. Exclusion criteria were as follows: patients requiring multi-organ transplant, patients treated with immunosuppressors (including patients with history of previous LT) and patients without underlying cirrhosis. This protocol is an ancillary study from EdMonHG study (N°ID-RCB 2019-A00954-53, CT identifier: NCT03995537).

Patients reported in this study were included from January 2022 to September 2022. Peripheral blood samples were collected once at enrolment (within 3 months before LT). Following LT, samples were collected twice a week for 1 month or until the occurrence of infection and/or acute cellular rejection. Post-LT time points were grouped as follows: day 1 to day 3 (D1-D3), day 4 to day 6 (D4-D6), day 7 to day 13 (D7-D13), day 14 to day 20 (D14-D20), day 21 to day 27 (D21-D27) and day 28 to day 31 (D28-D31). Before LT, all clinical data related to cirrhosis severity and aetiologies were collected. All relevant clinical and biological data occurring during and after transplant surgery were recorded. Acute decompensation (AD) of cirrhosis was defined by the acute development of one or more major complications of liver disease (i.e., ascites, hepatic encephalopathy, gastrointestinal haemorrhage and/or bacterial infections) (Moreau et al., 2013). ACLF stage in pre-LT patients were defined according to Moreau's criteria (Moreau et al., 2013). Pre-LT patients were divided into two groups according to Model of End stage Liver Disease (MELD) score, a validated chronic liver disease scoring system that predicts 3-month survival on liver waiting list. A cut-off of MELD score ≥30 was chosen to identify the most severe patients. In addition, patients were stratified according to the Child-Pugh score, which is a clinico-biological scoring system used to assess prognosis of cirrhotic patients. We compared Child-Pugh A or B patients (A/B) with Child-Pugh C patients (the most severe patients).

After LT, any event of acute cellular rejection or sepsis occurrence, according to the criteria of the American Society of Transplantation (Humar et al., 2006) stopped the immune monitoring (i.e., censured forthcoming results) since they both impact immune functions by themselves. Fifteen healthy volunteers (HV) served as controls (samples coming from French Blood Establishment). The median age of HV was 38 years and 33% were male.

Whole blood phenotyping

At each time point, in addition to leukocyte count, we assessed immature neutrophils (CD16^{low}) and LOX-1^{+} MDSC (CD15^{+}, CD45^{dim}, LOX-1^{+} polymorphonuclear cells) percentages as described by Coudereau et al. (2022) and immune checkpoint inhibitor (PD-1 and TIM-3) expression on CD3, CD4 and CD8 T lymphocytes. Cell staining was performed on fresh whole blood sample within 4 h after sampling. We used the following antibodies: CD45-PB, CD3-APC-AF750, CD4-FITC, CD8-Kro, CD14-PB, CD16-APC from BeckmanCoulter (Brea, CA) and: PD1-APC, TIM-3-PE-Dazzle, CD15-AF700, LOX1-PE from BioLegend (San Diego, CA). Isotype control antibodies (BioLegend) were used to determine the percentages of positive cells for PD-1, TIM-3 and LOX-1. Samples were run on Navios flow cytometer (Beckman Coulter). T lymphocytes subsets' absolute quantification was performed on Aquios flow cytometer (Beckman Coulter). Detailed protocols are presented in supplementary methods. Results were expressed as absolute counts for neutrophil subsets and T lymphocyte subsets (i.e., cells/mm3). Results were expressed as absolute cell counts for immature neutrophils and LOX-1^{+} MDSC. Immune checkpoint inhibitor expressions on T lymphocyte subsets were expressed as percentages of positive cells based on isotype controls.

Statistics

Statistical analyses were performed with the software RStudio (2021.09.2 + 382 version). Data are presented on boxplot graph with medians, interquartile ranges and individual values. Non-parametric Mann-Whitney, Fisher's exact test and $\chi 2$ tests were used to assess differences between groups. When appropriate, ANOVA test was used to assess differences between more than 2 independent groups. If ANOVA assumptions were not verified Kruskal–Wallis test was performed. Spearman coefficient was used to assess correlation between quantitative data. Statistical significance was assumed at $p < 0.05$. Due to relatively low number of transplanted patients, we did not perform statistical analysis after LT. Given the exploratory nature of the present observational study, no power analysis was performed.

Results

Patients' characteristics

During the study period, 28 cirrhotic patients were enrolled in this study. Clinical characteristics are presented in Table 1. Briefly, the median age was 58 years and 86% were male. Alcohol-related liver disease represented 53% of the cirrhosis aetiology. 7% of

TABLE 1 Patients characteristics of whole cohort and according to MELD score.

Patients characteristics	All patients (n = 28)	Patients with MELD <30 (n = 20)	Patients with MELD ≥30 (n = 8)	p
Demographic characteristics				
Age (years)	58 [37—68]	61.5 [48—68]	55 [37—61]	<0.01
Sex (male)	24 (86)	17 (85)	7 (88)	NS
Cirrhosis Aetiology				
Alcohol	15 (53)	10 (50)	5 (63)	NS
Dysmetabolic	2 (7)	2 (10)	0 (0)	
HCV	1 (4)	1 (5)	0 (0)	
Mixed cirrhosis				
Alcohol/dysmetabolic	5 (18)	5 (25)	0 (0)	
Alcohol/viruses	2 (7)	1 (5)	1 (13)	
Others	3 (11)	1 (5)	2 (25)	
Decompensation stages				<0.001
Compensated	8 (29)	8 (40)	0 (0)	
Chronic decompensation (CD)	8 (29)	8 (40)	0 (0)	
Acute decompensation (AD)	12 (43)	4 (20)	8 (100)	
Aetiology of AD				NS
Infection	8 (67)	4 (100)	4 (50)	
AAH	1 (8)	0 (0)	1 (13)	
HBV reactivation	1 (8)	0 (0)	1 (13)	
Wilson disease	1 (8)	0 (0)	1 (13)	
Alcohol intake	1 (8)	0 (0)	1 (13)	
Clinical parameters				
Active smokers	10 (36)	8 (40)	2 (25)	NS
Diabetes	9 (32)	9 (45)	0 (0)	0.03
HBP	12 (42)	10 (50)	2 (25)	NS
Chronic ascitis	10 (36)	7 (35)	3 (38)	NS
HE (at inclusion)	7 (25)	4 (20)	3 (38)	NS
AKF (at inclusion)	4 (14)	0 (0)	4 (50)	<0.001
Biologic markers				
Bilirubin	70 [5.8—679]	51.8 [5.8—330]	468 [71—679]	<0.001
ALP	108.5 [63—250]	113 [63—250]	69.5 [63—177]	NS
GGT	61.5 [21—273]	68 [21—273]	53 [28—246]	NS
ALT	36.5 [14—139]	33.5 [14—74]	61.5 [23—139]	0.02
AST	54 [15—286]	50.5 [15—130]	81.5 [54—286]	<0.001
Albumin	34.8 [21.5—45.7]	35.7 [22.5—45.7]	24.9 [21.5—39.4]	NS
Sodium	137 [128—142]	136.5 [130—142]	137.5 [128—140]	NS
PT	37.5 [14—100]	45.5 [26—100]	26.5 [14—52]	<0.01
INR	2.02 [1—5.5]	1.79 [1—2.99]	2.88 [1.65—5.5]	<0.01
Factor V	37 [10—123]	59 [21—123]	28.5 [10—76]	0.04
Creatinine	68 [36—275]	63.5 [36—127]	166 [41—275]	NS
Platelets (G/L)	82.5 [12—243]	108 [12—243]	64 [22—216]	NS
Hemoglobin (mg/dL)	9.5 [5.7—16.4]	10.6 [5.7—16.4]	8.7 [6.0—12.1]	0.03
CRP (mg/dL)	15.7 [0.5—50.9]	8.9 [0.5—50.9]	22,9 [19.6—44.7]	0.008
Pronostic scores				
MELD score	24 [6—40]	18 [6—27]	35 [30—40]	<0.001
Child-Pugh score	10 [5—14]	8 [5—13]	11 [10—14]	<0.01
Child-Pugh C	16 (57)	8 (40)	8 (100)	<0.01
SOFA score	4.5 [0—15]	3 [0—10]	8 [6—15]	<0.001
ACLF	10 (36)	2 (10)	8 (100)	<0.001
Immunologic parameters				
Neutrophils (G/L)	4.1 [1.6—20.9]	3.4 [1.6—8.5]	4.9 [2.8—20.9]	0.008
Monocytes (G/L)	0.65 [0.33–1.89]	0.72 [0.33—1.48]	0.59 [0.35—1.89]	NS

(Continued on following page)

TABLE 1 (*Continued*) Patients characteristics of whole cohort and according to MELD score.

Patients characteristics	All patients (n = 28)	Patients with MELD <30 (n = 20)	Patients with MELD ≥30 (n = 8)	p
T lymphocytes (cells/µL)	507 [79—1,479]	737 [79–1,479]	372 [204—1,395]	0.03
NLR	3.1 [1.6—40.2]	2.4 [1.6—8.2]	8.9 [4.5—40.2]	<0.001
Death on waiting list	3 (11)	0 (0)	3 (38)	NS

Quantitative data are presented as medians with minimum and maximum value within square brackets [min–max]. Qualitative data are presented as numbers of cases and percentage among the total population or subpopulation in brackets (%). Prognostic scores and immunologic parameters were calculated the day of patients' inclusion. *p*-values were calculated using Mann-Whitney, Fisher and χ2 tests when appropriate. AAH (acute alcoholic hepatitis). ACLF (acute on chronic liver failure). AD (acute decompensation of cirrhosis). AKF (acute kidney failure). ALP (alkaline phosphatase). ALT (alanine aminotransferase). AST (alanine aminotransferase). CD (chronic decompensation of cirrhosis). CRP (c-reactive protein). MELD (Model of End Stage Liver Disease). NLR (neutrophils to lymphocytes ratio). HBP (high blood pressure). HCV (hepatitis C virus). HE (hepatic encephalopathy). GGT (gamma glutamyl transferase). INR (international standardization ratio). PT (prothrombin time). SOFA (Sequential Organ Failure Assessment).

TABLE 2 Characteristics of transplanted patients.

Patients characteristics	Transplant patients (n = 13)
Decompensation stages	
Compensated	3 (23)
Chronic decompensation (CD)	3 (23)
Acute decompensation (AD)	7 (53)
Pronostic scores	
MELD score ≥30	4 (30)
Child-Pugh C	9 (69)
ACLF	6 (46)
Liver surgery	
Surgery time (minutes)	450 [248—525]
Cold ischaemia (minutes)	420 [278—560]
Red cells transfusion	2 [0—10]
Post-transplant outcomes (during the first month post LT)	
Intensive care length of stay (days)	6 [4—79]
Total duration of vasopressors (days)	0 [0—8]
Total duration of MV (days)	0 [0—29]
Surgical revision	4 (31)
Graft dysfunction at day 7*	6 (46)
Infectious event	5 (38)
Reject	1 (8)
One month survival	13 (100)

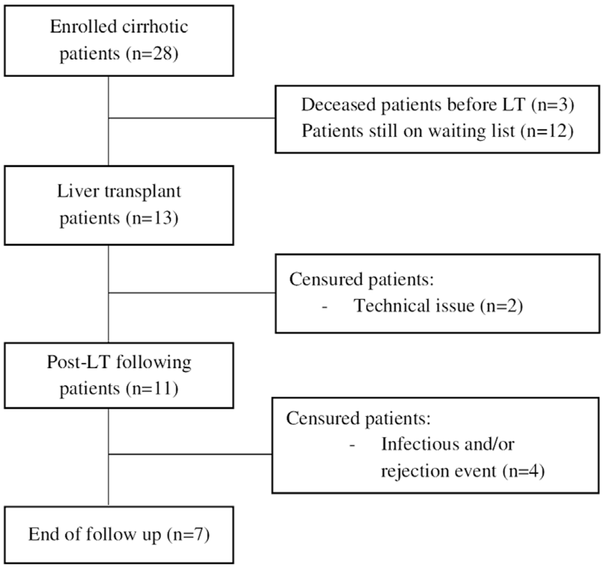

FIGURE 1
Flow chart.

patients had a dysmetabolic cirrhosis and 25% had a mixed cirrhosis (5 patients had a cirrhosis related to dysmetabolic syndrome and alcohol intake and 2 patients had a cirrhosis related to HCV or HBV infection and alcohol intake). One patient had a post hepatitis C cirrhosis. The two patients with background of hepatitis C obtained a viral clearance years before inclusion. The patient with hepatitis B had a patent HBV reactivation at inclusion. 26% of patients had MELD score ≥30 (n = 8) and 43% were in AD (n = 12). Among AD patients, 83% met ACLF criteria (n = 10). All the patients with a MELD score ≥30 were in AD and met ACLF criteria. The causes of AD were infections (n = 8), acute alcoholic hepatitis (AAH) (n = 1), alcohol consumption without AAH (n = 1), HBV reactivation (n = 1) and Wilson's disease exacerbation (n = 1). 38% of patients with a MELD score ≥30 died on waiting list (n = 3). In this cohort, 46% of patients (n = 13) underwent LT (table 2). Of them, 11 were monitored over post-LT period (2 were missing due to mistakes in protocol guidance). Seven patients completed the whole follow-up, 3 presented with sepsis, and last one presented both infection and rejection. Patient's flow chart is presented in Figure 1. Events of infection and reject are summarised in Table 3.

Pre-transplantation results

Total neutrophil count and neutrophil-lymphocyte ratio

Neutrophil count was not significantly different in pre-LT patients (whole cohort) in comparison to HV (Figure 2A). Nevertheless, increased neutrophils were associated with more severe cases according to MELD score (Figure 2B) and were associated with decompensation stages of cirrhosis (Figure 2C). NLR was significantly increased in pre-LT patients in comparison to HV (Figure 2D). Importantly, NLR was higher in patients with MELD score ≥30 and Child-Pugh score C (Figure 2E). Moreover, NLR was significantly associated with decompensation stages of cirrhosis as it was

TABLE 3 Infectious and graft rejection outcomes.

Patients	MELD score	ACLF	Clinical events	Identified germ	Post-transplant days	Intensive care unit stay	One month survival
1	19	No	Pneumoniae and acute cellular rejection	*Klebsiella pneumoniae*	D5 (infection) D6 (reject)	7	yes
2	30	No	Peritonitis	No	D12	72	yes
3	27	No	Pneumoniae	*Pseudomonas aeruginosa*	D10	68	yes
4	36	Yes	Infectious pleuritis	*Enterococcus* faecium	D17	still in ICU at Ms submission (i.e., 85 days)	yes

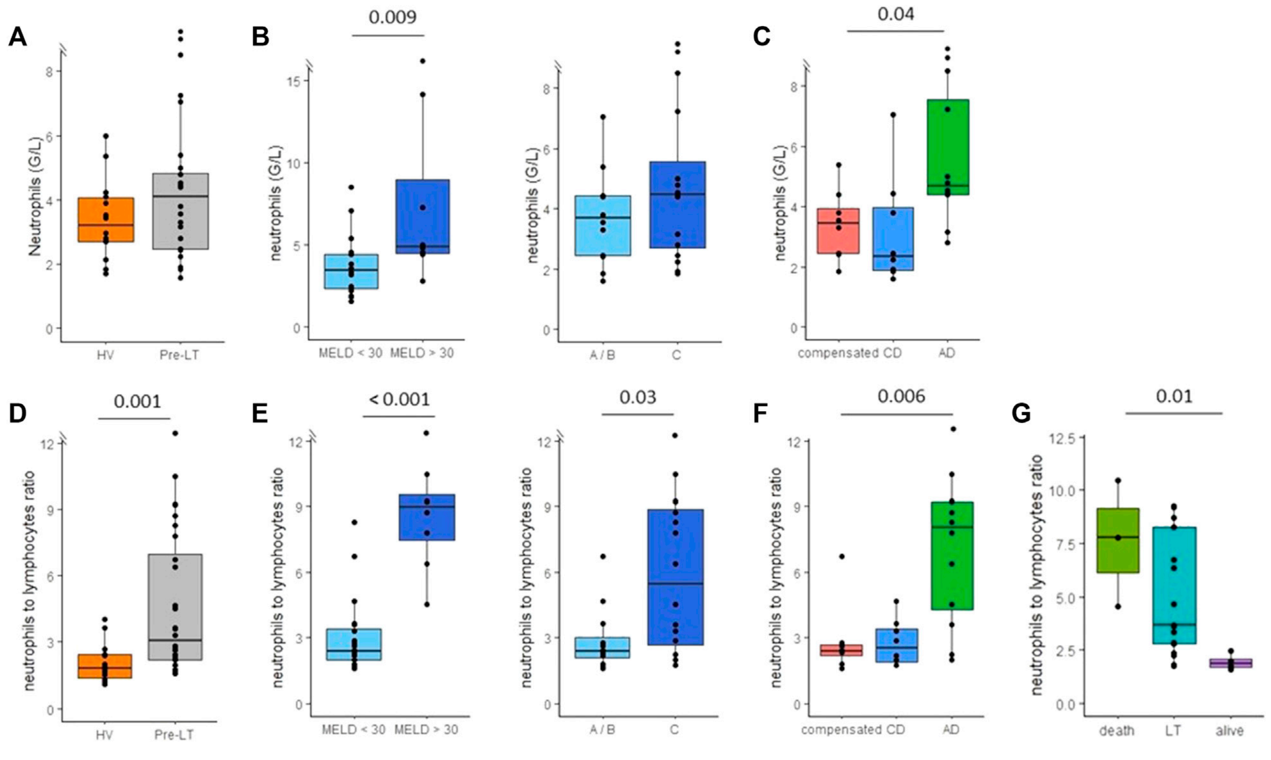

FIGURE 2
Monitoring of neutrophils count and neutrophils to lymphocytes ratio (NLR) in peripheral blood of pre-transplant patients. **(A)** Neutrophils count in healthy volunteers (HV, $n = 15$) and pre-transplant patients (pre-LT, $n = 28$). **(B)** Neutrophils count in patients with MELD score < 30 ($n = 20$) or with MELD score ≥ 30 ($n = 8$) and in patients with Child-Pugh score A or B (A/B, $n = 12$) or with Child-Pugh score C ($n = 16$). **(C)** Neutrophils count in patients with compensated cirrhosis ($n = 8$), chronic decompensated cirrhosis (CD, $n = 8$) and acute decompensated cirrhosis (AD, with $n = 10$ or without ACLF $n = 2$). **(D)** NLR in healthy volunteers (HV) and pre-transplant patients (pre-LT). **(E)** NLR in patients with or without MELD score < 30 and in patients with Child-Pugh A/B or C. **(F)** NLR in patients with compensated, chronic decompensated and acute decompensated cirrhosis. **(G)** NLR according to three months evolution post inclusion. The nonparametric Wilcoxon test was used to assess differences between patients and HV and between patients' subgroups determined by MELD and Child-Pugh scores. ANOVA or Kruskal-Wallis tests were used to assess differences between more than 2 independent groups.

predominantly increased in AD patients (Figure 2F). There was a positive correlation between NLR and MELD score (r = 0.7; $p < 0.001$) and between NLR and CRP (r = 0.74; $p = 0.001$). Interestingly, NLR was significantly associated with patients' survival 3 months after inclusion (Figure 2G). There was no transplant free survival in patients with NLR >4. The cause of death was multiple organ failure syndrome secondary to uncontrolled infection for the three patients who died on waiting list.

Neutrophil subsets

Immature CD16^{low} neutrophil counts were significantly increased in pre-LT patients in comparison with HV (Figure 3A). Increased immature neutrophils count was associated with cirrhosis severity according to MELD and Child-Pugh scores (Figure 3B). Moreover, AD patients tended to show increased immature neutrophils count in comparison with compensated and CD patients (Figure 3C). In addition, we found a positive and significant correlation between

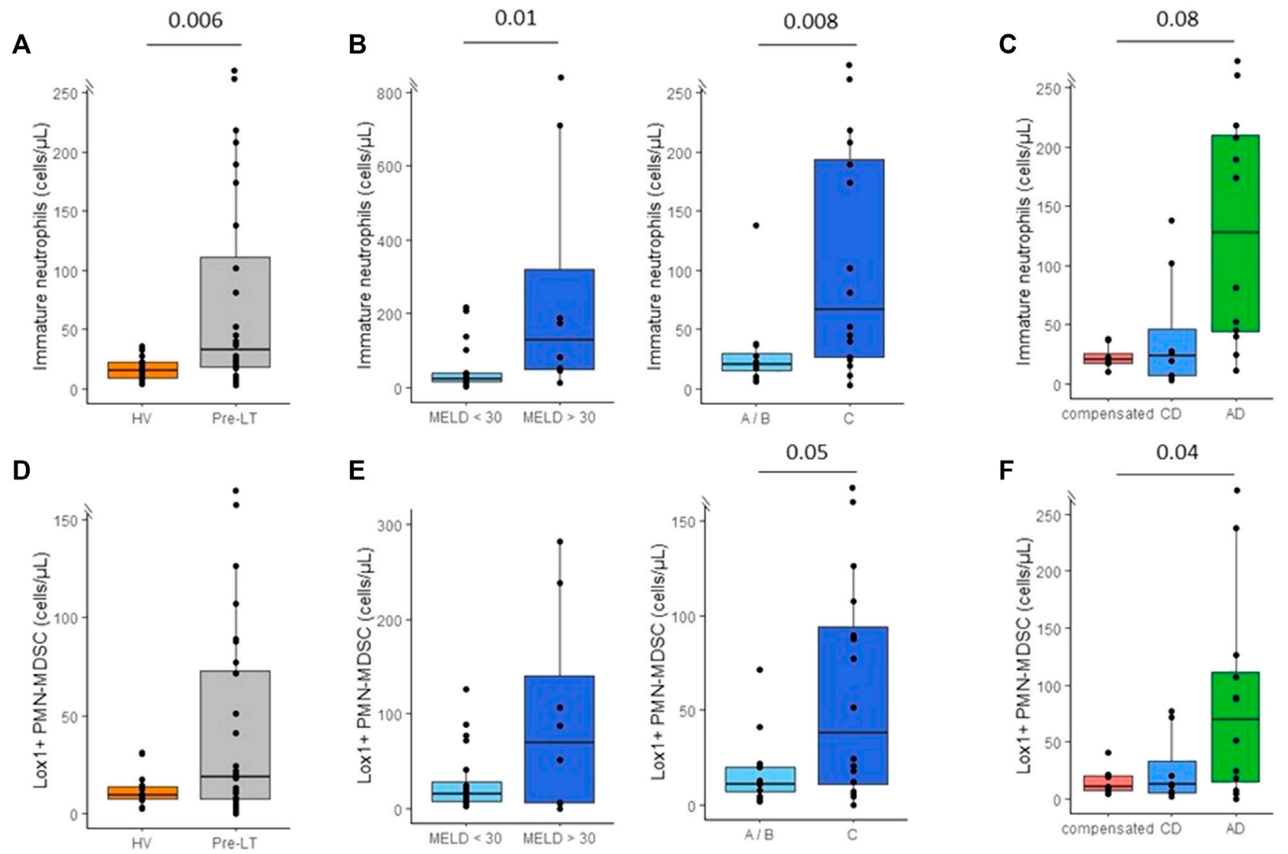

FIGURE 3
Monitoring of immatures neutrophils ($CD16^{low}$) and lectine-type oxidized LDL receptor 1 polymorphonuclear myeloid-derived suppressor cells (LOX1+ PMN-MDSC) in peripheral blood of pre-transplant patients. **(A)** Immature neutrophils count in healthy volunteers (HV, $n = 15$) and pre-transplant patients (pre-LT, $n = 28$). **(B)** Immature neutrophils count in patients with a MELD score < 30 ($n = 20$) or with a MELD score ≥ 30 ($n = 8$) and in patients with Child-Pugh score A or B (A/B, n=12) or with Child-Pugh score C (n=16). (C) Immature neutrophils count in patients with compensated cirrhosis ($n = 8$), chronic decompensated cirrhosis (CD, $n = 8$) and acute decompensated cirrhosis (AD, with $n = 10$ or without ACLF $n = 2$). **(D)** Number of LOX1+ MDSC in healthy volunteers (HV) and pre-transplant patients (pre-LT). **(E)** Number of LOX1+ MDSC in patients with or without MELD score < 30 and in patients with Child-Pugh A/B or C. **(F)** Number of LOX1+ MDSC in patients with compensated, chronic decompensated, and acute decompensated cirrhosis. The nonparametric Wilcoxon test was used to assess differences between patients and HV and between patients' subgroups determined by MELD and Child-Pugh score. Kruskal-Wallis test was used to assess differences between more than 2 independent groups.

immature neutrophil counts and CRP ($r = 0.60$, $p = 0.016$) and MELD score ($r = 0.56$, $p = 0.0039$). Although clearly elevated in some patients, LOX-1$^+$ MDSC counts were not significantly different between patients and HV (Figure 3D). Regarding association with pre-LT severity, solely AD patients presented with significantly elevated values (Figure 3F). Importantly, immature neutrophils and LOX-1$^+$ MDSC counts were significantly correlated to NLR ($r = 0.57$, $p = 0.002$; and $r = 0.4$, $p = 0.034$ respectively). Noteworthy, immature neutrophils and LOX-1$^+$ MDSC counts were not increased neither in patients with active hepatocellular carcinoma ($n = 4$) nor with patients transplanted for hepatocellular carcinoma ($n = 10$) (data not shown).

T lymphocyte counts

We observed a profound T lymphopenia in cirrhotic patients in comparison to HV. This affected both CD4$^+$ (median: 496 CD4$^+$ cells/mm^{3,} Figure 4A) and CD8$^+$ (median: 148 CD8$^+$ cells/mm^{3,} Figure 4D) T lymphocyte subsets in pre-LT patients. Lymphopenia was significantly more important in patients with MELD score ≥30 compared to patients with MELD score <30 (Figures 4B, E). Interestingly, CD8$^+$ T cells count was significantly decreased in compensated patients in comparison to HV ($p = 0.002$). Moreover, lymphopenia tented to accentuate during decompensated stages of cirrhosis (Figures 4C, F). CD3$^+$ T cells count was negatively correlated to CRP ($r = -0.73$; $p = 0.002$).

Immune checkpoint inhibitor expressions on T lymphocyte subsets

PD-1 and TIM3 expressions on CD3$^+$ T lymphocytes were not different between HV and pre-LT patients (Figures 5A, D). Overall, PD-1 and TIM3 expressions were not associated with cirrhosis severity according to MELD and Child-Pugh scores (Figures 5B, E) or with decompensation stages of cirrhosis (Figures 5C, F). These results were similar on CD8$^+$ and CD4$^+$ T cells (data not shown).

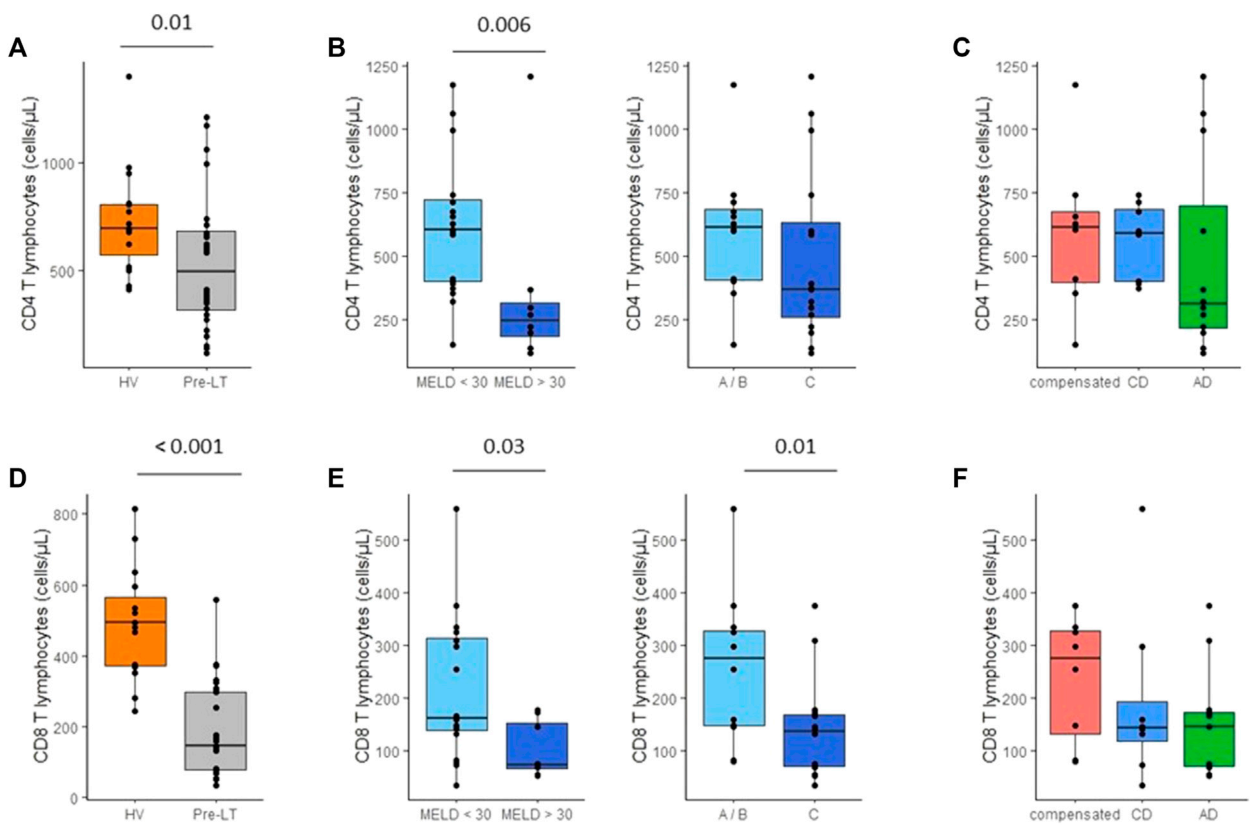

FIGURE 4
Monitoring of CD4+ and CD8+ T cell counts in peripheral blood of pre-transplant patients. **(A)** CD4+ T lymphocytes count in healthy volunteers (HV, *n* = 15) and pre-transplant patients (pre-LT, *n* = 28). **(B)** CD4+ T lymphocytes count in patients with a MELD score < 30 (*n* = 20) or with a MELD score ≥ 30 (*n* = 8) and in patients with Child-Pugh score A or B (A/B, *n* = 12) or with Child-Pugh score C (*n* = 16). **(C)** CD4= T lymphocytes count in patients with compensated cirrhosis (*n* = 8), chronic decompensated cirrhosis (CD, *n* = 8). **(D)** CD8+ T lymphocytes count in healthy volunteers (HV) and pre-transplant patients (pre-LT). **(E)** CD8+ T lymphocytes count in patients with or without MELD score < 30 and in patients with Child-Pugh A/B or C. **(F)** CD8+ T lymphocytes count in patients with compensated, chronic decompensated, and acute decompensated cirrhosis. The nonparametric Wilcoxon test was used to assess differences between patients and HV and between patients' subgroups determined by MELD and Child-Pugh score.

Importantly, as alcohol is able to induce PD-1 and TIM3 expressions *in vitro* (Markwick et al., 2015), we verified that immune checkpoint receptors were not differently expressed in alcohol consumer patients (n = 5) compared non-alcoholic and weaned patients (n = 23, data not shown). Moreover, as immune checkpoint receptors might be overexpressed in cancer, we addressed this aspect but noticed that PD-1 and TIM3 were not differently expressed in patients with active hepatocellular carcinoma (n = 4). In addition, there were no differences between patients enrolled on waiting list for hepatocellular carcinoma (n = 10) and patients without medical history of hepatocellular carcinoma (n = 18, data not shown).

Post-transplant results

Total neutrophil count, neutrophil-lymphocyte ratio and neutrophil subsets

After LT, we observed a tremendous increase of neutrophils count at D1-D3 post-LT. Then, neutrophils count decreased and reached pre-LT values during the third week post-LT (Figure 6A). In accordance, we observed an important rise of NLR at D1-D3 following LT (Figure 6B). However, this elevation was transitory and decreased at D4-D6 post-LT and remained stable until 1-month post-LT. However, throughout this follow-up, NLR remained higher than that from HV controls. According to total neutrophil count, immature neutrophils count peaked at D1-D3 after LT and then returned to pre-LT values at D4-D6 (Figure 7A). At the end of follow-up, immature neutrophils count remained slightly higher (median: 37 cells/mm^3) than HV value (median: 15 cells/mm^3). In contrast, LOX-1$^+$ MDSC count presented with a different kinetic. LOX-1$^+$ MDSC count remained stable during the first week after LT (Figure 7B) but reached a maximum during the second week post-LT (D7-D13). This elevation was transitory as LOX-1$^+$ MDSC rapidly went back down to low values (median: 20 cells/mm^3) similar to those observed in HV controls (median: 9 cells/mm^3 in HV).

T lymphocyte counts

Despite being already low before LT, lymphopenia amplified after transplantation (Figure 8A). Nadir was

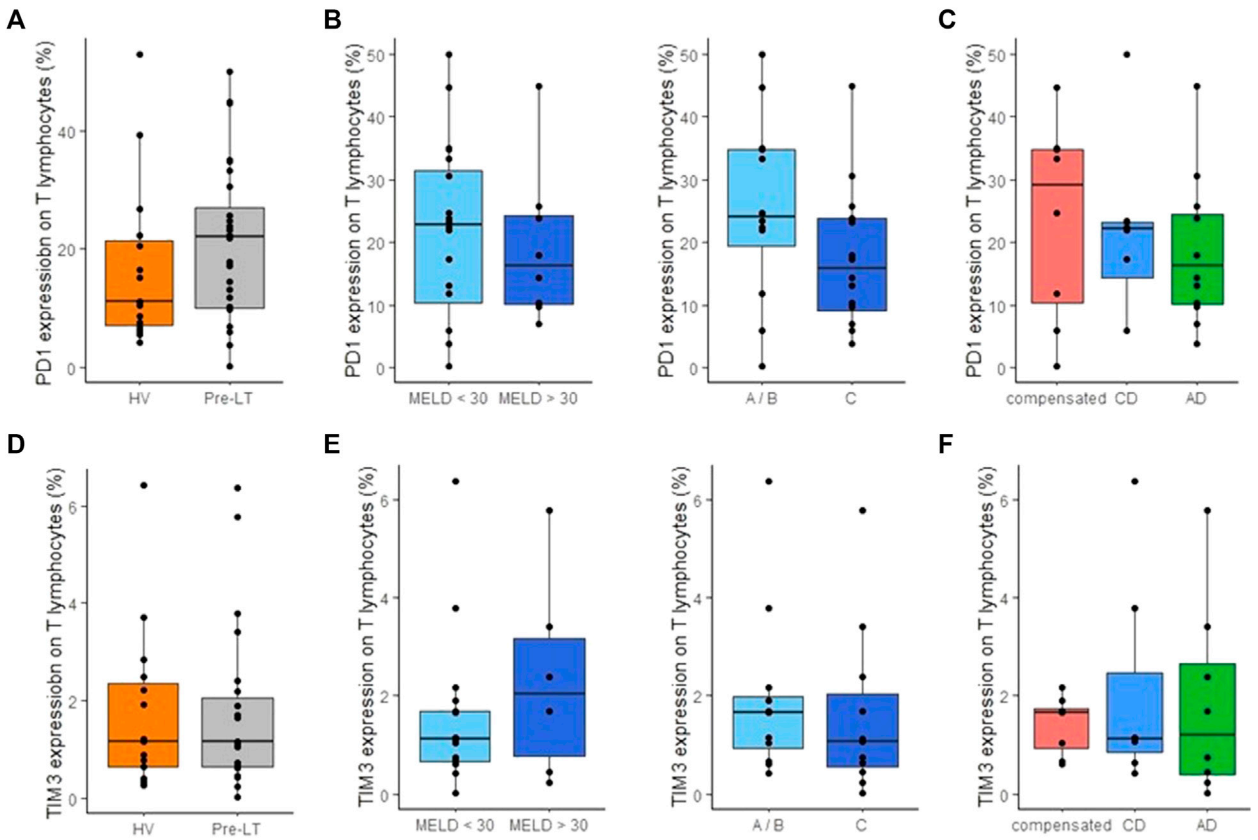

FIGURE 5
Monitoring of PD-1 and TIM3 expression on T cells in peripheral blood of pre-transplant patients. **(A)** Percentage of PD-1 expression on CD3+ T lymphocytes in healthy volunteers (HV, *n* = 15) and pre-transplant patients (pre-LT, *n* = 28). **(B)** Percentage of PD-1 expression on CD3+ T lymphocytes in patients with a MELD score < 30 (*n* = 20) or with a MELD score ≥ 30 (*n* = 8) and in patients with Child-Pugh score A or B (A/B, *n* = 12) or with Child-Pugh score C (*n* = 16). **(C)** Percentage of PD-1 expression on CD3+ T lymphocytes in patients with compensated cirrhosis (*n* = 8), chronic decompensated cirrhosis (CD, *n* = 8) and acute decompensated cirrhosis (AD, with *n* = 10 or without ACLF *n* = 2). **(D)** Percentage of TIM3 expression on CD3+ T lymphocytes in healthy volunteers (HV) and pre-transplant patients (pre-LT). **(E)** Percentage of TIM3 expression on CD3= T lymphocytes in patients with compensated, chronic decompensated, and acute decompensated cirrhosis. **(F)** Percentage of PD-1 expression on CD3+ T lymphocytes in patients with or without a MELD score < 30 and in patients with Child-Pugh A/B or C. The nonparametric Wilcoxon test was used to assess variations between patients and HV and between patients' subgroups determined by MELD and Child-Pugh score. Kruskal-Wallis test was used to assess differences between more than 2 independent groups.

observed at D1-D3 post-LT. This profoundly affected all T cells subsets (medians as follows: $CD3^+$ T cells: 192 cells/mm^3, $CD4^+$ T cells:131 cells/mm^3, $CD8^+$ T cells: 50 cells/mm^3). Thereafter, T lymphocytes increased at levels similar to pre-LT values during the second week post-surgery. However, at the end of follow-up, patients still presented with marked lymphopenia (Figure 8A). In parallel, we observed a progressive over expression of both TIM-3 and PD1 checkpoint inhibitor expressions on circulating T lymphocytes, TIM3 expression reached a maximum around 2–3 weeks post-LT and then remained stable (Figure 8B). Even if it was less clear, PD-1 tended to follow same pattern of expression (Figure 8C). Similar results were observed on both $CD4^+$ and $CD8^+$ T lymphocytes (data not shown).

Discussion

To the best of our knowledge, this preliminary study is the first to present a detailed neutrophils and T lymphocytes immune phenotyping overtime in cirrhotic patients before and after liver transplantation. These results provide valuable additional information and markers (LOX-1, TIM-3, PD-1) to complete previous results obtained in cirrhotic patients solely based on NLR.

NLR is believed to be associated with cirrhosis severity and mortality. Cai et al. reported that this parameter was an independent predictors of hospital-acquired bacterial infections in decompensated cirrhosis (Cai et al., 2017). They also demonstrated that cirrhotic patients presenting with NLR

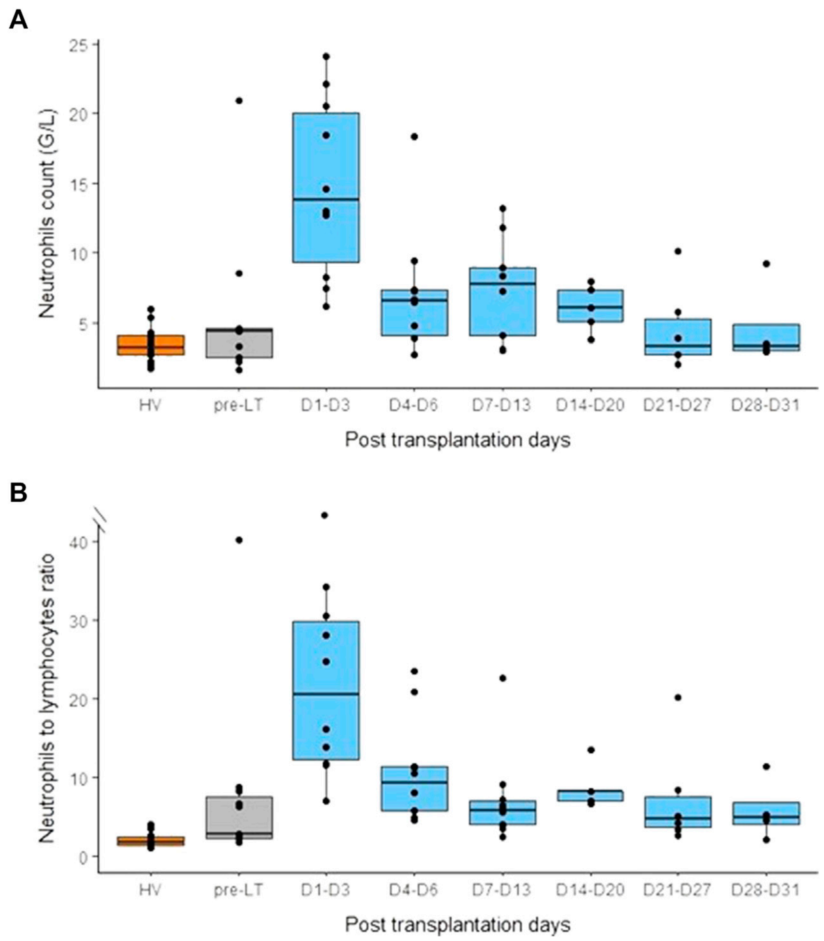

FIGURE 6
Monitoring of neutrophils count and neutrophils to lymphocytes ratio (NLR) before and after liver transplantation. **(A)** Neutrophils count in healthy volunteers (HV, n = 15), pre-transplant patients (pre-LT, n = 11) and after liver transplantation at different time points (day 1 to day 3, n = 10; day 4 to day 6, n = 10; day 7 to day 13, n = 10; day 14 to day 20, n = 5; day 21 to day 27, n = 6; day 28 to day 31, n = 4). **(B)** NLR in healthy volunteers (HV), pre-transplant patients (pre-LT) and following transplantation at different time points. Pre-transplant data only concern patients that benefited from transplantation.

superior or equal to 4.33 had a significantly lower survival. Others studies reported that NLR was associated with mortality in cirrhosis, both in patients with MELD score < 20 (Kalra et al., 2017) and in ACLF patients (Bernsmeier et al., 2020). The present results thus confirmed those previous findings. This composite biomarker reflects the balance between granulopoiesis induced by inflammation and lymphopenia. Whereas massive rise in neutrophils occurred in the most severe cirrhotic patients (i.e., at a time of tremendous inflammation), lymphopenia seems to be an earlier event in cirrhosis pathophysiology as it appeared in patients even at compensated stage of cirrhosis. Defect of thymopoiesis and activation-driven cell-death induced by bacterial translocation have been demonstrated to sustain this lymphopenic process (Lario et al., 2013). We extended these results by showing that mostly immature neutrophils and to a lower extent immunosuppressive LOX-1^{+} MDSC contributed to neutrophil rise before LT. This suggests that neutrophil and NLR rise before LT was mainly due to massive inflammatory response and emergency granulopoiesis (including immature cells) in ACLF patients. In contrast, MDSC, usually released in a more chronic manner are less elevated. This may explain why LOX-1^{+} MDSC are less correlated to severity than neutrophils (and subsequently NLR) and immature neutrophils. Overall, the present neutrophil results completed previous studies reporting on neutrophil dysfunction in cirrhotic patients including alterations of migration, oxidative burst and phagocytic capacity (Fiuza et al., 2000; Panasiuk et al., 2005; Tritto et al., 2011). Two studies also described reduced CD16 expression on neutrophils (Taylor et al., 2014; Markwick et al., 2015) which characterizes immature neutrophils, cells known to be less efficient in opsonisation and bacteria lysis (Drifte et al., 2013).

Consequently, as observed in sepsis, the most severe cirrhotic patients with marked neutrophil phenotypic may be at higher risk of infection. In line, we observed that patients who died due to sepsis occurrence before LT presented with significantly higher NLR compared with patients who

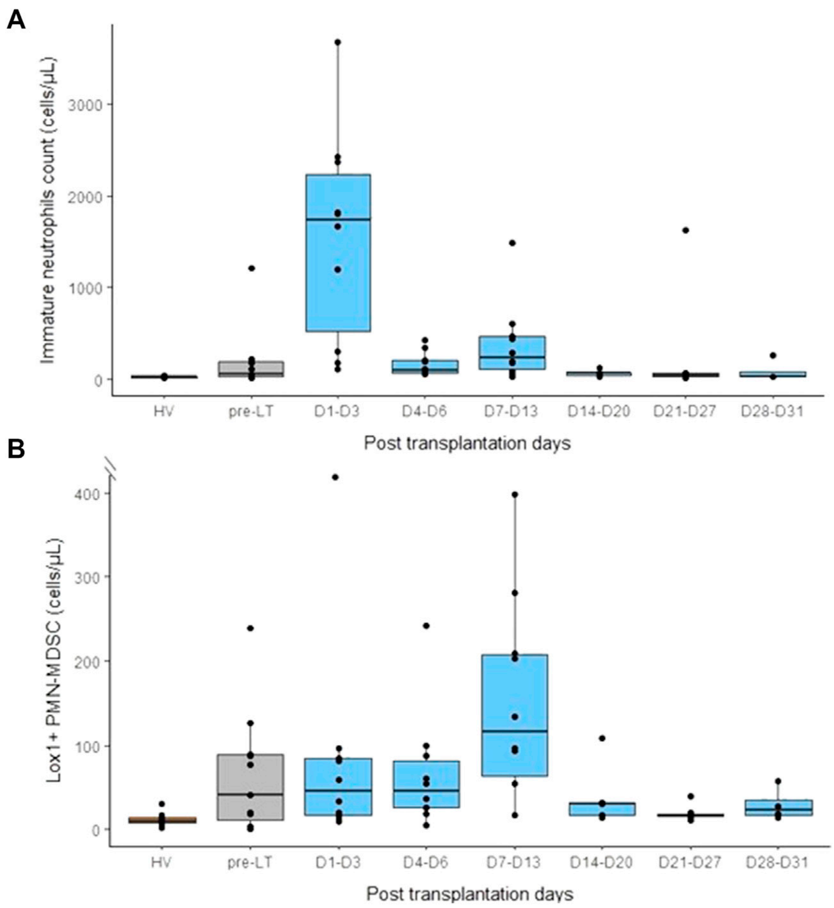

FIGURE 7
Monitoring of immature neutrophils (CD16low) and lectine-type oxidized LDL receptor 1 polymorphonuclear myeloid-derived suppressor cells (LOX1+ PMN-MDSC) in peripheral blood before and after liver transplantation. **(A)** Immature neutrophils count in healthy volunteers (HV, n = 15), pre-transplant patients (pre-LT, n = 11) and after liver transplantation at different time points (day 1 to day 3, n = 10; day 4 to day 6, n = 10; day 7 to day 13, n = 10; day 14 to day 20, n = 5; day 21 to day 27, n = 6; day 28 to day 31, n = 4). **(B)** Number of Lox1+ PMN-MDSC in healthy volunteers (HV), pre-transplant patients (pre-LT) and following transplantation at different time points. Pre-transplant data only concern patients that benefited from transplantation.

survived. In addition, we may hypothesize a role for LOX-1$^+$ MDSC. Indeed, MDSC are immature neutrophils with immunosuppressive properties as they are potent repressors of T-cell response (Gabrilovich, 2017). They expand under pathological conditions associated with acute or chronic inflammation such as sepsis (Schrijver et al., 2019), cancers (Cassetta et al., 2020), or chronic infections (Pallett et al., 2015). In these contexts, the presence of PMN-MDSC respectively promoted nosocomial infections, cancer progression and persistent viral infections. In the present work, we focused on LOX-1$^+$ MDSC since LOX-1 is the sole marker of granulocytic MDSC measurable in whole blood (Condamine et al., 2016; Coudereau et al., 2022). Thus, we likely underestimated the total number of MDSC. In hepatology, only one study reported of granulocytic MDSC in alcohol cirrhosis, especially in Child-Pugh B and C patients (Gao et al., 2019). In agreement, the present results showed increased LOX-1$^+$ MDSC in Child-Pugh C patients. More studies are required to assess the potential role of MDSC in the pathophysiology of cirrhosis associated immune suppression.

Immune checkpoint receptors are co-inhibitory molecules expressed on immune cells that downregulate the immune response in order to promote homeostasis after immune activation. Engagement of PD-1 and TIM3 pathways on T lymphocytes leads to the inhibition of the second signal of T cell activation. High and sustained expression of the co-inhibitory molecules during persistent antigen stimulation has been shown to promote immune cells exhaustion in cancer, sepsis (Rienzo et al., 2022) and chronic hepatitis B and C (Osuch et al., 2020; Li et al., 2022). Several studies described a slight increase in PD-1 and/or TIM-3 lymphocyte expressions in acute alcoholic hepatitis/cirrhosis (Markwick et al., 2015; Lebossé et al., 2019; Riva et al., 2021; Fadriquela et al., 2022). However, in the present work, PD-1 and TIM3 expressions on T lymphocytes were not significantly different between HV and pre-LT patients and were not associated with cirrhosis severity according to MELD and Child-Pugh scores or with decompensation stages of cirrhosis.

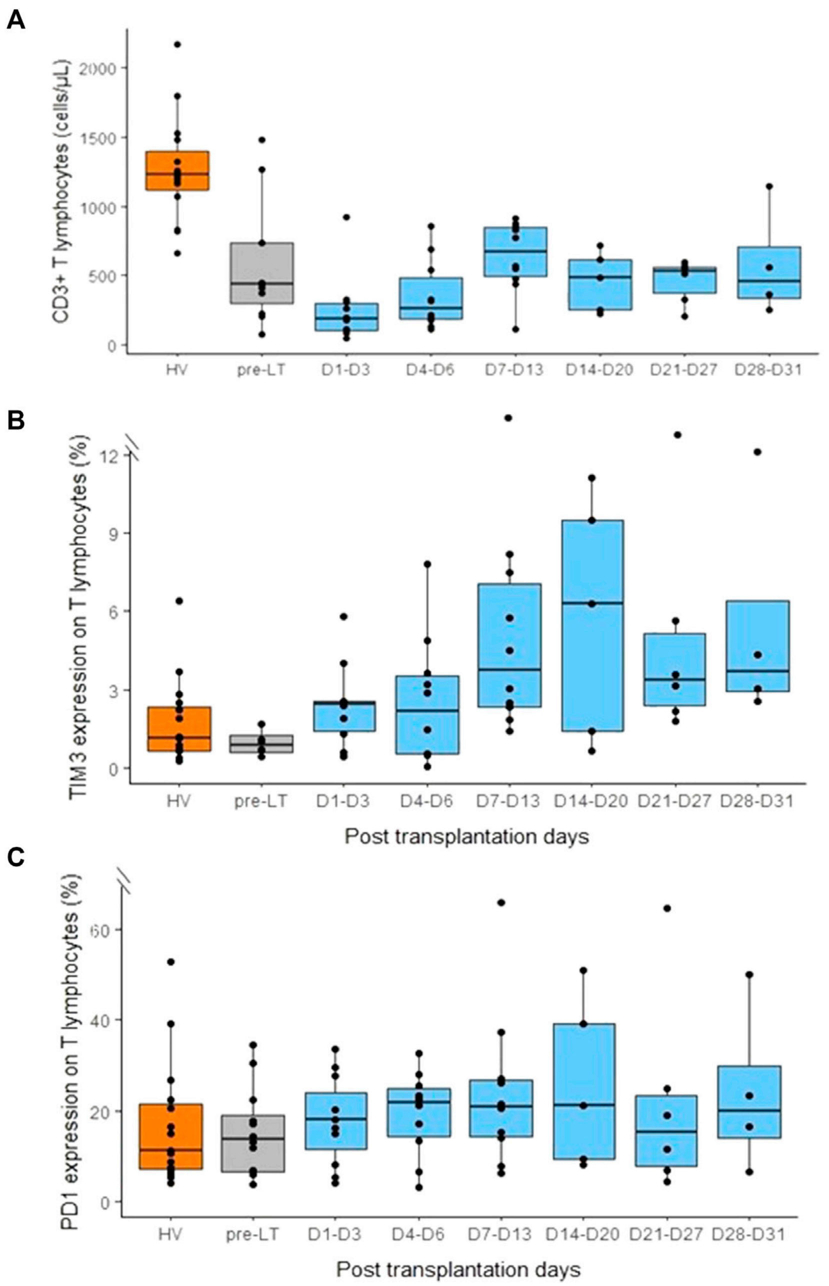

FIGURE 8
Monitoring of CD3+ T lymphocytes count and PD-1 and TIM3 expression on CD3+ T lymphocytes in peripheral blood before and after liver transplantation. **(A)** CD3+ T lymphocytes count in healthy volunteers (HV, *n* = 15), pre-transplant patients (pre-LT, *n* = 11) and following transplantation at different time points (day 1 to day 3, *n* = 10; day 4 to day 6, *n* = 10; day 7 to day 13, *n* = 10; day 14 to day 20, *n* = 5; day 21 to day 27, *n* = 6; day 28 to day 31, *n* = 4). **(B)** Percentage of TIM3 expression on CD3+ T lymphocytes in healthy volunteers (HV), pre-transplant patients (pre-LT) and following transplantation at different time points. **(C)** Percentage of PD-1 expression on CD3+ T lymphocytes in healthy volunteers (HV), pre-transplant patients (pre-LT) and following transplantation at different time points at different time points. Pre-transplant data only concern patients that benefited from transplantation.

Taken together, before LT, results indicated that out of viral induced cirrhosis, infectious risk in cirrhotic patients would be more induced by immature/suppressive neutrophil subsets and profound lymphopenia rather than by increased immune checkpoint inhibitors expressions.

Regarding post-LT results, the immediate augmentation of NLR after LT is most likely the sum of multiple causes mixing both inflammatory signals and accentuated lymphopenia induced by immunosuppressant regimen, surgery, ischemia-reperfusion injury and per operative bleeding. This point needs further explorations including a larger number of patients in order to perform multiparametric analyses. As immature neutrophil count rapidly decreased after LT, it most likely does not participate to post-LT infection risk. Interestingly, LOX-1^{+} MDSC count increased 1 week after surgery. Condamine et al. revealed that these cells accumulated as the result of two groups of signals: those promoting myelopoiesis (mainly by inflammatory cytokines) and suppressive signals as occurring after transplantation (Condamine et al., 2016). In addition, as MDSC have a role in tissue repair, we may hypothesize that hepatic recruitment of these cells may contribute to counteract liver damage due to ischemia-reperfusion injury. Further exploration would be of utmost interest to associate these observations with liver dysfunction/rejection after transplantation. Not surprisingly, lymphopenia worsened days after transplantation and remained at low values throughout follow-up. Most importantly, we observed a progressive over expression of checkpoint inhibitor expressions on both $CD4^+$ and $CD8^+$ T cells. TIM3 expression reached a maximum around 2–3 weeks post-LT and then remained stable. In line, Mysore et al. showed that patients who developed infection during the first year post-LT had elevated co-expressions of PD-1 and TIM3 on T lymphocytes 30 days after LT (Mysore et al., 2018). Accordingly, another study revealed that PD-1 expression on CMV-specific CD8 T cells was elevated preceding CMV reactivation in LT patients (La Rosa et al., 2008). One the opposite side, checkpoint inhibitors might also contribute to immune tolerance in order to prevent graft rejection (Gong et al., 2017). Noteworthy, we noticed that during post-LT follow-up, LOX-1^+ MDSC count and TIM-3 expression tended to peak at the same time (around 2 weeks after LT). One may hypothesize a common inducer for both mechanisms which remained to be investigated. Overall, the current preliminary data deserve further evaluations as they may provide novel understanding of immunosuppression occurring after LT.

Although the present study presents novelties regarding NLR by concomitantly assessing neutrophil ($CD16^{low}$, $LOX1^+$) and T lymphocyte (PD-1, TIM-3) subsets before and after transplantation, we acknowledge some limitations of this study. First, as a preliminary study, the number of included patients was low, especially in post-transplant period which did not allow us to associate immune parameters with clinical events after LT (sepsis, rejection). Second, only one single sample was performed pre-LT sample whereas elapsed time until transplantation was heterogeneous. This aspect should be better controlled in forthcomings studies. Lastly, along with cell count and checkpoint inhibitor expression, T cell and neutrophil functionality testing was not performed but may contribute to better understanding of post-LT immunosuppression.

In conclusion, the present study showed that NLR, immature neutrophils and LOX-1^+ MDSC counts along with T lymphocyte count and checkpoint inhibitor expression were altered in cirrhotic patients before and after LT. These data illustrate the potential interest of immune monitoring of cirrhotic patients in the context of LT in order to better define risk of sepsis or rejection. For this purpose, larger cohorts of patients, including phenotypic and functional testing, are now necessary in order to move forward a more personalised care of LT 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 author.

Ethics statement

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes Ile de France XI. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MC-D, FV, FL, and GM, conceived the original idea. SP, TA, FV, FZ, J-YM, JD, FL, and AR included patients. AR, MH, MC-D, and RC performed all flow cytometry staining and analysis. AR, FV, FL, and GM wrote the manuscript. All authors contributed to the article and approved submitted version.

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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/fphys.2023.1095723/full#supplementary-material

References

Abensur Vuillaume, L., Le Borgne, P., Alamé, K., Lefebvre, F., Bérard, L., Delmas, N., et al. (2021). Neutrophil-to-Lymphocyte ratio and early variation of NLR to predict in-hospital mortality and severity in ED patients with SARS-CoV-2 infection. *J. Clin. Med.* 10 (12), 2563. doi:10.3390/jcm10122563

Albillos, A., de Gottardi, A., and Rescigno, M. (2020). The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J. Hepatol.* 72 (3), 558–577. doi:10.1016/j.jhep.2019.10.003

Albillos, A., Martin-Mateos, R., Van der Merwe, S., Wiest, R., Jalan, R., and Álvarez-Mon, M. (2021). Cirrhosis-associated immune dysfunction. *Nat. Rev. Gastroenterol. Hepatol.* 19, 112–134. doi:10.1038/s41575-021-00520-7

Anthony, P. P., Ishak, K. G., Nayak, N. C., Poulsen, H. E., Scheuer, P. J., and Sobin, L. H. (1977). The morphology of cirrhosis: Definition, nomenclature, and classification. *Bull. World Health Organ* 55 (4), 521–540.

Arvaniti, V., D'Amico, G., Fede, G., Manousou, P., Tsochatzis, E., Pleguezuelo, M., et al. (2010). Infections in patients with cirrhosis increase mortality four-fold and should Be used in determining prognosis. *Gastroenterology* 139 (4), 1246–1256. doi:10.1053/j.gastro.2010.06.019

Bernsmeier, C., Cavazza, A., Fatourou, E. M., Theocharidou, E., Akintimehin, A., Baumgartner, B., et al. (2020). Leucocyte ratios are biomarkers of mortality in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. *Aliment. Pharmacol. Ther.* 52 (5), 855–865. doi:10.1111/apt.15932

Boomer, J. S., Shuherk-Shaffer, J., Hotchkiss, R. S., and Green, J. M. (2012). A prospective analysis of lymphocyte phenotype and function over the course of acute sepsis. *Crit. Care Lond Engl.* 16 (3), R112. doi:10.1186/cc11404

Cai, Y. J., Dong, J. J., Dong, J. Z., Yang, N. B., Song, M., Wang, Y. Q., et al. (2017). Neutrophil-lymphocyte ratio predicts hospital-acquired bacterial infections in decompensated cirrhosis. *Clin. Chim. Acta* 469, 201–207. doi:10.1016/j.cca.2017.04.011

Cassetta, L., Bruderek, K., Skrzeczynska-Moncznik, J., Osiecka, O., Hu, X., Rundgren, I. M., et al. (2020). Differential expansion of circulating human MDSC subsets in patients with cancer, infection and inflammation. *J. Immunother. Cancer* 8 (2), e001223. doi:10.1136/jitc-2020-001223

Condamine, T., Dominguez, G. A., Youn, J. I., Kossenkov, A. V., Mony, S., Alicea-Torres, K., et al. (2016). Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci. Immunol.* 1 (2), aaf8943. doi:10.1126/sciimmunol.aaf8943

Coudereau, R., Waeckel, L., Cour, M., Rimmele, T., Pescarmona, R., Fabri, A., et al. (2022). Emergence of immunosuppressive LOX-1+ PMN-MDSC in septic shock and severe COVID-19 patients with acute respiratory distress syndrome. *J. Leukoc. Biol.* 111 (2), 489–496. doi:10.1002/JLB.4COVBCR0321-129R

Drifte, G., Dunn-Siegrist, I., Tissières, P., and Pugin, J. (2013). Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. *Crit. Care Med.* 41 (3), 820–832. doi:10.1097/CCM.0b013e318274647d

Fadriquela, A., Kim, C. S., Lee, K. J., Kang, S. H., and Lee, J. H. (2022). Characteristics of immune checkpoint regulators and potential role of soluble TIM-3 and LAG-3 in male patients with alcohol-associated liver disease. *Alcohol Fayettev N.* 98, 9–17. doi:10.1016/j.alcohol.2021.10.002

Finkenstedt, A., Nachbaur, K., Zoller, H., Joannidis, M., Pratschke, J., Graziadei, I. W., et al. (2013). Acute-on-chronic liver failure: Excellent outcomes after liver transplantation but high mortality on the wait list. *Liver Transpl.* 19 (8), 879–886. doi:10.1002/lt.23678

Fiuza, C., Salcedo, M., Clemente, G., and Tellado, J. M. (2000). *In vivo* neutrophil dysfunction in cirrhotic patients with advanced liver disease. *J. Infect. Dis.* 182 (2), 526–533. doi:10.1086/315742

Gabrilovich, D. I. (2017). Myeloid-derived suppressor cells. *Cancer Immunol. Res.* 5 (1), 3–8. doi:10.1158/2326-6066.CIR-16-0297

Gao, M., Huang, A., Sun, Z., Sun, Y., Chang, B., Zhang, J. Y., et al. (2019). Granulocytic myeloid-derived suppressor cell population increases with the severity of alcoholic liver disease. *J. Cell. Mol. Med.* 23 (3), 2032–2041. doi:10.1111/jcmm.14109

Gong, J., Cao, D., Chen, Y., Li, J., Gong, J., and Zeng, Z. (2017). Role of programmed death ligand 1 and Kupffer cell in immune regulation after orthotopic liver transplantation in rats. *Int. Immunopharmacol.* 48, 8–16. doi:10.1016/j.intimp.2017.04.009

Guignant, C., Lepape, A., Huang, X., Kherouf, H., Denis, L., Poitevin, F., et al. (2011). Programmed death-1 levels correlate with increased mortality, nosocomial infection and immune dysfunctions in septic shock patients. *Crit. Care Lond Engl.* 15 (2), R99. doi:10.1186/cc10112

Humar, A., and Michaels, M.AST ID Working Group on Infectious Disease Monitoring (2006). American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. *Am. J. Transpl.* 6 (2), 262–274. doi:10.1111/j.1600-6143.2005.01207.x

Kalra, A., Wedd, J. P., Bambha, K. M., Golden-Mason, L., Collins, C., Rosen, H. R., et al. (2017). Neutrophil-to-lymphocyte ratio correlates with proinflammatory neutrophils and predicts death in low model for end-stage liver disease patients with cirrhosis. *Liver Transpl.* 23 (2), 155–165. doi:10.1002/lt.24702

La Rosa, C., Krishnan, A., Longmate, J., Martinez, J., Manchanda, P., Lacey, S. F., et al. (2008). Programmed death-1 expression in liver transplant recipients as a prognostic indicator of cytomegalovirus disease. *J. Infect. Dis.* 197 (1), 25–33. doi:10.1086/523652

Lario, M., Muñoz, L., Ubeda, M., Borrero, M. J., Martínez, J., Monserrat, J., et al. (2013). Defective thymopoiesis and poor peripheral homeostatic replenishment of T-helper cells cause T-cell lymphopenia in cirrhosis. *J. Hepatol.* 59 (4), 723–730. doi:10.1016/j.jhep.2013.05.042

Lebossé, F., Gudd, C., Tunc, E., Singanayagam, A., Nathwani, R., Triantafyllou, E., et al. (2019). CD8+T cells from patients with cirrhosis display a phenotype that may contribute to cirrhosis-associated immune dysfunction. *EBioMedicine* 49, 258–268. doi:10.1016/j.ebiom.2019.10.011

Li, S., Li, N., Yang, S., Deng, H., Li, Y., Wang, Y., et al. (2022). The study of immune checkpoint inhibitors in chronic Hepatitis B virus infection. *Int. Immunopharmacol.* 109, 108842. doi:10.1016/j.intimp.2022.108842

Liu, J., Li, H., Xia, J., Wang, X., Huang, Y., Li, B., et al. (2021). Baseline neutrophil-to-lymphocyte ratio is independently associated with 90-day transplant-free mortality in patients with cirrhosis. *Front. Med.* 8, 726950. doi:10.3389/fmed.2021.726950

Lorente, L., Martín, M. M., Ortiz-López, R., Alvarez-Castillo, A., Ruiz, C., Uribe, L., et al. (2022). Association between neutrophil-to-lymphocyte ratio in the first seven days of sepsis and mortality. *Enfermedades Infecc. Microbiol. Clin.* 40, 235–240. doi:10.1016/j.eimce.2020.11.022

Magalhães, R. D. S., Magalhães, J., Sousa-Pinto, B., Cúrdia Gonçalves, T., Rosa, B., and Cotter, J. (2021). Neutrophil-to-lymphocyte ratio: An accurate method for diagnosing infection in cirrhosis. *Postgrad. Med.* 133 (6), 613–618. doi:10.1080/00325481.2021.1916258

Markwick, L. J. L., Riva, A., Ryan, J. M., Cooksley, H., Palma, E., Tranah, T. H., et al. (2015). Blockade of PD1 and TIM3 restores innate and adaptive immunity in patients with acute alcoholic hepatitis. *Gastroenterology* 148 (3), 590–602.e10. doi:10.1053/j.gastro.2014.11.041

Moreau, R., Jalan, R., Gines, P., Pavesi, M., Angeli, P., Cordoba, J., et al. (2013). Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 144 (7), 1426–1437. doi:10.1053/j.gastro.2013.02.042

Mysore, K. R., Ghobrial, R. M., Kannanganat, S., Minze, L. J., Graviss, E. A., Nguyen, D. T., et al. (2018). Longitudinal assessment of T cell inhibitory receptors in liver transplant recipients and their association with posttransplant infections. *Am. J. Transpl.* 18 (2), 351–363. doi:10.1111/ajt.14546

Osuch, S., Metzner, K. J., and Caraballo Cortés, K. (2020). Reversal of T Cell exhaustion in chronic HCV infection. *Viruses* 12 (8), 799. doi:10.3390/v12080799

Pallett, L. J., Gill, U. S., Quaglia, A., Sinclair, L. V., Jover-Cobos, M., Schurich, A., et al. (2015). Metabolic regulation of Hepatitis B immunopathology by myeloid-derived suppressor cells. *Nat. Med.* 21 (6), 591–600. doi:10.1038/nm.3856

Panasiuk, A., Wysocka, J., Maciorkowska, E., Panasiuk, B., Prokopowicz, D., Zak, J., et al. (2005). Phagocytic and oxidative burst activity of neutrophils in the end stage of liver cirrhosis. *World J. Gastroenterol.* 11 (48), 7661–7665. doi:10.3748/wjg.v11.i48.7661

Rehman, F. U., Khan, A., Aziz, A., Iqbal, M., Mahmood, S. B. Z., and Ali, N. (2020). Neutrophils to lymphocyte ratio: Earliest and efficacious markers of sepsis. *Cureus* 12 (10), e10851. doi:10.7759/cureus.10851

Rienzo, M., Skirecki, T., Monneret, G., and Timsit, J. F. (2022). Immune checkpoint inhibitors for the treatment of sepsis:insights from preclinical and clinical development. *Expert Opin. Investig. Drugs* 31, 885–894. doi:10.1080/13543784.2022.2102477

Riva, A., Palma, E., Devshi, D., Corrigall, D., Adams, H., Heaton, N., et al. (2021). Soluble TIM3 and its ligands galectin-9 and CEACAM1 are in disequilibrium during alcohol-related liver disease and promote impairment of anti-bacterial immunity. *Front. Physiol.* 12, 632502. doi:10.3389/fphys.2021.632502

Schrijver, I. T., Théroude, C., and Roger, T. (2019). Myeloid-derived suppressor cells in sepsis. *Front. Immunol.* 10, 327. doi:10.3389/fimmu.2019.00327

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., et al. (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315 (8), 801–810. doi:10.1001/jama.2016.0287

Sundaram, V., Mahmud, N., Perricone, G., Katarey, D., Wong, R. J., Karvellas, C. J., et al. (2020). Longterm outcomes of patients undergoing liver transplantation for acute-on-chronic liver failure. *Liver Transpl.* 26 (12), 1594–1602. doi:10.1002/lt.25831

Taylor, N. J., Manakkat Vijay, G. K., Abeles, R. D., Auzinger, G., Bernal, W., Ma, Y., et al. (2014). The severity of circulating neutrophil dysfunction in patients with cirrhosis is associated with 90-day and 1-year mortality. *Aliment. Pharmacol. Ther.* 40 (6), 705–715. doi:10.1111/apt.12886

Tranah, T. H., Kronsten, V. T., and Shawcross, D. L. (2022). Implications and management of cirrhosis-associated immune dysfunction before and after liver transplantation. *Liver Transpl.* 28 (4), 700–716. doi:10.1002/lt.26353

Tritto, G., Bechlis, Z., Stadlbauer, V., Davies, N., Francés, R., Shah, N., et al. (2011). Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *J. Hepatol.* 55 (3), 574–581. doi:10.1016/j.jhep.2010.11.034

Venet, F., Demaret, J., Gossez, M., and Monneret, G. (2021). Myeloid cells in sepsis-acquired immunodeficiency. *Ann. N. Y. Acad. Sci.* 1499 (1), 3–17. doi:10.1111/nyas.14333

Venet, F., and Monneret, G. (2018). Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat. Rev. Nephrol.* 14 (2), 121–137. doi:10.1038/nrneph.2017.165

Wasmuth, H. E., Kunz, D., Yagmur, E., Timmer-Stranghöner, A., Vidacek, D., Siewert, E., et al. (2005). Patients with acute on chronic liver failure display « sepsis-like » immune paralysis. *J. Hepatol.* 42 (2), 195–201. doi:10.1016/j.jhep.2004.10.019

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Prognostic significances of PD-L1- and CTLA-4-positive T cells and positive correlations of immunosuppressive marker expression between cancer tissue and peripheral blood in patients with gastric cancer

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Introduction: Although tumor, node, metastasis (TNM) staging has been used for prognostic assessment of gastric cancer (GC), the prognosis may vary among patients with the same TNM stage. Recently, the TNM-Immune (TNM-I) classification staging system has been used for prognostic assessment of colorectal cancer based on intra-tumor T-cell status, which is a superior prognostic factor compared with the American Joint Committee on Cancer staging manual. However, an immunoscoring system with prognostic significance for GC has not been established.

Method: Here, we evaluated immune phenotypes in cancer and normal tissues, then examined correlations between tissues and peripheral blood. GC patients who underwent gastrectomy at Seoul St. Mary's Hospital between February 2000 and May 2021 were included. We collected 43 peripheral blood samples preoperatively and a pair of gastric mucosal samples postoperatively, including normal and cancer mucosa, which did not influence tumor diagnosis and staging. Tissue microarray samples of GC were collected from 136 patients during surgery. We investigated correlations of immune phenotypes between tissues and peripheral blood using immunofluorescence imaging and flow cytometry, respectively. GC mucosa exhibited an increased number of $CD4^{+}$ T cells, as well as increased expression levels of immunosuppressive markers (e.g., programmed death-ligand-1 [PD-L1], cytotoxic T lymphocyte antigen-4 [CTLA-4], and interleukin-10), in CD4+ T cells and non-T cells.

Result: The expression levels of immunosuppressive markers were significantly increased in cancer tissues and peripheral blood mononuclear cells. In gastric mucosal tissues and peripheral blood of GC patients, similar immunosuppression phenotypes were observed, including increased numbers of PD-L1- and CTLA-4-positive T cells.

Discussion: Therefore, peripheral blood analysis may be an important tool for prognostic assessment of GC patients.

KEYWORDS

gastric cancer, tumor microenvironment, programmed death-ligand-1, cytotoxic T lymphocyte antigen-4, interleukin-10

Introduction

Gastric cancer (GC) is one of the most common cancers in East Asia, which is ranks 5th in incidence and was the 4th leading cause of death among all solid cancers in South Korea excluding non-melanoma skin cancer in 2020 (1). In South Korea, new patients of gastric cancer (26,662 cases) ranked 4th (10.8%), followed by thyroid cancer (11.8%), lung cancer (11.7%), and colorectal cancer (11.2%), with a slight difference in 2020, according to the report of the Korea Central Cancer Registry (2, 3). In South Korea, early diagnosis of GC is common because esophagogastroduodenoscopy is widely performed for screening, and the proportion of patients with advanced GC (AGC) is decreasing (4). However, GC diagnosis and prognostic prediction can only be conducted using invasive methods, such as endoscopic biopsy. Although tumor markers (e.g., carcinoembryonic antigen and cancer antigen 19-9) are commonly used, they have limited utility in GC because of their low sensitivity and specificity (5, 6).

The Korean Practice guidelines for GC state that tumor, node, metastasis (TNM) staging is a useful indicator of cancer patient prognosis; treatment should be determined on the basis of the stage (7). Although TNM staging has been used for prognostic assessment of GC, the prognosis and clinical outcomes significantly vary among patients with the same TNM stage (8). The classification system provides limited prognostic information and does not predict the treatment response (9). Recently, the TNM-Immune (TNM-I) classification staging system has been used for prognostic assessment of colorectal cancer based on intra-tumor T-cell status, which is a superior prognostic factor compared with the American Joint Committee on Cancer staging manual (10).

Several recent studies have revealed relationships of immune-related markers with the treatment response, prognosis, and survival rate in GC treated with chemotherapy. The addition of molecular markers to TNM staging provides additional information regarding GC (11–13). Cancer progression depends on crosstalk between cancer cells and the immune system (14). GC characteristics (e.g., metastasis, treatment resistance, and disease recurrence) are associated with a tumor subpopulation known as GC stem cells (14). GC patients have reduced cancer suppression function in immune cells around cancer tissues. Honjo and Allison were awarded the 2018 Nobel Prize for their discovery of programmed death-ligand-1 (PD-L1) and cytotoxic T lymphocyte antigen-4 (CTLA-4), co-stimulatory factors that regulate cancer and autoimmune diseases (15, 16). Interleukin (IL)-10, which exhibits carcinogenic behavior, is a marker of GC and a potential therapeutic target (17). In the treatment of AGC patients, molecular markers are targeted *via* monoclonal antibodies, such as nivolumab and pembrolizumab; this constitutes a molecular approach for the treatment of AGC (18). Factors that decrease immune function (e.g., PD-L1, CTLA-4, and IL-10) are significantly increased in the immune and cancer cells in cancer tissues (17, 19–21). Immune cells activated or produced locally in gastric mucosa may reach systemic circulation and be detected in peripheral blood samples (22). However, the correlations and interactive effects of these cells in GC have not been elucidated.

In the present study, we evaluated differences in immune phenotypes between cancer and normal tissues, then examined correlations of immune phenotypes between GC tissues and peripheral blood.

Materials and methods

Study population

This study enrolled patients with gastric adenocarcinoma diagnosed preoperatively on endoscopic biopsy. All patients underwent conventional radical gastrectomy with curative intent, in accordance with the Korean Gastric Cancer Treatment Guidelines at Seoul St. Mary's Hospital between February 2000 and May 2021. Patients with early GC (EGC) underwent D1+ lymph node dissection, whereas patients with locally advanced cancer underwent D2 or D2+ lymph node dissection. In total, 43 peripheral blood samples and gastric mucosal tissue samples were collected. Furthermore, a pair of gastric mucosal samples was obtained preoperatively, including normal and cancer mucosa, which did not influence tumor diagnosis and staging. Tissue microarray samples of GC were collected from 136 patients during surgery. The pathological stage of GC was classified in accordance with the criteria of the eighth American Joint Committee on Cancer. Patients with stage I and II disease were included in the EGC group, whereas patients with stage III disease were included in the AGC group. This study protocol was approved by the Institutional Review Board of the College of Medicine, Catholic University of Korea (KC20TISI0985). Patient records were anonymized before analysis.

Intracellular staining and flow cytometry

Human peripheral blood mononuclear cells were isolated from blood samples of GC patients using Ficoll-Paque (GE Healthcare, Chicago, IL, USA), then stimulated with 25 ng/mL phorbol myristate acetate and 250 ng/mL ionomycin (Sigma-Aldrich, St. Louis, MO, USA) in the presence of GolgiStop (BD Biosciences, San Jose, CA, USA) for 4 h. Surface staining was performed with surface Alexa Fluor® 700-conjugated anti-CD4$^+$ (BD Pharmingen, Franklin Lakes, NJ, USA), allophycocyanin-C7-conjugated anti-CD8$^+$ (BD Pharmingen), phycoerythrin-conjugated anti-CTLA-4, and fluorescein isothiocyanate-conjugated anti-PD-L1 (Biolegend, San Diego, CA, USA) antibodies. Samples were analyzed using FACSCalibur (BD Pharmingen) and a fluorescence-activated cell sorting instrument. Data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

Immunofluorescence analysis

Mucosa from GC patients was fixed in 10% formalin and embedded in paraffin. Paraffin-embedded sections were probed with anti-CD4$^+$ (Novus Biologicals, Littleton, CO, USA), anti-CD8$^+$ (Novus Biologicals), anti-PD-L1 (Invitrogen, Carlsbad, CA, USA), and anti-CTLA-4 (Invitrogen) primary antibodies at 4°C overnight. They were then stained with secondary antibodies conjugated with fluorescein isothiocyanate (Santa Cruz Biotechnology, Santa Cruz, CA, USA), allophycocyanin (Invitrogen), and phycoerythrin (Southern Biotech, Birmingham, AL, USA) at room temperature for 2 h. Nuclei were stained with 4,'6-diamidino-2-phenylindole (DAPI; Invitrogen). Immunofluorescence images were obtained using an LSM 700 confocal microscope (Zeiss, Oberkochen, Germany) at 200× magnification. Images were analyzed using ZEN 2 (blue edition) (Zeiss).

Statistical analysis

Data are shown as means ± standard errors of the mean. Statistical analyses were performed using GraphPad Prism software (version 8; GraphPad Software, San Diego, CA, USA). Normally distributed continuous data were analyzed using Student's *t*-test. Differences in means among groups were evaluated using one-way analysis of variance. $P < 0.05$ was considered indicative of statistical significance.

Results

Patient characteristics

The participants' clinicopathological characteristics are shown in Table 1. The mean patient age was 59.2 years, and 68.4% of the participants were men. There were 47 and 89 patients with EGC (stage I and II) and AGC (stage III), respectively. There were significant differences between patients with EGC and AGC in terms of the extent of resection (subtotal gastrectomy, 85.1% and 55.1%, respectively; p = 0.001), Lauren classification subtype (intestinal type, 57.4% and 33.7%, respectively; p = 0.008), tumor size (4.2 ± 2.4 and 6.7 ± 2.8cm, respectively; p < 0.001), and positive lymph node ratio (0.04 ± 0.06 and 0.18 ± 0.13, respectively; p < 0.001). Lymphatic and neural invasion were significantly more common in AGC patients than in EGC patients (lymphatic invasion, 48.9% and 97.8%, respectively; p < 0.001; neural invasion, 17.0% and 67.4%, respectively; p < 0.001).

Analysis of peripheral blood and gastric mucosal samples from GC patients

Flow cytometry revealed higher expression levels of immunosuppressive markers, such as PD-L1 and CTLA-4, in CD4$^+$ and CD8$^+$ T cells from peripheral blood among AGC patients than among EGC patients, although a statistically significant difference was only observed for CTLA-4$^+$ CD8$^+$ T cells (Figure 1A). Immunofluorescence images showed higher numbers of CD4$^+$ and CD8$^+$ T cells in GC mucosal tissue than in normal mucosal tissue. Additionally, expression levels of immunosuppressive markers on CD4$^+$ and CD8$^+$ T cells were greater in cancer mucosa tissue than in normal mucosa tissue (Figures 1B, C).

Analysis of GC mucosal tissue according to cancer stage

Immunofluorescence images showed higher expression levels of immunosuppressive markers, such as PD-L1 and CTLA-4, in CD4$^+$ and CD8$^+$ T cells from cancer mucosa of GC patients as the cancer stage increased (Figure 2A). The proportion of CD4$^+$ T cells was significantly greater in stage III cancer than in stages I or II, whereas there was no significant difference in the number of CD8$^+$ T cells according to cancer stage. The numbers of PD-L1$^+$ CD4$^+$T, CTLA-4$^+$ CD4$^+$T, PD-L1$^+$ CD8$^+$ T, and CTLA-4$^+$ CD8$^+$ T cells increased as the cancer stage increased. The expression levels of immunosuppressive markers in CD4$^+$ T cells increased with increasing CD4$^+$ T cell infiltration into cancer mucosa. Therefore, the percentages of PD-L1 and CTLA-4 expression in CD4$^+$ T cells did not differ according to cancer stage. The number of infiltrating CD8$^+$ T cells in cancer

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TABLE 1 Clinicopathologic characteristics of patients with gastric cancer according to pStages.

		pStage		
Characteristics	Total (n=136)	I, II (n=47)	III, IV (n=89)	*p*-value
Age, mean ± SD (yrs)	59.2 ± 11.0	58.9 ± 9.5	59.2 ± 11.8	0.823
Sex				0.267
male	93 (68.4%)	35 (74.5%)	58 (62.5%)	
female	45 (31.6%)	12 (25.5%)	31 (34.8%)	
Approach of surgery				0.585
Open	134 (98.5%)	47 (100%)	87 (97.8%)	
Laparoscopic	2 (1.5%)	0	2 (2.2%)	
Extent of resection				0.001
TG	47 (34.6%)	7 (14.9%)	40 (44.9%)	
STG	89 (65.4%)	40 (85.1%)	49 (55.1%)	
LN dissection				0.559
<D1+	25 (18.4%)	7 (14.9%)	18 (20.2%)	
>D2	110 (80.9%)	40 (85.1%)	70 (78.7%)	
others	1 (0.7%)	0	1 (1.1%)	
R0 resection	121 (89.0%)	45 (95.7%)	76 (85.4%)	0.067
Differentiation				0.127
Differentiated	49 (36.0%)	21 (44.7%)	28 (31.5%)	
Undifferentiated	87 (64.0%)	26 (55.3%)	61 (68.5%)	
Lauren classification				0.008
Intestinal	57 (41.9%)	27 (57.4%)	30 (33.7%)	
Diffuse/mixed	79 (58.1%)	20 (42.6%)	59 (66.3%)	
Tumor size (cm)	5.9 ± 2.9	4.2 ± 2.4	6.7 ± 2.8	<0.001
Retrieved LN (number)	42.6 ± 14.9	38.2 ± 13.4	45.0 ± 15.2	0.012
Positive LN ratio	0.14 ± 0.13	0.04 ± 0.06	0.18 ± 0.13	<0.001
pT				<0.001
1	22 (16.2%)	22 (46.8%)	0	

(Continued)

TABLE 1 Continued

		pStage		
Characteristics	Total (n=136)	I, II (n=47)	III, IV (n=89)	*p*-value
2	10 (7.4%)	7 (14.9%)	3 (3.4%)	
3	37 (27.2%)	17 (36.2%)	20 (22.5%)	
4	67 (49.3%)	1 (2.1%)	66 (74.2%)	
pN				<0.001
0	23 (16.9%)	23 (48.9%)	0	
1	33 (24.3%)	15 (31.9%)	18 (20.2%)	
2	30 (22.1%)	7 (14.9%)	23 (25.8%)	
3	48 (35.3%)	2 (4.3%)	48 (53.9%)	
Lymphatic invasion, yes	110 (80.9%)	23 (48.9%)	87 (97.8%)	<0.001
Venous invasion, yes	21 (15.4%)	4 (8.5%)	17 (19.1%)	0.195
Neural invasion, yes	68 (50.0%)	8 (17.0%)	60 (67.4%)	<0.001

SD, Standard deviation; TG, Total gastrectomy; STG, Subtotal gastrectomy; LN, Lymph node.
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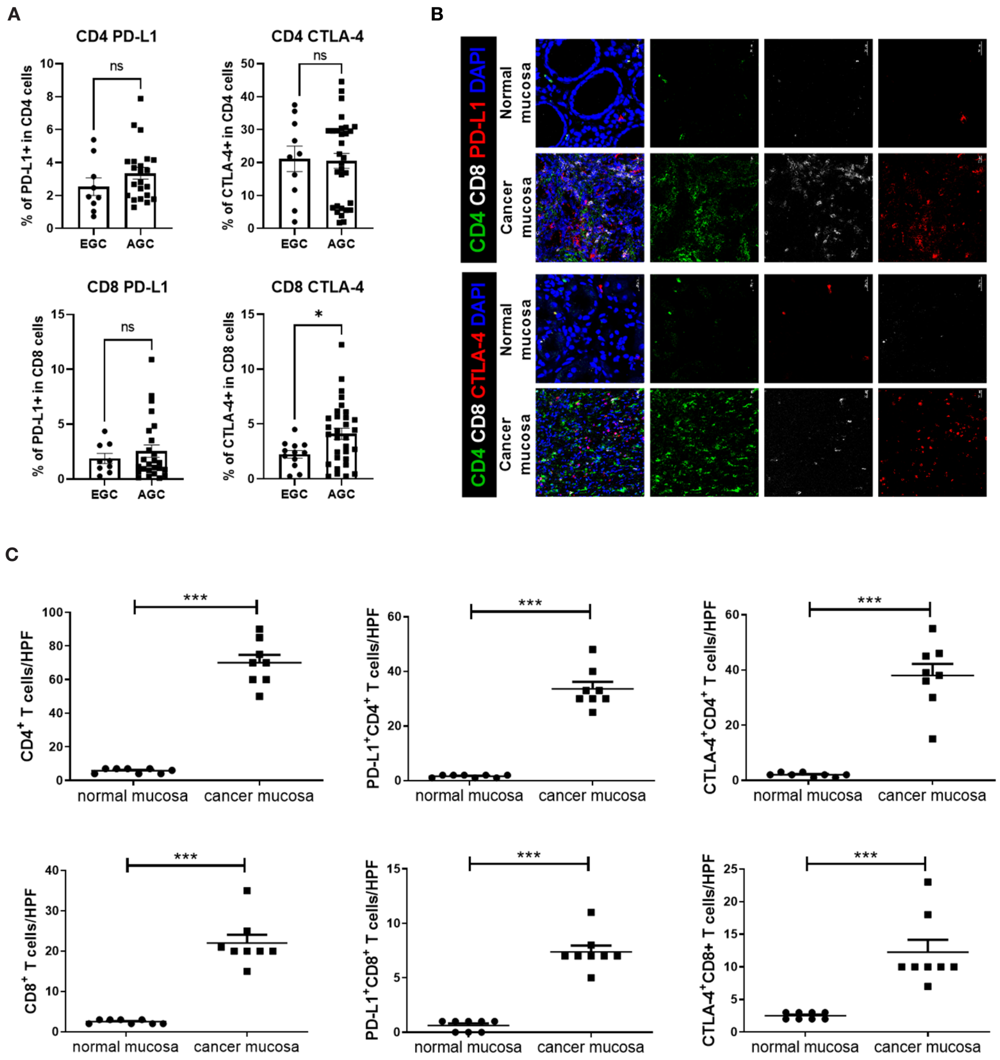

FIGURE 1
Expression levels of immunosuppressive markers, such as PD-L1 and CTLA-4, on T cells were higher in blood and cancer tissue from GC patients. Peripheral blood mononuclear cells from GC patients were stimulated with phorbol myristate acetate and ionomycin for 4 h, followed by GolgiStop for an additional 2 (h) Normal and cancer mucosa were harvested from GC patients, then stained with CD4+, CD8+, PD-L1, CTLA-4, and DAPI. **(A)** Bar graphs show percentages of PD-L1$^+$ CD4$^+$ T cells (top and left), CTLA-4$^+$ CD4$^+$ T cells (top and right), PD-L1$^+$ CD8$^+$ T cells (bottom and left), and CTLA-4$^+$ CD8$^+$ T cells (bottom and right) in peripheral blood mononuclear cells from early GC (EGC) and advanced GC (AGC) patients. **(B)** Representative confocal images showing PD-L1$^+$ CD4$^+$, CTLA-4$^+$ CD4$^+$, PD-L1$^+$ CD8$^+$, and CTLA-4$^+$ CD8$^+$ T cells in normal (n = 8) and mucosa (n = 8) mucosa. **(C)** Bar graphs show mean number of cells per high-power field (HPF) in normal and cancer mucosa. Scale bar = 20 μm. Data are means ± standard errors of the mean (*$p < 0.05$, ***$p < 0.001$).

mucosa did not significantly differ according to cancer stage; however, the levels of PD-L1 and CTLA-4 expression were increased in CD8$^+$ T cells (Figure 2B). Our results suggest that immunosuppression in cancer mucosa increases with increasing cell number and increasing proportions of immunosuppressive marker-positive CD4$^+$ and CD8$^+$ cells, respectively.

Correlations of immunosuppressive markers in CD4$^+$ and CD8$^+$ T cells from cancer tissue of GC patients

We investigated correlations of immunosuppressive markers (e.g., PD-L1, CTLA-4, and IL-10) in CD4$^+$ and CD8$^+$ T cells from cancer mucosa of GC patients. There were significant correlations involving the numbers of PD-L1$^+$ CD4$^+$ T cells/high-power field (HPF) and CTLA-4$^+$ CD4$^+$ T cells/HPF (Figure 3A), the number of PD-L1$^+$ CD4$^+$ T cells/HPF and CTLA-4$^+$ CD8$^+$ T cells/HPF (Figure 3B), the numbers of PD-L1$^+$ CD4$^+$ T cells/HPF and IL-10$^+$ CD4$^+$ T cells/HPF (Figure 3C), the numbers of CTLA-4$^+$ CD4$^+$ T cells/HPF and CTLA-4$^+$ CD8$^+$ T cells/HPF (Figure 3D), the numbers of CTLA-4$^+$ CD4$^+$ T cells/HPF and IL-10$^+$ CD4$^+$ T cells/HPF (Figure 3E), and the numbers of CTLA-4$^+$ CD8$^+$ T cells/HPF and IL-10$^+$ CD4$^+$ T cells/HPF (Figure 3F). These results showed that the numbers of immunosuppressive CD4$^+$ and CD8$^+$ T cells were correlated with each other in cancer mucosa from GC patients.

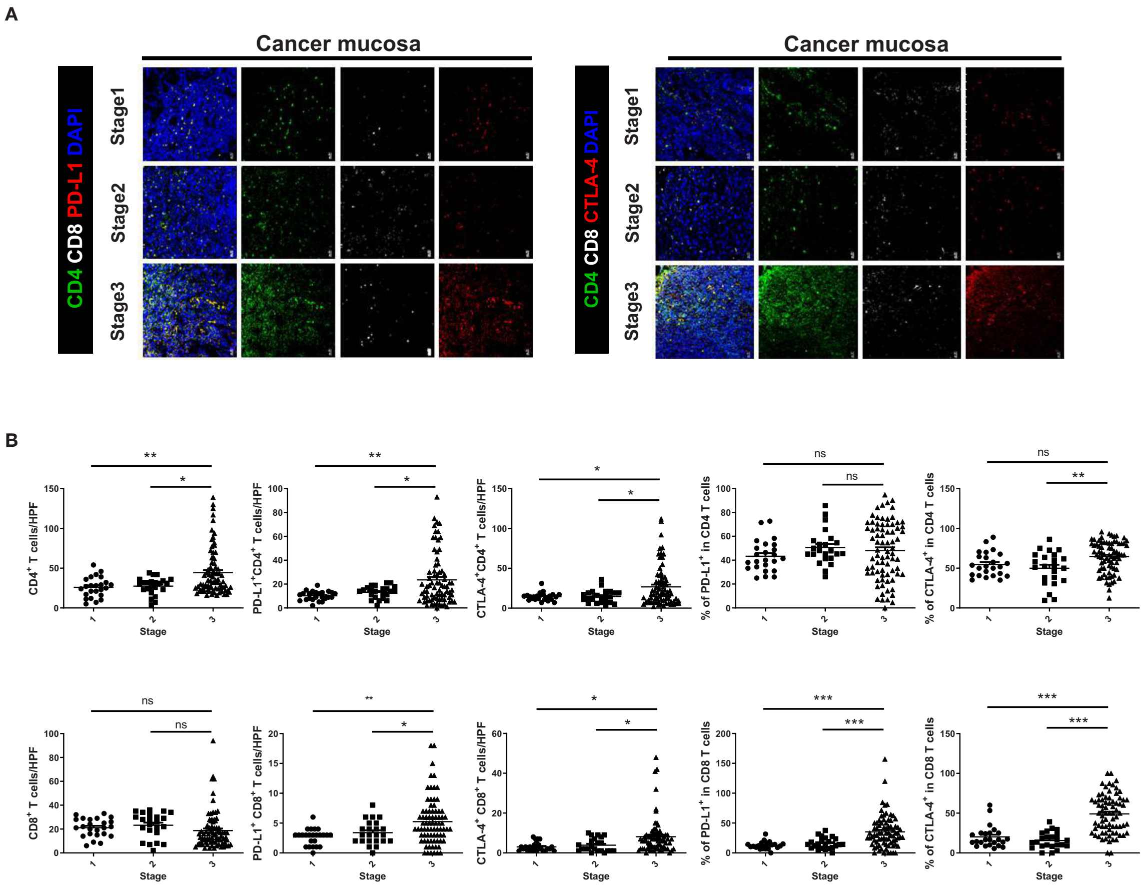

FIGURE 2
Expression levels of immunosuppressive markers on CD4+ and CD8+ T cells were increased in cancer tissue from GC patients with increasing TNM stage. **(A)** Confocal microscopic analysis of cancer mucosa from GC patients. Representative confocal images showing PD-L1+ CD4+, CTLA-4+ CD4+, PD-L1+ CD8+, and CTLA-4+ CD8+ T cells in cancer tissues. **(B)** Bar graphs show mean number of cells per HPF in cancer tissues according to cancer stage (Stages I–III, n = 24, = 23, and = 83, respectively). Scale bar = 20 µm. Data are means ± standard errors of the mean (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Correlations of immunosuppressive markers in CD4+ T cells, CD8+ T cells, and macrophages from cancer tissue of GC patients

We evaluated IL-10-producing CD68+ tumor-associated macrophages (TAMs) in cancer tissue from GC patients. There were significant correlations involving the numbers of PD-L1+ CD4+ T cells/HPF and IL-10+ CD68+ TAMs/HPF (Figure 4A), the numbers of CTLA-4+ CD4+ T cells/HPF and IL-10+ CD68+ TAMs/HPF (Figure 4B), the numbers of CTLA-4+ CD8+ T cells/HPF and IL-10+ CD68+ TAMs/HPF (Figure 4C), and the numbers of IL-10+ CD4+ T cells/HPF and IL-10+ CD68+ TAMs/HPF (Figure 4D). These results showed that the numbers of immunosuppressive CD4+ and CD8+ T cells were also correlated with the numbers of IL-10-producing CD68+ TAMs in cancer mucosa from GC patients.

Discussion

In this study, we evaluated whether immune cells (CD4+ and CD8+ T cells) and immunosuppressive markers (PD-L1, CTLA-4, and IL-10) were present in peripheral blood and cancer tissues from GC patients, then investigated whether those findings were correlated with each other. Several recent studies have revealed correlations of immunosuppressive markers with GC (22–24). Our results showed that the number of CTLA-4+ CD8+ T cells in peripheral blood was significantly greater among AGC patients than among EGC patients. The numbers of CD4+ and CD8+ T cells, as well as the expression levels of their immunosuppressive markers, were greater in cancer mucosa than in normal mucosa. There were also significant differences among cancer stages. The number of CD4+ T cells was greater in stage III than in other stages, whereas the number of CD8+ T cells did not differ according to cancer stage. The numbers of PD-L1+ CD4+ T, CTLA-4+ CD4+ T, PD-L1+ CD8+

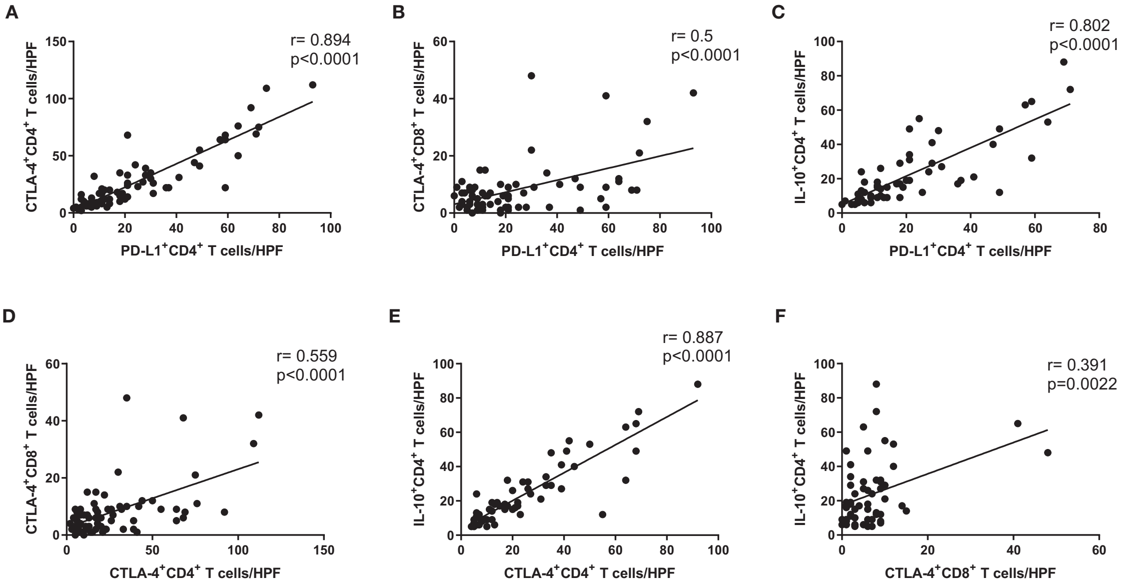

FIGURE 3
Expression levels of immunosuppressive markers on CD4+ and CD8+ T cells were correlated with each other in cancer tissue from GC patients. Correlation analysis of PD-L1+ CD4+ T cells with **(A)** CTLA-4+ CD4+ T cells, **(B)** CTLA-4+ CD8+ T cells, and **(C)** IL-10+ CD4+ T cells in cancer tissue from stage III cancer patients. Correlation analysis of CTLA-4+ CD4+ T cells with **(D)** CTLA-4+ CD8+ T cells and **(E)** IL-10+ CD4+ T cells in cancer tissue from stage III cancer patients. Correlation analysis of CTLA-4+ CD8+ T cells with **(F)** IL-10+ CD4+ T cells in cancer tissue from stage III cancer patients.

T, and CTLA-4+ CD8+ T cells increased with increasing disease stage. The expression levels of immunosuppressive markers in CD4+ T cells from cancer mucosa increased with increasing cancer stage. Therefore, the percentages of PD-L1- and CTLA-4-positive CD4+ T cells did not differ according to cancer stage. In contrast, the infiltration of CD8+ T cells did not significantly differ with cancer progression; however, the percentages of PD-L1- and CTLA-positive CD8+ T cells were increased. Therefore, the levels of immunosuppressive markers in CD8+ T cells increased with cancer progression. Our results suggest that the levels of

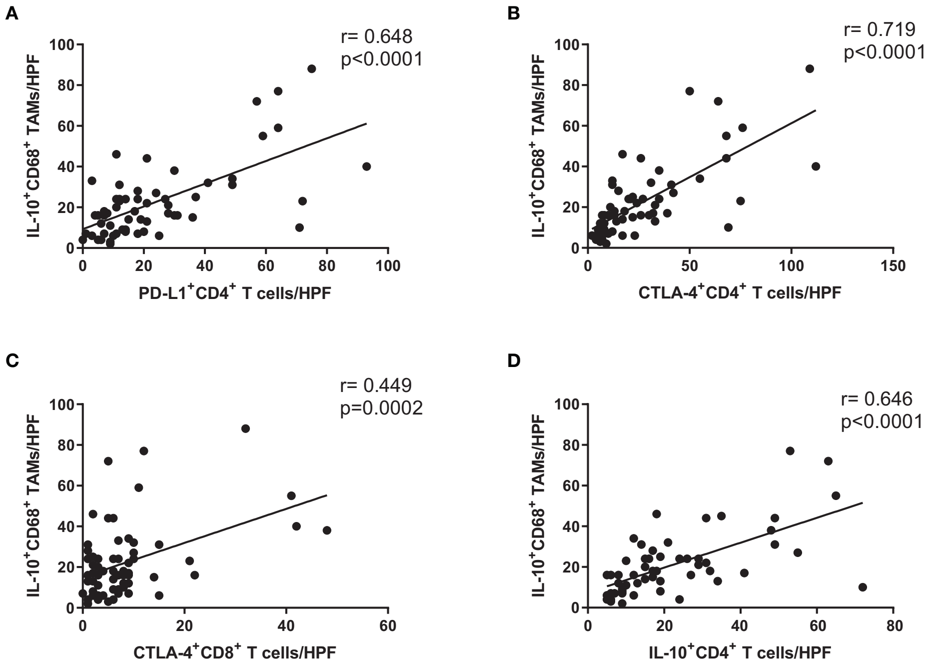

FIGURE 4
Expression levels of immunosuppressive markers on CD4+ and CD8+ T cells were correlated with number of IL-10-producing CD68+ macrophages in cancer tissue from GC patients. Correlation analysis of **(A)** PD-L1+ CD4+ T cells, **(B)** CTLA-4+ CD4+ T cells, **(C)** CTLA-4+ CD8+ T cells, and **(D)** IL-10+ CD4+ T cells with number of IL-10+ CD68+ TAMs in cancer tissue from stage III GC patients.

immunosuppressive markers in immune cells are closely related to GC, and the distribution patterns of circulating markers in GC tissues are correlated with the patterns of markers in peripheral blood. Although it is unclear whether immunosuppression is a cause or consequence of GC, our results showed that peripheral blood sampling may be useful in prognostic prediction for GC patients.

There are increasing numbers of immunological and molecular studies focused on GC. Sánchez-Zauco et al. (25) performed a comparative analysis of circulating markers between GC patients and healthy controls. *Helicobacter pylori* activates a specific signaling cascade, thereby inducing several cytokines and chemokines that lead to GC (26–28). In a study of blood samples collected from patients before surgery, interferon-γ and IL-10 were identified as diagnostic markers for EGC; IL-1β, IL-8, and macrophage chemotactic protein-1 were identified as diagnostic markers for AGC. In the present study, we also analyzed markers present in the cancer mucosa, which were excluded from analysis in previous studies. The strength of our study is that we identified a correlation between immune markers in cancer tissue and peripheral blood from GC patients.

This study had some limitations. First, it was a single-center study with a small sample size. Moreover, disease biomarkers are influenced by ethnicity, country, environment, and lifestyle (29–32). Thus, it is difficult to generalize our results to other institutions or countries. Therefore, future studies should evaluate the utilities of biomarkers for various ethnicities, countries, and cultures. Second, despite substantial efforts to identify cancer biomarkers over the past 15 years, only a few markers have been identified with utility in cancer diagnosis and monitoring (33). Because of variations in molecular characteristics, the utility of a candidate biomarker cannot be determined. Mechanisms underlying the roles of specific markers may differ according to cancer type and tumor microenvironment. Therefore, further studies are needed to explore molecular mechanisms that underlie biomarkers and their effects. In conclusion, there were similar immunosuppression phenotypes in gastric mucosal tissues and peripheral blood from GC patients. We found correlations between disease severity and the expression levels of immunosuppressive markers. These findings suggest that peripheral blood analysis can be used as a prognostic tool and facilitate the development of anti-cancer therapy directed against immune cells.

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

The studies involving human participants were reviewed and approved by the institutional review board of the College of Medicine, Catholic University of Korea (KC20TISI0985). The patients/participants provided their written informed consent to participate in this study.

Author contributions

KHL, M-LC and KYS conceived and designed the study. KHL, SJK, JSW, JM and YJJ wrote the manuscript and performed the data analysis. KHL, SJK, JSW, SYL, and JYJ were responsible for data collection and reviewing the data analysis. M-LC and KYS reviewed the manuscript and provided feedback. All authors discussed the results and contributed to the final version of the manuscript.

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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. World Health Organization. (2020). WHO report on cancer: setting priorities, investing wisely and providing care for all. World Health Organization.

2. Hong S, Won YJ, Lee JJ, Jung KW, Kong HJ, Im JS, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2018. *Cancer Res Treat* (2021) 53(2):301–15. doi: 10.4143/crt.2021.291

3. Park SH, Kang MJ, Yun EH, Jung KW. Epidemiology of gastric cancer in Korea: trends in incidence and survival based on Korea central cancer registry data (1999-2019). *J Gastric Cancer.* (2022) 22(3):160–8. doi: 10.5230/jgc.2022.22.e21

4. Korean Gastric cancer association-led nationwide survey on surgically treated gastric cancers in 2019. *J Gastric Cancer* (2021) 21(3):221–35. doi: 10.5230/jgc.2021.21.e27

5. Chen YY, Feng Y, Mao QS, Ma P, Liu JZ, Lu W, et al. Diagnostic and prognostic value of the peripheral natural killer cell levels in gastric cancer. *Exp Ther Med* (2020) 20(4):3816–22. doi: 10.3892/etm.2020.9101

6. Tian SB, Yu JC, Kang WM, Ma ZQ, Ye X, Cao ZJ, et al. Combined detection of CEA, CA 19-9, CA 242 and CA 50 in the diagnosis and prognosis of resectable gastric cancer. *Asian Pac J Cancer Prev* (2014) 15(15):6295–300. doi: 10.7314/APJCP.2014.15.15.6295

7. Korean Practice guideline for gastric cancer 2018: an evidence-based, multi-disciplinary approach. *J Gastric Cancer.* (2019) 19(1):1–48. doi: 10.5230/jgc.2019.19.e8

8. Galon J, Pagès F, Marincola FM, Angell HK, Thurin M, Lugli A, et al. Cancer classification using the immunoscore: a worldwide task force. *J Transl Med* (2012) 10:205. doi: 10.1186/1479-5876-10-205

9. Mranda GM, Xue Y, Zhou X-G, Yu W, Wei T, Xiang Z-P, et al. Revisiting the 8th AJCC system for gastric cancer: a review on validations, nomograms, lymph nodes impact, and proposed modifications. *Ann Med Surgery.* (2022) 75:103411. doi: 10.1016/j.amsu.2022.103411

10. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol* (2014) 232(2):199–209. doi: 10.1002/path.4287

11. Grassadonia A, De Luca A, Carletti E, Vici P, Di Lisa FS, Filomeno L, et al. Optimizing the choice for adjuvant chemotherapy in gastric cancer. *Cancers.* (2022) 14 (19):4670. doi: 10.3390/cancers14194670

12. He Y, Song H, Jiang Y, Ren W. Identification of immune-related prognostic markers in gastric cancer. *J Healthcare Engineering.* (2022) 2022:7897274. doi: 10.1155/2022/7897274

13. Kim JH, Ryu M-H, Park YS, Ma J, Lee SY, Kim D, et al. Predictive biomarkers for the efficacy of nivolumab as ≥ 3rd-line therapy in patients with advanced gastric cancer: a subset analysis of ATTRACTION-2 phase III trial. *BMC Cancer.* (2022) 22 (1):378. doi: 10.1186/s12885-022-09488-2

14. Becerril-Rico J, Alvarado-Ortiz E, Toledo-Guzmán ME, Pelayo R, Ortiz-Sánchez E. The cross talk between gastric cancer stem cells and the immune microenvironment: a tumor-promoting factor. *Stem Cell Res Ther* (2021) 12(1):498. doi: 10.1186/s13287-021-02562-9

15. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* (1992) 11(11):3887–95. doi: 10.1002/j.1460-2075.1992.tb05481.x

16. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science.* (1996) 271(5256):1734–6. doi: 10.1126/science.271.5256.1734

17. Chen L, Shi Y, Zhu X, Guo W, Zhang M, Che Y, et al. IL–10 secreted by cancer –associated macrophages regulates proliferation and invasion in gastric cancer cells *via* c–Met/STAT3 signaling. *Oncol Rep* (2019) 42(2):595–604. doi: 10.3892/or.2019.7206

18. Kawazoe A, Shitara K, Boku N, Yoshikawa T, Terashima M. Current status of immunotherapy for advanced gastric cancer. *Jpn J Clin Oncol* (2021) 51(1):20–7. doi: 10.1093/jjco/hyaa202

19. Gu L, Chen M, Guo D, Zhu H, Zhang W, Pan J, et al. PD-L1 and gastric cancer prognosis: a systematic review and meta-analysis. *PloS One* (2017) 12(8):e0182692. doi: 10.1371/journal.pone.0182692

20. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *J Exp Clin Cancer Res* (2019) 38(1):255. doi: 10.1186/s13046-019-1259-z

21. Wang B, Qin L, Ren M, Sun H. Effects of combination of anti-CTLA-4 and anti-PD-1 on gastric cancer cells proliferation, apoptosis and metastasis. *Cell Physiol Biochem* (2018) 49(1):260–70. doi: 10.1159/000492876

22. Suh KJ, Kim JW, Kim JE, Sung JH, Koh J, Kim KJ, et al. Correlation between tumor infiltrating immune cells and peripheral regulatory T cell determined using methylation analyses and its prognostic significance in resected gastric cancer. *PloS One* (2021) 16(6):e0252480. doi: 10.1371/journal.pone.0252480

23. Eto S, Yoshikawa K, Nishi M, Higashijima J, Tokunaga T, Nakao T, et al. Programmed cell death protein 1 expression is an independent prognostic factor in gastric cancer after curative resection. *Gastric Cancer.* (2016) 19(2):466–71. doi: 10.1007/s10120-015-0519-7

24. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* (2018) 24(9):1449–58. doi: 10.1038/s41591-018-0101-z

25. Sánchez-Zauco N, Torres J, Gómez A, Camorlinga-Ponce M, Muñoz-Pérez L, Herrera-Goepfert R, et al. Circulating blood levels of IL-6, IFN-γ, and IL-10 as potential diagnostic biomarkers in gastric cancer: a controlled study. *BMC Cancer.* (2017) 17 (1):384. doi: 10.1186/s12885-017-3310-9

26. Li X, Pan K, Vieth M, Gerhard M, Li W, Mejías-Luque R. JAK-STAT1 signaling pathway is an early response to helicobacter pylori infection and contributes to immune escape and gastric carcinogenesis. *Int J Mol Sci* (2022) 23(8):4147. doi: 10.3390/ijms23084147

27. Pereira-Marques J, Ferreira RM, Pinto-Ribeiro I, Figueiredo C. Helicobacter pylori infection, the gastric microbiome and gastric cancer. *Adv Exp Med Biol* (2019) 1149:195–210. doi: 10.1007/5584_2019_366

28. Schulz C, Schütte K, Mayerle J, Malfertheiner P. The role of the gastric bacterial microbiome in gastric cancer: helicobacter pylori and beyond. *Therap Adv Gastroenterol* (2019) 12:1756284819894062. doi: 10.1177/1756284819894062

29. Ye F, Han X, Shao Y, Lo J, Zhang F, Wang J, et al. Identification of novel biomarkers differentially expressed between African-American and Caucasian-American prostate cancer patients. *Am J Cancer Res* (2022) 12(4):1660–70.

30. Peres LC, Colin-Leitzinger CM, Teng M, Dutil J, Alugubelli RR, DeAvila G, et al. Racial and ethnic differences in clonal hematopoiesis, tumor markers, and outcomes of patients with multiple myeloma. *Blood Adv* (2022) 6(12):3767–78. doi: 10.1182/bloodadvances.2021006652

31. Javadian P, Xu C, Sjoelund V, Borden LE, Garland J, Benbrook DM. Identification of candidate biomarker and drug targets for improving endometrial cancer racial disparities. *Int J Mol Sci* (2022) 23(14):7779. doi: 10.3390/ijms23147779

32. Smith KER, Brown JT, Wan L, Liu Y, Russler G, Yantorni L, et al. Clinical outcomes and racial disparities in metastatic hormone-sensitive prostate cancer in the era of novel treatment options. *Oncologist.* (2021) 26(11):956–64. doi: 10.1002/onco.13848

33. Diamandis EP. Cancer biomarkers: can we turn recent failures into success? *J Natl Cancer Inst* (2010) 102(19):1462–7. doi: 10.1093/jnci/djq306