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Trabectedin, lurbinectedin, and other marine-derived anticancer alkaloids on solid cancer: Mechanisms of action, clinical impact, and future perspectives

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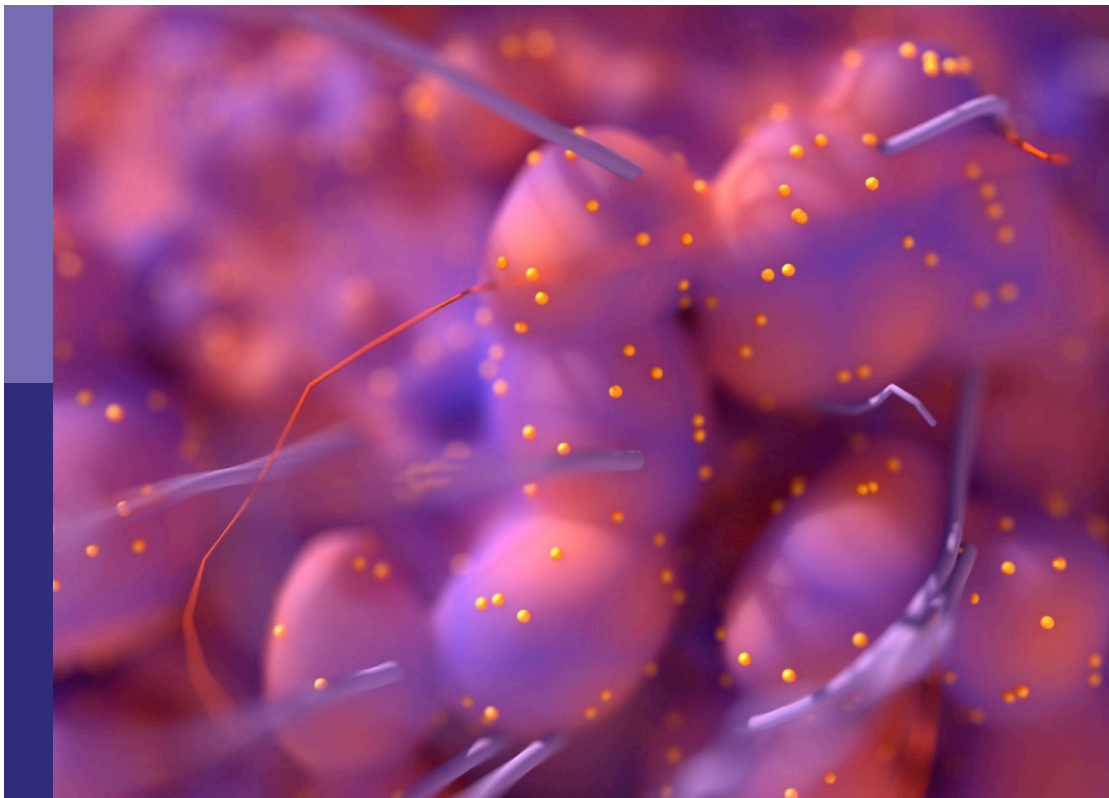

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Trabectedin, lurbinectedin, and other marine-derived anticancer alkaloids on solid cancer: Mechanisms of action, clinical impact, and future perspectives

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Editorial: Trabectedin, lurbinectedin, and other marine-derived anticancer alkaloids on solid cancer: Mechanisms of action, clinical impact, and future perspectives

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Editorial on the Research Topic

Trabectedin, lurbinectedin, and other marine-derived anticancer alkaloids on solid cancer: Mechanisms of action, clinical impact, and future perspectives

The growing knowledge of the molecular pathogenesis of many diseases allows designing drugs able to bind or inhibit specific molecular targets with high specificity. As far as cancer, this approach has been very successful and several drugs eventually have been developed as inhibitors of oncogenes and antagonist of aberrantly deregulated pathways, in specific human neoplasms. The Hallmarks of cancer - recently overviewed by Hanahan - are multiple and complex (1). They involve cell proliferation signaling, evading cell-growth suppressors, resisting cell death, enabling replicative immortality, inducing neo-angiogenesis, activating invasion and metastases, reprogramming of cell metabolism and avoiding anti-cancer immune engagement.

The explosion of knowledge on cancer biology has offered tremendous opportunities to develop novel synthetic compounds or antibodies acting by inhibiting different cancer targets. However, the results achieved with these target therapies have been frequently less impressive than expected, deriving only in few cases a meaningful improvement in patients' overall survival (2). This is probably due to the fact that for some human malignancies no drugable driving-targets have been eventually identified, whereas for others rapid occurrence of resistance is observed because of genomic instability and high biological heterogeneity, as crucial features of most human advanced malignancies.

In this context, the identification of new natural products with antitumor activity is far from being an obsolete strategy and in particular marine chemistry provides access to a large number of compounds with unique chemical structure and potentially exploitable biological and pharmacological properties. Indeed, the exploration of the seas with myriads of microenvironments has played an important role in our understanding of the adaptation of life to hostile environment, mainly trough the production of compounds that in some cases have turned out to be useful drugs in the fight against cancer.

In this special issue entitled *"Trabectedin, Lurbinectedin, and other Marine-Derived Anticancer Alkaloids"* basic scientists and clinical researchers provide some interesting examples of marine compounds that are already part of the therapeutic armamentarium of the medical oncologists (i.e. trabectedin and eribulin) or under preclinical and clinical investigation, as in the case of lurbinectedin.

Trabectedin was one of the first marine-derived anti-neoplastic drugs approved in solid tumor, in particular for the treatment of soft tissue sarcomas and relapsed platinum-sensitive ovarian cancer. Its unique mechanism of action, related to transcription regulation, DNA-repair machinery interference and direct effects on tumor microenvironment, is paving the way for new investigations in the field, as highlighted in the papers published in this Research Topic of *Frontiers in Oncology*, that reports a mixture of updated reviews and original contributions. In the paper by Merlini et al. a potential predictive biomarker of response to trabectedin combined with the PARP inhibitor olaparib is proposed in advanced bone and soft tissue sarcomas. In the paper by Allavena et al. the unique effects of trabectedin and lurbinectedin on tumor microenvironment are carefully described showing that both drugs can potentiate immunotherapy with checkpoint inhibitors by reducing the immune escape and the number and function of the immunosuppressant tumor associated macrophages (TAM). Namely, lurbinectedin shares some mechanistic properties of trabectedin but has also some distinct pharmacokinetic/pharmacodynamic features that are relevant for the encouraging toxicity profile and large spectrum of activity, as overviewed in the papers by Gadducci and Cosio, and by Musacchio et al. this latter mainly focusing on ovarian cancer. The paper by Heredia-Soto et al. shows the marine antimitotic agent Plocabulin has a preclinical potent anti-proliferative activity and migration in several ovarian cancer cell lines. Although the data presented were preliminary and no conclusion can be definitively drawn on the therapeutic index of Plocabulin, nevertheless the drug appears to be equally effective in platinum sensitive/resistant ovarian cancer cells, a finding of potential clinical relevance.

The papers by Nakamura and Sudo and by Phillips et al. provide updated overviews on the activity of trabectedin and eribulin in different histotypes of soft tissue sarcomas. The paper by Sanctis et al. highlights the therapeutic activity of several marine anticancer drugs - including trabectedin, lurbinectedin and eribulin - in breast cancer. In addition, the paper overviews the recent data obtained with the very potent marine compound monomethyl auristatin bound to specific antibodies, with high selectivity for breast cancer. As illustrated, these novel antibody-drug conjugates (ADC) show promising activity in breast cancer and likely in others cancer types.

Several papers of the issue report the available data on the pharmacological properties and specific toxicities of marine natural products, such as trabectedin and eribulin that have been used in the clinic for a long. In particular, the paper by Keritam et al. focuses on the toxicity associated to the extravasation of trabectedin and provides preclinical original data to assess this type of undesired effects.

Certainly, the papers published on this issue can be an important source of references for researchers and clinical oncologists and may fuel further interest in marine products that we feel will be not only important for relevant biological discoveries but will also contribute to improve the therapy of many human tumors, in the current era of molecular medicine.

Author contributions

AZ and MD equally contributed to the manuscript conceptualization, writing, review, and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

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REVIEW
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The Role of Trabectedin in Soft Tissue Sarcoma

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Background: Systemic chemotherapy for advanced disease is another therapeutic option in the management of metastases in soft tissue sarcoma (STS). Doxorubicin either alone or in combination with ifosfamide has been used as first-line chemotherapy. Furthermore, in the past decade, new drugs have been shown to be effective in the treatment of advanced STS after the failure of first-line anthracycline-based chemotherapy: trabectedin, pazopanib and eribulin. However, the appropriate usage of these agents has not been established.

Methods: We summarized clinical trials of trabectedin focusing on the efficacy and toxicity of trabectedin in the treatment of STS.

Results: Trabectedin can be administered safely and effectively to the patients with advanced STS at second line setting or later. Although trabectedin may be effective as first-line treatment in selected patients, anthracycline-based chemotherapy should be recommended because no regimen in addition to trabectedin has proved to be unequivocally superior to doxorubicin as the first-line treatment for locally advanced or metastatic STS. Nucleotide excision repair (NER) and homologous recombination (HRe) repair may be of particular importance as efficacy of trabectedin.

Conclusion: Trabectedin has shown a favorable toxicity profile and is an alternative therapeutic option in patients with advanced STS.

Keywords: trabectedin, soft tissue sarcoma, clinical trials, progression-free survival, advanced soft tissue sarcoma

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INTRODUCTION

Soft tissue sarcoma (STS) is a rare, heterogeneous group of tumors (Clark et al., 2005; Bourcier et al., 2019). The incidence of STS is fewer than six per 100,000 cancer cases, which represents 1–2% cases of all cancer in adults (Clark et al., 2005). Lung metastasis from STS occur in 20–50% of these patients (Nevala et al., 2019; Nakamura et al., 2021). Metastasectomy is the standard treatment for improving survival in patients with lung metastasis from STS (Marulli et al., 2017; Nakamura et al., 2017; Stamenovic et al., 2021). Recently, radiofrequency ablation (RFA) of the lung has also proved to be a useful option which promise a similar outcome to metastasectomy (Nakamura et al., 2009; Nakamura et al., 2017; Tetta et al., 2021). However, even after a seemingly complete resection of metastatic tumors, metastasis will recur in 40–80% of the patients (Weiser et al., 2000). Systemic chemotherapy for advanced disease is another therapeutic option in the management of metastases (Bramwell et al., 2003; Judson et al., 2014; Ratan and Patel, 2016; Smrke et al., 2020). Doxorubicin either alone or in combination with ifosfamide has been used as first-line chemotherapy (Judson et al., 2014; Smrke et al., 2020). Furthermore, in the past decade, new drugs have been shown to be

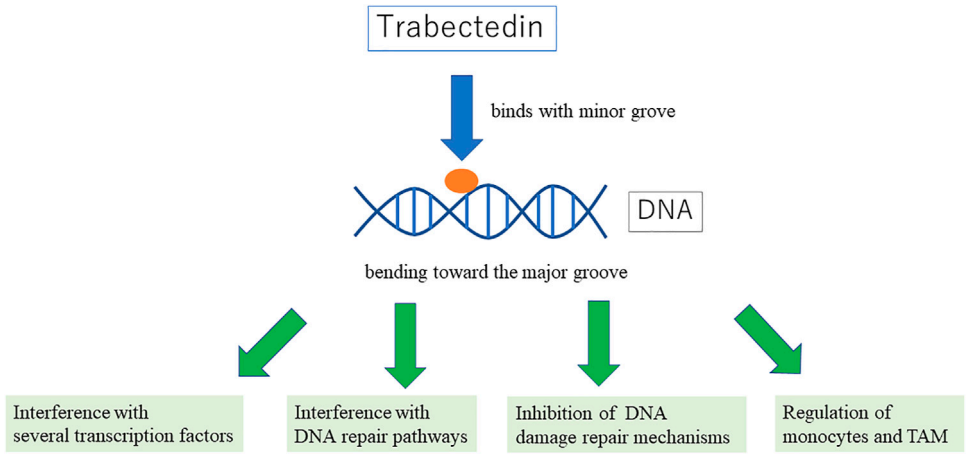

FIGURE 1 | showing the mechanism of trabectedin.

effective in the treatment of advanced STS after the failure of first-line anthracycline-based chemotherapy: trabectedin, pazopanib and eribulin. However, the appropriate usage of these agents has not been established because of the rarity of STS and difficulty of large study.

Trabectedin is a synthetic, marine-derived anticancer alkaloids derived from the Caribbean tunicate, Ecteinascidia turbinate (Carter and Keam, 2007; Cuaves and Francesch, 2009). The success of trabectedin in preliminary clinical trials for STSs has led to the approval of the drug in European countries in 2007 for the treatment of patients with advanced STS after the failure of therapy with doxorubicin either alone or in combination with ifosfamide (European Medical Agency). In 2015, Food and Drug Administration (FDA) approved trabectedin for the treatment of patients with unresectable or metastatic liposarcoma or leiomyosarcoma who received a prior anthracycline-containing regimen (Barone et al., 2017). Approval was based on the results of a randomized phase III study (ET743-SAR-3007, ClinicalTrials.gov Identifier; NCT01343277) comparing the safety and efficacy of trabectedin 1.5 mg/m2 as a 24-h continuous intravenous (IV) infusion once every 3 weeks with dacarbazine 1,000 mg/m2 IV once every 3 weeks (Demetri et al., 2016). Furthermore, in 2015, trabectedin was approved in Japan for the treatment of patients with STS after a clinical trial targeting translocation-related sarcoma (TRS) (Kawai et al., 2015).

Although the detailed indication of trabectedin is different in the world, several studies were conducted for finding the characteristics of trabectedin in the field of STS. The purpose of this review is to summarize the efficacy and toxicity of trabectedin in the treatment of STS.

How Does Trabectedin Worked (Figure.1)?

Trabectedin is a tetrahydroisoquinoline alkaloid derived from the Caribbean marine tunicate, Ecteinascidia turbinata, and is currently produced synthetically (Carter and Keam, 2007; Cuaves and Francesch, 2009). Trabectedin interacts with the minor groove of DNA double helix and alkylates guanine at the N2 position, which bends toward the major groove (D'Incalci and Galmarini, 2010; D'Incalci et al., 2014; Larsen et al., 2016), triggering a cascade of events that interferes with several transcription factors, DNA binding proteins, and DNA repair pathways, resulting in a delayed S phase progression and accumulation of cells in G2 phase and ultimately apoptosis (D'Incalci and Galmarini, 2010). Furthermore, the pattern of sensitivity observed in cells deficient in DNA damage repair (DDR) mechanisms is different. In the case of trabectedin, nucleotide excision repair (NER) and homologous recombination (HRe) repair are of particular importance (Soares et al., 2007; Italiano et al., 2011; Schoffski et al., 2011; Laroche-Clay et al., 2015). In contrast to other DNA-damaging agents such as cisplatin, NER-deficient cells are two to ten times less sensitive to trabectedin (Damia et al., 2001; Brodowicz 2014). On the other hand, cells deficient in HRe repair are sensitive to trabectedin (Avila-Arroyo et al., 2015). Therefore, DDR-related genes might be potential predictive biomarkers for this drug. Trabectedin seems to be more active in the context of high levels of expression of NER gene (ERCC1 and ERCC5) and low expression levels of HRe genes (BRCA1). Trabectedin selectively targets monocytes and tumor associated macrophages and downregulates the production of inflammatory mediators such as IL-6 and CCL2, which may underlie the strong association between chronic inflammation and cancer progression (D'Incalci et al., 2014; Germano et al., 2013).

Trabectedin also has a specific mechanism against some translocation-related sarcomas. Trabectedin blocks the *trans*-activating ability of chimaeras by displacing the oncogenic fusion protein FUS-CHOP from its target promoters in myxoid liposarcoma (Di Giandomenico et al., 2014; Forni et al., 2009). Recently, Genomic analysis in murine models of human myxoid liposarcoma showed that prolonged treatment causes losses in 4p15.2, 4p16.3 and 17q21.3 cytobands leading to acquired-resistance against trabectedin (Mannarino et al., 2021).

TABLE 1 | clinical trials for advanced STS.

Study design/Trial registration	Setting	Patients	Regimen	ORR (%)	PFS (mos)	OS
Phase 2 (Yovine et al., 2004)	the second-line setting or later	STS (n = 54)	A. T 1.5 mg/m2 24-h IV q3ws	4.0	1.9	12.8 months
Phase 2 (Garcia-Carbonero et al., 2004)	the second-line setting or later	STS (n = 36)	A. T 1.5 mg/m2 24-h IV q3ws	8.0	1.7	12.1 mos
Phase 2 (Demetri et al., 2009)	the second-line setting or later	LPS or LMS (n = 270)	A. T 1.5 mg/m2 24-h IV q3ws	5.6	3.7	13.9 months
R (1:1)			B. T 0.58 mg/m2 3-h IV weekly	1.6	2.3	10.8 months
Phase 2 (Kawai et al., 2015)	the second-line setting or later	TRS (n = 76)	A. T 1.2 mg/m2 24-h IV q3ws	11.0	5.6	Not reached
R (1:1)			B. Best supportive care	0.0	0.9	8 months
JapicCTI-121850						
phase 3 (Demetri et al., 2016; Patel et al., 2019)	the second-line setting or later	LPS or LMS (n = 518)	A. T 1.5 mg/m2 24-h IV q3ws	9.9	4.2	13.7 months
R (2:1)			B. Dac 1 g/m2 20- to 120-min q3ws	6.9	1.5	13.1 mos
NCT01343277						
phase 3 (Le Cesne et al., 2021)	the second-line setting or later	STS (n = 103)	A. T 1.5 mg/m2 24-h IV q3ws	13.7	3.1	13.6 months
R (1:1)			B. Best supportive care	0.0	1.5	10.8 months
NCT02672527						
phase 3 (Blay et al., 2014)	the first-line setting	TRS (n = 121)	A. T 1.5 mg/m2 24-h IV q3ws	5.9		16.1
R (1:1)			B. D 75 mg/m2 q3ws or D 60 mg/m2 + I 6–9 g/m2 q3ws	27.0	8.8	
NCT00796120						
phase 2 (Bui-Nguyen et al., 2015)	the first-line setting	STS (n = 133)	A. T 1.5 mg/m2 24-h IV q3ws	4.7	3.1	
R (1:1:1)			B. T 1.3 mg/m2 3-h q3ws	14.8	2.8	
NCT01189253			C. D 75 mg/m2 q3ws	25.6	5.5	
phase 2 (Martin-Broto et al., 2016)	the first-line setting	STS (n = 115)	A. T 1.1 mg/m2 3-h plus D 60 mg/m2 q3ws		5.5	13.3 months
R (1:1)			B. D 75 mg/m2 q3ws		5.7	13.7 months
NCT01104298						
phase 2 (Grosso et al., 2020)	the first-line setting	STS (n = 24)	A. T 1.3–1.5 mg/m2 24-h IV q3ws		4	12 months
NCT02066675						
phase 2 (Pautier et al., 2015; Pautier et al., 2021)	the first-line setting	STS (n = 62)	T 1.1 mg/m2 3-h plus D 60 mg/m2 q3ws		12.9	38.7 months
NCT02131480						

Abbreviations: R, randomized study; STS, soft tissue sarcoma; A, group A; B, group B; C, group C; T, trabectedin; D, doxorubicin; I, ifosfamide; Dac, dacarbazine; q3ws, every 3 weeks; h, hour; ORR, objective response rate according to Response Evaluation Criteria In Solid Tumors criteria; PFS, progression-free survival; OS, overall survival; mos, months; LPS, liposarcoma; LMS, leiomyosarcoma; TRS, translocation-related soft tissue sarcoma.

Trabectedin Treatment After the Failure of Prior Chemotherapy in Advanced STS (Table 1)

Two phase II trials in 2004 provided the initial analysis of trabectedin in STSs (Garcia-Carbonero et al., 2004; Yovine et al., 2004). Trabectedin was administered at a dose of 1.5 mg/m^2, 24-h IV infusion every 3 weeks. The first of these studies was conducted in 54 advanced or metastatic STS patients with failure of prior chemotherapy (Yovine et al., 2004). The objective response rate was 4%, although the disease control rate at 6 months was 24%. The median progression-free survival (PFS) and overall survival (OS) were 1.9 and 12.8 months, respectively. The second phase II trials reported a response rate of 8% in 36 recurrent or metastatic STS patients with disease progression despite prior chemotherapy (Garcia-Carbonero et al., 2004). The median PFS and OS were 1.7 and 12.1 months, respectively.

In addition to the efficacy of trabectedin 1.5 mg/m^2 24-h IV infusion every 3 weeks, a weekly trabectedin schedule (0.58 mg/m^2 3-h IV infusion for 3 consecutive weeks in a 4-weeks cycle) was demonstrated to have substantial anticancer activity in pretreated ovarian cancer (Krasner et al., 2007). To assess the efficacy and safety of these two schedules in STS, a randomized, open-label, phase II trial was conducted in patients with advanced and/or metastatic liposarcomas or leiomyosarcomas after the failure of standard therapies (Demetri et al., 2009). The time to progression was the primary endpoint. The 24-h IV q3ws demonstrated a superior time to progression of 3.7 vs 2.3 months (hazard ratio (HR), 0.734; 95% confidential interval (CI), 0.554–0.974; p = 0.0302). The median PFS was 3.3 vs 2.3 months (HR, 0.755; 95% CI, 0.574–0.992; p = 0.0418). The median OS was 13.9 vs 11.8 months (HR, 0.843; 95% CI, 0.653–1.090; p = 0.1920). After these results, trabectedin 1.5 mg/m2 24-h IV infusion every 3 weeks is common schedule of trabectedin treatment.

A recent phase II study in the second-line setting or later has been reported (Kawai et al., 2015). This study was a randomized phase II study of trabectedin monotherapy vs best supportive care

(BSC) in patients with translocation-related sarcoma subtypes. The patients were randomized (1:1) to receive trabectedin (1.2 mg/m2 24-h IV infusion every 3 weeks) or best supportive care. The trabectedin dose of this trial was 1.2 mg/m^2 according to the results of a phase I study in Japanese patients with STSs, in which two of three patients had dose-limiting toxicity at 1.5 mg/m^2 (Ueda et al., 2014). The primary endpoint of this trial was the PFS. The median PFS of the trabectedin group was 5.6 months and that of the BSC group was 0.9 months (HR, 0.07; 95% CI, 0.03–0.16; $p < 0.0001$). The success of trabectedin in this clinical trial for STSs has led to the approval of the drug in Japan.

In 2015, trabectedin has been approved by the FDA based on the result of an open-label, randomized (2:1) phase III trial of trabectedin (n = 345) vs dacarbazine (n = 173) in patients with metastatic liposarcoma or leiomyosarcoma (ET743-SAR-3007, ClinicalTrials.gov Identifier; NCT01343277) (Demetri et al., 2016). In the final analysis of PFS, trabectedin administration resulted in a 45% reduction in the risk of disease progression or death compared with dacarbazine. The median PFS was 4.2 vs 1.5 months (HR, 0.55; 95% CI, 0.44–0.70; $p < 0.001$).

After the analysis of PFS in 2016, the final overall survival (OS) results in an open-label, randomized (2:1) phase III trial of trabectedin (n = 384) vs dacarbazine (n = 193) in 577 patients with metastatic liposarcoma or leiomyosarcoma (ET743-SAR-3007, ClinicalTrials.gov Identifier; NCT01343277) was published in 2019 (Patel et al., 2019). Despite improved disease control by trabectedin, no improvement in OS was observed. The median OS for trabectedin and dacarbazine was 13.7 and 13.1 months, respectively ($p = 0.49$). Trabectedin prolonged time to starting any post-study anticancer therapy in the trabectedin arm (median 6.8 months) compared with the dacarbazine arm (3.5 months).

As a subgroup analysis of phase III study (ET743-SAR-3007, ClinicalTrials.gov Identifier; NCT01343277), 131 elderly patients were collected for evaluating the safety and efficacy in elderly patients with metastatic liposarcoma or leiomyosarcoma (Jones et al., 2018). Among 131 patients (trabectedin = 94; dacarbazine = 37), elderly patients treated with trabectedin (median age = 69 years) showed significantly improved PFS (4.9 versus 1.5 months, respectively; HR 0.40; $p = 0.0002$) but no significant improvement in OS (15.1 vs 8.0 months, respectively; HR = 0.72, $p = 0.18$). The safety profile for elderly trabectedin-treated patients was comparable to that of the overall trabectedin-treated study.

The French Sarcoma Group assessed the efficacy, safety, and quality of life of trabectedin versus BSC in patients with advanced STS (ClinicalTrials.gov Identifier; NCT02672527) (Le Cesne et al., 2021). This study was a randomized phase III study. The patients were randomized (1:1) to receive trabectedin (1.5 mg/m^2 24-h IV infusion every 3 weeks) or BSC. The primary endpoint of this trial was the PFS. The median PFS of the trabectedin group (n = 52) was 3.1 months and that of the BSC group (n = 51) was 1.5 months (HR, 0.39; 95% CI, 0.24–0.64; $p < 0.0001$). Trabectedin demonstrates superior disease control to BSC. In this study, the health-related quality of life (QOL) was assessed using the 30-item core European Organization for the Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (EORTC QLQ-C30). Compliance to EORTC QLQ-30 was good in both arm at baseline and after 8 months decreased to 59% in the trabectedin arm and 63% in the BSC arm. Therefore, trabectedin demonstrated superior disease control to BSC without impairing QOL.

Clinical Trial as First-Line Chemotherapy in Advanced STS

Generally, trabectedin is considered to be administered for the patients with advanced STS after the failure of first-line chemotherapy. Some clinical trials aimed to develop the trabectedin treatment as first-line chemotherapy. One phase III study in the first-line setting has been reported (ClinicalTrials.gov Identifier; NCT00796120). (Blay et al., 2014). This study was a randomized, phase III study of first-line trabectedin vs doxorubicin-based chemotherapy in patients with TRS subtypes. The primary endpoint was PFS. Patients were randomized (1:1) to receive trabectedin (1.5 mg/m^2 24-h IV infusion every 3 weeks), doxorubicin (75 mg/m^2 IV every 3 weeks), or doxorubicin (60 mg/m^2 IV) plus ifosfamide (range, 6–9 g/m^2 IV) every 3 weeks. There was no difference in the median PFS or OS between the groups ($p = 0.9573$ and $p = 0.3659$, respectively). The response rate according to the RECIST (Response Evaluation Criteria In Solid Tumors) criteria was significantly higher in the chemotherapy arm (27%) compared to the trabectedin arm (5.9%). In contrast, the response rate according to the Choi criteria showed fewer differences between the chemotherapy arm (45.9%) and trabectedin arm (37.3%).

Recently, results from randomized, multicenter, prospective dose-selection phase IIb trials to evaluate whether trabectedin as first-line chemotherapy for advanced/metastatic STS prolongs the PFS, compared to doxorubicin, were published (ClinicalTrials.gov Identifier; NCT01189253) (Bui-Nguyen et al., 2015). One hundred and thirty-three patients were randomized (1:1:1) to doxorubicin, trabectedin (3-h [T3h arm] infusion every 3 weeks), or trabectedin (24-h [T24h arm] infusion every 3 weeks). The median PFS was 2.8 months in the T3h arm, 3.1 months in the T24h arm, and 5.5 months in the doxorubicin arm. No significant improvement in the PFS was observed in the trabectedin arms as compared to the doxorubicin arm (T24h vs doxorubicin: HR 1.13; 95% CI 0.67–1.90, $p = 0.675$; T3h vs doxorubicin: HR 1.50, 95% CI 0.91–2.48, $p = 0.944$).

Spanish group conducted randomized, phase II clinical trial for comparing the clinical outcome of trabectedin plus doxorubicin with doxorubicin as first line treatment of advanced STS (ClinicalTrials.gov Identifier; NCT01104298) (Martin-Broto et al., 2016). The primary endpoint was PFS. One hundred and fifteen patients were randomized (1:1) to trabectedin (1.1 mg/m2 in a 3-h infusion) plus doxorubicin (60 mg/m2) as the experimental arm or doxorubicin (75 mg/m2) as control arm. PFS was 5.5 months in the control arm and 5.7 months in the experimental arm (HR, 1.16; 95% CI, 0.79–1.71, $p = 0.45$). The proportion of patients with grade 3 or 4 thrombocytopenia, asthenia, and liver toxicity was significantly higher in the experimental arm. Trabectedin plus doxorubicin did

not show superiority over doxorubicin alone as first-line treatment of advanced STS.

Italian Sarcoma Group reported a phase II single-arm study for investigating trabectedin as a first-line treatment in elderly patients with advanced STS who were inoperable and were unfit to receive standard anthracycline-based chemotherapy (TR1US study, ClinicalTrials.gov Identifier; NCT02066675) (Grosso et al., 2020). The primary endpoint was PFS at 3 months and the rate of clinically limiting toxicities (CLTs). With a median age of 79 years, 24 patients were enrolled. progression-free survival at 3 months was 71%. Median PFS and OS were 4 and 12 months, respectively. There were no significant differences in trabectedin pharmacokinetics compared with younger populations.

Although trabectedin may be effective as first-line treatment in selected patients, anthracycline-based chemotherapy should be recommended because no regimen in addition to trabectedin has proved to be unequivocally superior to doxorubicin as the first-line treatment for locally advanced or metastatic STS (Seddon et al., 2017).

Interestingly, French Sarcoma Group performed a single-arm, multicentre, phase II study (LMS-02, ClinicalTrials.gov Identifier; NCT 02131480) of doxorubicin combined with trabectedin as first-line treatment in patients with uterine leiomyosarcoma and STS (Pautier et al., 2015; Pautier et al., 2021). Patients received 60 mg/m2 IV doxorubicin followed by trabectedin 1.1 mg/m2 as a 3 h infusion on day 1 and pegfilgrastim on day 2, every 3 weeks, up to six cycles. Median PFS in 62 patients with STS was 12. 9 months (95%CI 9.2–14.1 months). The median OS was 38. 7 months (95%CI 31–52.9 months). Now, LMS04 trial (ClinicalTrials.gov Identifier; NCT02997358), a randomized phase III study comparing the doxorubicin plus trabectedin combination versus doxorubicin alone in first-line therapy in metastatic leiomyosarcoma are pending.

Combination Therapy With Radiotherapy in Metastatic STS

Spanish groups assessed the combined use of trabectedin and radiotherapy in patients with metastatic STS as phase I/II clinical trial (ClinicalTrials.gov Identifier; NCT02275286) (Martin-Broto et al., 2020). Trabectedin was administered every 3 weeks in a 24-h infusion. Radiotherapy (3 Gy/day for 10 days) was required to start within 1 h after completion of the first trabectedin infusion. In phase 1, recommended dose of trabectedin for this combination treatment was 1.5 mg/m2. In phase 2, among 25 patients, the overall response rate was 72% for local assessment and 60% for central assessment. Overall response rate was calculated as the proportion of patients who achieved a partial or complete RECIST response during therapy.

Clinical Trial as Neoadjuvant Chemotherapy

One phase II clinical trial in the neoadjuvant setting in patients with advanced localized myxoid liposarcoma has been previously reported ClinicalTrials.gov Identifier; NCT00579501) (Gronchi et al., 2012). The treatment consisted of trabectedin 1.5 mg/m^2 given as 24-h IV infusion every 3 weeks. Twenty-nine patients received a minimum of three and a maximum of six cycles before surgery. Of 23 patients who could be evaluated by the pathological response, three patients achieved a pathological complete response. Another 12 of 23 had at least a good regression rate (>50% regression). Of 29 patients, seven patients (24%) had a partial response and 21 patients had SD according to the RECIST criteria. One patient died prior to the evaluation due to rhabdomyolysis with hepatic and renal failure after the second trabectedin cycle.

In 2017, phase III clinical trial for evaluating the superiority of neoadjuvant chemotherapy of histotype-tailored regimen to standard chemotherapy (ISG-STS 1001, ClinicalTrials.gov Identifier; NCT01710176) (Gronchi et al., 2017). The STS was non-metastatic, high-risk (high malignancy grade, 5 cm or longer in diameter, and deeply located according to the investing fascia) at extremities or trunk wall and belonging to one of five histological subtypes: high-grade myxoid liposarcoma, leiomyosarcoma, synovial sarcoma, malignant peripheral nerve sheath tumor, and undifferentiated pleomorphic sarcoma. Trabectedin (1.3 mg/m^2 via 24-h IV infusion) was administered in patients with high-grade myxoid liposarcoma. Patients were randomly assigned (1:1) to receive three cycles of full-dose standard chemotherapy (epirubicin 60 mg/m^2 per day [short infusion, days 1 and 2] plus ifosfamide 3 g/m^2 per day [days 1, 2, and 3], repeated every 3 weeks) or histotype-tailored chemotherapy. In the exploratory subgroup analyses according to histology, the difference in disease-free survival favoring standard chemotherapy was consistently seen in all strata, with the exception of high-grade myxoid liposarcoma, in which disease-free survival in the two groups were similar (HR, 1.03; 95%CI, 0.24–4.39).

In addition to previous studies (Gronchi et al., 2016.; Tanaka et al., 2019), the results of clinical trials suggested that preoperative chemotherapy with anthracycline and ifosfamide might be highly effective for treating high-risk STS.

TABLE 2 | Safety profile of trabectedin (NCI-CTC Grade3 or 4 toxicity).

Adverse events	Demetri et al. (2016)	Le Cesne et al. (2013)
	n = 340 (%)	n = 350 (%)
Neutropenia	37	47.6
ALT elevation	26	44.6
Thrombocytopenia	17	13.5
Anemia	14	12.3
AST elevation	13	34.4
Fatigue	6	8.3
CPK elevation	5.3	4.1
Nausea	5	6.3
Vomiting	5	5.1
Rhabdomyolysis	1.2	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; NCI-CTC, National Cancer Institute Common Toxicity Criteria.

Safety (Table 2)

Trabectedin was well tolerated in a phase III randomized clinical trial (ET743-SAR-3007, ClinicalTrials.gov Identifier; NCT01343277) (Demetri et al., 2016). The most frequently reported grade 3/4 adverse events were neutropenia (37%) and

elevated serum levels of AST/ALT (13%/26%). Less often, grade3/4 creatine phosphokinase elevations (5.3%) and rhabdomyolysis (1.2%) were seen. Deaths associated with drug-related adverse events were infrequent (2.1%). These events were consistent with the well characterized safety and toxicity profiles of trabectedin (Le Cesne, et al., 2013).

The subgroup analysis of the elderly population of ET743-SAR-3007 showed tolerability of trabectedin in elderly patients (Jones et al., 2018). The safety profile for elderly trabectedin-treated patients was comparable to that of the overall trabectedin-treated study. Among 94 patients, the most frequently reported grade 3/4 adverse events were neutropenia (40%) and elevated serum levels of AST/ALT (15%/24%). No unique or unexpected adverse events were noted.

Transaminase increase was the most frequent cause of dose reductions (Calvo et al., 2018). The post hoc analyses of ET743-SAR-3007 confirmed that transaminase elevations were typically highest in the first 2 cycles and mostly transient, non-cumulative, and without clinical consequences, even in patients with grade3/4 transaminase elevations (Calvo et al., 2018). These liver laboratory abnormalities could be managed through dose reduction and delays.

A recurring pattern was observed with increased transaminase levels, typically reaching a peak between days 5 and 7 of each cycle and resolving to grade ≤1 by day 15 without implication for the patient (Brodowicz. 2014). Steroid pretreatment is an effective way of reducing the extent of hepatotoxicity, and steroids are now given routinely before trabectedin administration. Premedication with 20 mg of dexamethasone IV 30 min prior to trabectedin was shown to provide hepatoprotective effects beyond its antiemetic effect (Grosso et al., 2006; Amart et al., 2015).

Prognostic Factors for the Treatment of Trabectedin

Previous *in vitro* studies have demonstrated that trabectedin cytotoxicity depends on the status of both NER and HR DNA repair pathway (Soares et al., 2007; Italiano et al., 2011; Schoffski et al., 2011; Laroche-Clay et al., 2015). Moreover, DNA-damage biding proteins, which are known components of NER pathway have been described to be a part of the CUL4A ubiquitin ligase complex (Iovine et al., 2011; Moura et al., 2020). The expression of CUL4A could be an indicator of NER pathway integrity and trabectedin efficacy. One prospective translational analysis was performed as a correlative study within the comparative phase II trial that compared trabectedin plus doxorubicin versus doxorubicin alone as first line of advanced STS (Moura et al., 2020). The cases included for gene (n = 66) and protein expression (n = 85). In the group of trabectedin plus doxorubicin (n = 32), overexpression of CUL4A, ERCC1, and ERCC5 significantly correlated with better median PFS, although BRCA1 expression did not correlated with PFS. None of these genes were statistically significant correlated with OS in trabectedin plus doxorubicin group. Furthermore, in the study of phase IIb trial (41), genotype status was available for 60 patients. There was no significant association between BRCA1 haplotype and PFS (Italiano et al., 2018).

CONCLUSION

Trabectedin can be administered effectively to patients, but it is important to note that evidence is available for different types of cancer although belonging to the group of advanced STS. Also, trabectedin can be administered safely to the patients although all evidence is limited and future studies should be necessary.

AUTHOR CONTRIBUTIONS

(I) Conception and design: All authors; (II) Administrative support: AS (III) Provision of study materials: TN (IV) Collection and assembly of data: TN (V) Data analysis and interpretation: TN (VI) Manuscript writing: TN (VII) Final approval of manuscript: All authors.

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REVIEW
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Preclinical and Clinical Evidence of Lurbinectedin in Ovarian Cancer: Current Status and Future Perspectives

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Lurbinectedin is an antitumor agent belonging to the natural marine-based tetrahydroisoquinoline family which has shown very promising clinical activity with a favorable safety profile in many types of cancer. Preclinical evidence showed that lurbinectedin inhibits active transcription and binds to GC-rich sequences, leading to irreversible degradation of RNA polymerase II and generation of single- and double-strand DNA breaks and, as a consequence, apoptosis of tumor cells. In addition, lurbinectedin has demonstrated modulation of the tumor microenvironment and activity against cancer cells harboring homologous recombination DNA repair deficiency. Although considerable improvements have been made in the treatment of epithelial ovarian cancer, most patients with advanced disease experience recurrence with a dismal prognosis due to chemotherapy (mainly platinum) resistance. Platinum-resistant/refractory ovarian cancer remains a difficult-to-treat setting of disease, and currently, the exploration of new therapeutic approaches represents a main field of interest. Although the CORAIL phase III study did not meet its primary endpoint, the results suggest that lurbinectedin might be a valid alternative for patients that have exhausted therapeutic options. This article will focus on the clinical evidence, the most recent investigations, and the future perspective regarding the use of lurbinectedin in ovarian cancer.

Keywords: ovarian cancer, lurbinectedin, platinum-resistant ovarian cancer, marine-derived drugs, DNA minor groove

INTRODUCTION

Ovarian cancer (OC) is the eighth most common malignancy in women, with an estimated 313,959 new cases and 207,252 new deaths in 2020 (1). Due to a lack of early-stage detection, about 70% of patients present with advanced disease (FIGO stage III–IV) at diagnosis, and the mainstay of treatment is represented by radical surgery and platinum/paclitaxel chemotherapy. In the last few years, the

introduction of anti-angiogenic agents and poly(ADP-ribose) polymerase inhibitors (PARPi) reshaped completely the outcomes of these patients, with remarkable improvements in terms of progression-free survival (PFS) (2–8) and overall survival (OS) (5). Despite the initial effectiveness of this approach, unfortunately, a large proportion of patients will experience disease relapse or progression. Treatment of recurrent OC for several years has been selected based only on the progression-free interval (PFI) after platinum-based chemotherapy. Recently, other considerations, i.e., the toxicity profile of the drugs, the genomic characteristics of the disease, the number of previous treatment lines, etc., play a role, in combination with PFI, in defining the most appropriate treatment at recurrence (9). Former platinum-resistant/refractory patients (patients who develop progressive disease during or within 6 months from platinum treatment completion) are usually managed with single-agent, non-platinum, chemotherapy between weekly paclitaxel, pegylated liposomal doxorubicin (PLD), gemcitabine, or topotecan with response rates (RR) around 10% and median OS of about 12 months (9). Therefore, the development of new agents in this setting represents a challenging field of interest. Lurbinectedin, a synthetic alkaloid originally derived from the marine tunicate *Ectenaiscidia turbinata*, has shown promising activity against platinum-resistant OC in preclinical models; in addition, some studies suggest that it possesses the capability to modulate the tumor microenvironment and to evoke anticancer immunity. Based on these lines of evidence and after the completion of a phase I trial assessing its tolerability and efficacy in advanced solid tumors (10), lurbinectedin was evaluated in platinum-resistant and platinum-refractory OC in a two-stage controlled phase II study, where a remarkable antitumor activity with a 23% overall response rate (ORR) (95% CI, 13%–37%) was reported (11). Based on these results, a randomized, controlled phase III trial of lurbinectedin or standard chemotherapy (PLD or topotecan) in platinum-resistant OC was designed. However, the primary endpoint of the CORAIL trial was not met, with no difference in PFS between the lurbinectedin arm and the standard chemotherapy arm (3.5 months, 95% CI 2.1–3.7 months vs. 3.6 months, 95% CI 2.7–3.8 months for lurbinectedin and standard chemotherapy, respectively). Moreover, compared with PLD or topotecan, lurbinectedin did not show a significant prolongation in OS nor a significant increase in ORR with a manageable toxicity profile (12). Although negative, the results of this trial suggest that lurbinectedin may be a reasonable therapeutic alternative in the management of platinum-resistant OC when other treatment options have been exploited. To explore its potential synergism and to enhance its therapeutic activity, lurbinectedin is currently being evaluated in several trials partnering with other agents such as PARPi (13).

BACKGROUND AND PRECLINICAL INVESTIGATION

Lurbinectedin is a novel synthetic alkaloid structurally and functionally related to trabectedin, a marine-derived product of the ecteinascidin family, which is currently approved for the treatment of relapsed platinum-sensitive epithelial OC in combination with PLD (14). These two compounds share an analogous mechanism of action, which consists in the formation of a covalent bond with central guanines in specific nucleoside triplets located in the minor groove of the DNA molecules. These interactions lead to the formation of lurbinectedin–DNA adducts that eventually induce double-strand breaks (DSBs) in cancer cells and perturbations in the cell cycle; in *in-vitro* models, the exposure to lurbinectedin determines increased apoptotic rates in cancer cells, which occurs with a caspase-dependent pathway. As a result of these processes, lurbinectedin exerts strong *in-vitro* cytotoxic activities against multiple cancer cell lines, including OC, which has been confirmed *in vivo* through xenograft models of different human cancers (lung, ovary, colon, and stomach) (15).

It has been demonstrated that the cytotoxic activity of the members of the ecteinascidin family is dependent upon the mechanism of DNA nucleotide excision repair (NER). Selected cancer cell lines which are resistant to trabectedin show deficient xeroderma pigmentosum (XPG/ERCC4) gene expression, which is implicated in the NER pathway. Takebayashi et al. have shown that sensitivity to trabectedin can be restored by complementation with wild-type XPG, thus suggesting that trabectedin-induced cytotoxicity requires an intact NER mechanism. These authors propose that the adducts generated by the binding of trabectedin to the minor groove of DNA are recognized by the NER system, which ultimately leads to the formation of irreversible single-strand breaks and, consequently, to cell death (16). Interestingly, they observed that cisplatin-resistant cell lines display enhanced NER activity, which makes them more sensitive to trabectedin cytotoxicity.

The relationship between the NER system, platinum resistance, and the activity of trabectedin and lurbinectedin has been investigated in a preclinical model. After exposure to UV irradiation, which generates adducts in DNA molecules, platinum-resistant cell lines showed an increase in NER activity; nevertheless, when the same cell lines were treated with trabectedin or lurbinectedin, no cross-resistance to these agents was detected. Furthermore, a synergistic activity of the combination of lurbinectedin and cisplatin in cisplatin-resistant cell lines was observed (17). These findings have provided the bases to investigate the role of lurbinectedin in the setting of platinum-resistant OC.

The potential activity of lurbinectedin in platinum-resistant OC has been assessed in a preclinical model using a perpetuable orthotopic graft of a patient-derived epithelial ovarian cancer. In this murine model study (18), a cisplatin-resistant tumor graft was generated by serial cisplatin treatments and subsequent implantation of the post-treatment-derived tumor mass in mice. After the implant, mice were randomized to receive placebo, cisplatin, lurbinectedin, or the combination of the two agents. The results of this study showed that lurbinectedin is more effective than cisplatin in platinum-resistant OC and that the combination of cisplatin and lurbinectedin was more active than either single therapy in the context of platinum-resistant

OC, again suggesting a synergistic activity of these two drugs. Of note, in cisplatin-sensitive OC, no significant benefit of the combined treatment was observed. The antitumor activity of lurbinectedin in OC was further corroborated by the analysis of the histological changes in tumor population: the highest grade of histopathological tumor regression was observed in the combination arm in both cisplatin-sensitive and cisplatin-resistant tumors.

Notably, lurbinectedin showed significant antitumor activity also against clear cell carcinoma (CCC) of the ovary, a relatively platinum-resistant OC subtype (19). In the study by Takahashi et al. (20), lurbinectedin inhibited tumor growth of platinum-resistant CCC cells both *in vitro* and *in vivo*; furthermore, when tested in combination with other antineoplastic agents, lurbinectedin showed synergistic activity with irinotecan, while its antitumor effects were enhanced when given in combination with the mammalian target of rapamycin-1 (mTORC1) inhibitor everolimus, which may represent a druggable target in CCC (21).

Along with its antitumor activity, lurbinectedin has also tumor microenvironment modulation properties which have been assessed in preclinical studies. When tested against human monocytes from healthy donors, lurbinectedin induces monocyte apoptosis *in vitro* and hampers proinflammatory activity by inhibiting the production of inflammatory chemokines such as CCL2 and CXCL8, which translates into a diminished monocyte migration. Furthermore, lurbinectedin exerts an anti-angiogenetic effect by inhibiting the generation of the vascular endothelial growth factor (VEGF). These effects have also been observed in cell lines and in *in-vivo* models, where treatment with lurbinectedin markedly reduced the amount of tumor-associated macrophages (TAM) and tumor vascularization (22). Tumor-associated inflammation is a well-recognized hallmark of cancer which contributes to tumor growth and survival; therefore, the activity of lurbinectedin in contrasting the tumor-associated proinflammatory cells makes this molecule of particular interest.

In addition, it has been recently shown that lurbinectedin may evoke anticancer immunity by inducing immunogenic cell death (ICD). In a preclinical study by Xie et al. conducted on osteosarcoma cell lines, treatment with lurbinectedin was associated with the stimulation of ICD as demonstrated by multiple cell modifications, such as the translocation of calreticuline (CALR) at the cell surface, the generation of an autocrine and paracrine response mediated by type I interferons, and the release of nuclear high mobility group box 1 (HMGB1), which is involved in tumor antigen recognition. Given these immunomodulatory effects, it has subsequently been investigated whether lurbinectedin may act in a synergistic fashion with immunotherapy in xenograft models, by sensitizing cancer cells to immune checkpoint inhibitors (ICIs). Treatment with a combination of both an anti-PD-1 and an anti-CTLA-4 antibody after exposure to lurbinectedin significantly extended the survival in murine models when compared with single ICI therapies; moreover, tumor-free mice that were rechallenged with the same cancer type show tumor rejection, indicating that the combination of lurbinectedin and immunotherapy may generate immunological memory (23).

PUBLISHED CLINICAL DATA

A recent phase I trial investigated the recommended phase II dose (RP2D) of cisplatin administered in combination with lurbinectedin, with or without aprepitant (group A and group B, respectively) in patients with advanced solid tumors, including OC. The secondary objectives of the study were the characterization of safety profile, pharmacokinetics, and preliminary antitumor activity. All patients were treated with 60 mg/m^2 cisplatin intravenous (i.v.) infusion followed by lurbinectedin i.v. infusion at escalating doses on day 1 every 3 weeks (q3wk). For patients in group A, the recommended dose was cisplatin 60 mg/m^2 plus lurbinectedin 1.1 mg/m^2, while for group B, the recommended dose was cisplatin 60 mg/m^2 plus lurbinectedin 1.4 mg/m^2. The most frequent grade ≥3 adverse events were hematological [neutropenia (41%), lymphopenia (35%), leukopenia (24%), thrombocytopenia (18%)] and fatigue (35%) in group A (n = 17) and neutropenia (50%), leukopenia (42%), lymphopenia (29%), and fatigue (13%) and nausea (8%) in group B (n = 24). Four patients (2 in each group) had a partial response and 14 patients (4 in group A and 10 in group B) achieved a stable response. No signs of activity were reported in the cohort of OC patients as well as in the group of patients receiving aprepitant, and the combination of lurbinectedin and cisplatin was considered highly toxic (24). Another multicenter, open-label, phase I study evaluated the recommended dose (RD) of the combination of lurbinectedin and gemcitabine in patients with advanced solid tumors. Forty-five patients were treated between May 2011 and May 2013 and received lurbinectedin 3.5 mg flat dose (FD)/gemcitabine 1,000 mg/m^2. Dose-limiting toxicities (DLTs) were mostly hematological and resulted in the expansion of a lower dose level (lurbinectedin 3.5 mg FD/gemcitabine 800 mg/m^2); 19 patients at this dose level were evaluable but >30% reported DLT and >20% had febrile neutropenia. On the contrary, DLT was observed in 11 patients treated with lurbinectedin 3.0 mg FD/gemcitabine 800 mg/m^2, which was defined as the RD. Nine of 38 patients were evaluable for response according to RECIST 1.1, with 3% complete responses and 21% partial responses, with an ORR of 24% (95% CI, 12%–40%). Eleven patients (29%) had disease stabilization for at least or more than 4 months. The median duration of response was 8.5 months and the median PFS was 4.2 months (95% CI, 2.7–6.5 months). This schedule is generally well tolerated and has reported antitumor activity in several advanced solid tumors (25). Recently, the results of a phase I study, designed to evaluate the safety and toxicity of lurbinectedin in combination with olaparib in patients with advanced solid tumors without standard therapeutic alternatives, were published. In total, 20 patients with OC, endometrial cancer, and uterine leiomyosarcoma were enrolled in this 3 + 3 dose-escalation study. The RP2D was lurbinectedin 1.5 mg/m^2 on day 1 and olaparib capsules 250 mg BID on days 1–5 of a 21-day cycle. The study did not report complete or partial responses, but disease control rate was achieved in 60% of patients. The most common, mainly grade 1–2, adverse events were asthenia (55%), nausea (55%), vomiting (50%), constipation (45%), abdominal pain (40%), neutropenia (35%),

and anemia (35%) (13). The safety and the efficacy of single-agent lurbinectedin were also evaluated in a two-stage, controlled, randomized, multicenter phase II study trial. The primary endpoint was ORR by the RECIST and/or GCIG criteria. The exploratory first stage (n = 22 patients) confirmed the activity of lurbinectedin as a single agent at 7.0 mg flat dose q3wk. The second stage (n = 59) was randomized and controlled versus topotecan on days 1–5 q3wk (1.50–0.75 mg/m^2) or weekly (4.0–2.4 mg/m^2). The ORR was 23% (95% CI, 13%–37%) for lurbinectedin with a median duration of response of 4.6 months (95% CI, 2.5–6.9 months), with 23% (95% CI, 0%–51%) of the responses lasting 6 months or more. Ten of the 12 confirmed responses were reported in the 33 platinum-resistant patients [ORR = 30% (95% CI, 16%–49%)]. No responses were reported among the 29 patients treated with topotecan. The median PFS was 4.0 months (95% CI, 2.7–5.6 months) for all lurbinectedin-treated patients and 5.0 months (95% CI, 2.7–6.9 months) for patients with platinum-resistant disease. Specifically, in the second randomized stage, the median PFS was significantly longer with lurbinectedin 3.9 months (95% CI, 2.5–5.7 months) versus 2.0 months (95% CI, 1.4–2.8 months) with topotecan (P = 0.0067). The median OS was 9.7 months (95% CI, 7.7–19.3 months) with lurbinectedin and 8.5 months (95% CI, 3.3–15.6 months) with topotecan (P = 0.2871).

Myelosuppression was the most frequent adverse event (AE). In the lurbinectedin arm, grade 3/4 neutropenia and thrombocytopenia were observed in 85% and 33% of patients, respectively, while in the topotecan arm, grade 3/4 neutropenia occurred in 38% of patients and grade 3/4 thrombocytopenia in 24% of patients (11).

Recently, the results of a phase III, multicenter, randomized trial, evaluating the efficacy of lurbinectedin with respect to PLD or topotecan in platinum-resistant ovarian cancer patients, were published. In this trial, patients were randomized in a 1:1 ratio to receive lurbinectedin 3.2 mg/m^2 i.v. infusion q3wk in the experimental arm or PLD 50 mg/m^2 i.v. infusion q4wk or topotecan 1.50 mg/m^2 i.v. infusion days 1–5 q3wk in the control arm. Performance status (PS) (0 vs. ≥1), prior PFI (1–3 vs. >3 months), and prior chemotherapy lines (1–2 vs. 3) were the stratification factors. The primary endpoint was PFS evaluated by an independent review committee according to RECIST 1.1. Two hundred and twenty-one women were randomized in the lurbinectedin arm and 221 patients in the control arm (127 of them received PLD and 94 patients were treated with topotecan). With a median follow-up of 25.6 months, the median PFS was 3.5 months (95% CI, 2.1–3.7) in the lurbinectedin arm and 3.6 months (95% CI, 2.7–3.8) in the control arm (stratified log-rank P = 0.6294; HR = 1.057), respectively. The safety of lurbinectedin was considered manageable: grade ≥3 treatment-related AEs were the most frequent in the control arm: 64.8% vs. 47.9% (P = 0.0005), mainly due to hematological toxicities. The most common non-hematological grade ≥3 AEs were fatigue (7.3% of patients) and nausea (5.9%) in the experimental arm, while mucosal inflammation (8.5%) and fatigue (8.0%) were the most common non-hematological grade ≥3 toxicity in the control arm (12).

CONCLUSION

Platinum-resistant and platinum-refractory OCs have a dismal prognosis and treatment options in these patients are limited (9). Lurbinectedin demonstrated antitumor activity in the phase II study by Poveda et al., with an ORR of 23% (95% CI, 13%–37%), a median PFS of 4.0 months (95% CI, 2.7–5.6 months), and a median OS of 10.6 months (95% CI, 9.5–19.1 months) (11). Unfortunately, the phase III, randomized, multicenter CORAIL study failed to demonstrate the superiority of this agent in terms of PFS when compared with topotecan and PLD in platinum-resistant OC patients (stratified long-rank P = 0.6294; HR = 1.057) (13). However, some consideration may help in the interpretation of the CORAIL trial results. In the CORAIL trial, the patients were older with respect to the previously reported phase II trial (patients ≥65 years, 43% vs. 27%), more heavily pretreated (three prior chemotherapy lines in 23% vs. 12%), had a shorter median PFI (3.9 vs. 4.6 months), and had reported fewer responses to the last platinum therapy (31% vs. 76%). Additionally, a larger proportion of patients presented ascites (27% vs. 18%), which seems to abolish the activity of lurbinectedin, inhibiting its cellular uptake (26). Moreover, the dosages of the two standard arm regimens were poorly used in clinical practice because of unmanageable toxicity, and in particular, the dosage and schedule of topotecan was different in the two trials, with more patients treated with the less effective weekly regimen in the phase II than in the phase III. Regardless of the results, the phase III trial reported an activity of lurbinectedin at least overlapping to that registered with the most used standard regimens, with a better toxicity profile. In this context, in our opinion, it remains unclear the real benefit of lurbinectedin for these patients and the place, if any, of lurbinectedin in the treatment armamentarium of platinum-resistant OC disease.

Moreover, lurbinectedin induces the generation of double-strand DNA breaks, with consequent cell apoptosis, and reduces tumor-associated macrophages and the inflammatory microenvironment through inhibition of inflammatory factors (27).

Since these DNA double-stand breaks are processed through homologous recombinant repair (HRR), lurbinectedin is associated with activity in HRR-deficient cells, and the molecular data in the CORAIL trial seem to suggest that patients with tumor harboring BRCA mutations had a longer survival compared with those without BRCA mutations when treated with lurbinectedin. Additionally, a recent multicenter phase II trial showed a notable response and survival advantage in BRCA 1/2 breast cancer patients treated with lurbinectedin (28). Based on these results, the combination of olaparib, an inhibitor of DNA damage repair (DDR), with a DNA-damaging agent such as lurbinectedin seems an interesting approach to maximize the effect of DNA damage, and in a phase I study, the combination of these agents showed antitumor activity with 60% of DCR in patients with solid tumors (13).

Preclinical evidence has suggested that the simultaneous inhibition of multiple DNA repair mechanisms, along with the DNA damage induced by ectenaiscidins, might enhance

theactivity of these drugs. Lima et al. have shown that the treatment with lurbinectedin and trabectedin activates both ATM and ATR pathways in OC cell lines (29). The combination of an ectenaiscidin with an inhibitor of ATM or ATR did not provide a significant increase of the cytotoxicity of lurbinectedin; however, the simultaneous inhibition of both ATM and ATR resulted in a marked increase of lurbinectedin-induced cell death (29). This seems to suggest that the two mechanisms may, at the same time, be overlapping and complementary and that the dual inhibition of these pathways may significantly enhance the activity of ectenaiscidins. In addition, Riabinska et al. have demonstrated that, in human and murine cancer cells treated with genotoxic chemotherapy, ATM depletion leads to strong addiction on DNA-PKcs.

In their paper, Riabinska et al. showed that the inhibition of DNA-PK in ATM-defective cells leads to apoptotic death (30). These lines of evidence may provide the basis to combine lurbinectedin with other inhibitors of the DNA repair machinery such as berzosertib, an ATR inhibitor, which is currently been tested in a phase I trial in advanced solid tumors.

In conclusion, lurbinectedin showed antitumor activity in platinum-resistant OC patients with a favorable safety profile, suggesting that this agent should continue to be considered as an option in this setting of disease. Moreover, recent studies have shown that combining lurbinectedin with DNA repair inhibitors, i.e., PARPi, seems particularly promising in HRR ovarian and breast cancer patients.

AUTHOR CONTRIBUTIONS

Conceptualization: DL. Data curation: LM and CC. Investigation: LM and CC. Methodology: DL and LM. Project administration: DL. Supervision: DL. Writing—original draft: LM, CC, and DL. All authors acquired the data, revised the manuscript, and approved the final version of the manuscript.

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REVIEW
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Effects of the Anti-Tumor Agents Trabectedin and Lurbinectedin on Immune Cells of the Tumor Microenvironment

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Immune cells in the tumor micro-environment (TME) establish a complex relationship with cancer cells and may strongly influence disease progression and response to therapy. It is well established that myeloid cells infiltrating tumor tissues favor cancer progression. Tumor-Associated Macrophages (TAMs) are abundantly present at the TME and actively promote cancer cell proliferation and distant spreading, as well as contribute to an immune-suppressive milieu. Active research of the last decade has provided novel therapeutic approaches aimed at depleting TAMs and/or at reprogramming their functional activities. We reported some years ago that the registered anti-tumor agent trabectedin and its analogue lurbinectedin have numerous mechanisms of action that also involve direct effects on immune cells, opening up new interesting points of view. Trabectedin and lurbinectedin share the unique feature of being able to simultaneously kill cancer cells and to affect several features of the TME, most notably by inducing the rapid and selective apoptosis of monocytes and macrophages, and by inhibiting the transcription of several inflammatory mediators. Furthermore, depletion of TAMs alleviates the immunosuppressive milieu and rescues T cell functional activities, thus enhancing the anti-tumor response to immunotherapy with checkpoint inhibitors. In view of the growing interest in tumor-infiltrating immune cells, the availability of antineoplastic compounds showing immunomodulatory effects on innate and adaptive immunity deserves particular attention in the oncology field.

Keywords: tumor-associated macrophages, trabectedin, lurbinectedin, tumor micro-environment, immunity

INTRODUCTION

Trabectedin is a registered anti-tumor agent originally extracted from the marine organism *Ecteinascidia turbinate*, now synthetically produced by PharmaMar (Spain) (1). Trabectedin is used in the clinic for the second line treatment of soft tissue sarcoma (STS), especially liposarcoma and leiomyosarcoma, and for relapsed platinum-sensitive ovarian cancer, in combination with

pegylated liposomal doxorubicin (2–5). Trabectedin was selected for its potent activity to kill cancer cells and efficiently block their proliferation by directly interacting with DNA. Its mechanism of action is complex and different from that of other anticancer agents: by binding to the minor grove, trabectedin directly interferes with activated transcription to poison the transcription-coupled nucleotide excision repair system and generates double-strand DNA breaks (6–14).

Further studies demonstrated that it mediates the displacement of oncogenic transcription factors from their target promoters, thereby affecting oncogenic signalling addiction (6, 13, 15, 16).

Besides its direct activity on cancer cells, a remarkable feature of trabectedin is its effects on the tumor micro-environment, in particular on cells of the mononuclear phagocyte system (monocytes/macrophages) as well as on the blood vessels. In this review we will focus on the peculiar tropism of trabectedin and of its analogue lurbinectedin on monocytes, macrophages and Tumor-Associated Macrophages (TAMs), and will discuss how these stromal-centered activities impact on their clinical anti-tumor efficacy.

CYTOTOXIC EFFECT OF TRABECTEDIN ON MONONUCLEAR PHAGOCYTES: *IN VITRO* AND *IN VIVO* STUDIES

A distinguishing feature of trabectedin is its cytotoxic effect on mononuclear phagocytes. To distinguish the inhibitory activity of trabectedin on the cell cycle of proliferating cells from that on transcription factors, some years ago trabectedin was tested on non-proliferating immune cells. Circulating blood human monocytes were used as cells of choice, based on the fact that the transcription factor NF-Y, known to be inhibited by trabectedin (15) is expressed in monocytes and considered of major importance for their differentiation to mature macrophages (17, 18).

Quite surprisingly, monocytes exposed *in vitro* to nM concentrations of trabectedin proved to be highly affected and rapidly underwent apoptosis in a time frame of 24-48 hours. Other chemotherapeutic agents used in parallel as comparison (cisplatin and doxorubicin) had no such cytotoxic effect (19). Even more remarkably, this cytotoxic effect was highly selective for monocytes and macrophages, as neutrophils or T lymphocytes were not affected (19).

This finding stimulated a series of experiments to explain the selectivity of trabectedin for mononuclear phagocytes. It turned out that trabectedin rapidly triggers a caspase-dependent apoptosis where caspase-8 is activated within few hours (20). Caspase-8 is downstream of death membrane receptors, such as TRAIL receptors. The expression of TRAIL-R in the different leukocyte subsets was very informative to decipher the mechanism of trabectedin-induced apoptosis. TRAIL-R1 and R2 receptors were highly expressed in monocytes but not in neutrophils and T lymphocytes which, in turn, mainly expressed the non-signalling TRAIL-R3 (or decoy receptor) (21). Thus, the prevalent expression of functional TRAIL receptors in monocytes explained why only monocytes were susceptible to trabectedin, while neutrophils and T cells were spared by the decoy TRAIL-R3 that prevents activation of caspase-8 (**Figure 1**).

The analysis of TRAIL-R expression in leukocytes from human tissues revealed that in normal spleen and lungs, TRAIL-Rs were barely detectable, but in human tumor tissues from hepatic and mammary carcinoma, TRAIL-R2 was expressed in the majority of macrophages while it was absent in tumor infiltrating lymphocytes and neutrophils (21).

TRAIL-R molecules form a trimer with an internal space to lodge their ligand. Trabectedin is a small compound and likely is not directly binding to the trimer. However, it is well-known that some molecules, for instance the natural compounds Palmitate, Quercetin and some snail venoms are able to activate TRAIL-Rs and caspase-8 in a ligand-independent manner, through the upregulation and/or aggregation of death receptors (21–23).

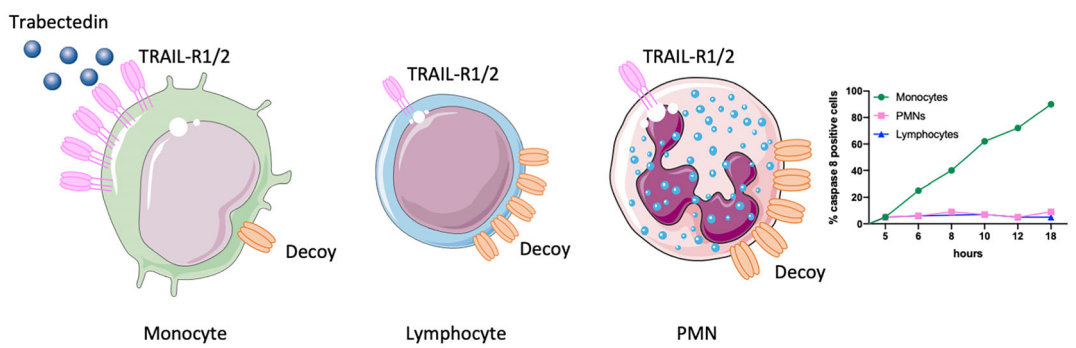

FIGURE 1 | Selectivity of trabectedin for mononuclear phagocytes. TRAIL-R1 and R2 receptors are highly expressed in monocytes but not in T lymphocytes and neutrophils (PMNs) which indeed mainly express the non-signalling decoy receptor (TRAIL-R3). Thus, only monocytes are susceptible to trabectedin, which activates TRAIL-R1 and R2 and triggers a caspase 8-dependent apoptosis. This figure was made with Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0. Unported License (https://smart.servier.com).

Indeed, we found that *in vitro* treatment with trabectedin significantly upregulated the expression of TRAIL-R2 in monocytes and induced their aggregation into lipid rafts (21).

In view of these peculiar effects of trabectedin on mononuclear phagocytes it was of interest to demonstrate whether this compound was able to kill macrophages also *in vivo*, in particular Tumor-Associated Macrophages (TAMs) in experimental mouse tumor models. This issue bears particular importance because of the ambiguous liaison that TAMs have in the tumor tissue. In fact, it is now recognized that in established tumors myeloid cells of the innate immunity (especially macrophages) promote tumor progression and produce immunosuppressive factors that inhibit anti-tumor immune responses (24–28).

Using different pre-clinical tumor models, such as: fibrosarcoma, lung and ovarian cancer, the *in vivo* administration of trabectedin significantly and selectively reduced the number of blood monocytes, and that of macrophages in the tumor tissue (20). Interestingly, the only functional TRAIL-R expressed in mice (DR5) was selectively expressed on murine monocytes and TAMs and was virtually absent in neutrophils and lymphocytes. Therefore, the pattern of TRAIL-R expression in mice perfectly mimics that of human leukocytes (21).

Of interest, the percentage of splenic F4/80+ macrophages was also significantly decreased after treatment with trabectedin (20). Considering the population of MDSCs that expands in tumor-bearing animals, the monocytic component (M-MDSCs: GR1+ $Ly6C^{high}$) was numerically reduced after treatment, while the granulocytic component (PMN-MDSCs: GR1+ $Ly6C^{low}$) was not (20). This finding underlines - once more - the peculiar selectivity of trabectedin for the monocytic lineage. The phenotype analysis of MDSCs GR1+ in mouse tumor tissues revealed lower levels of DR5 compared to macrophages F4/80+ (21); however, others have reported that mouse and human MDSCs express functional TRAIL-Rs and are sensitive to TRAIL-mediated killing (29).

The ability of trabectedin to selectively kill macrophage *in vivo* in mice has been confirmed by several groups. In a mouse model of orthotopic pancreatic cancer, trabectedin strongly reduced the number of TAMs and of circulating monocytes, while neutrophils were not significantly affected (30). Other recent studies reported similar findings in mouse models of orthotopic osteosarcoma, melanoma and in skeletal metastasis from prostate cancer (31–33). In Ewing sarcoma, treatment with trabectedin alone had no efficacy on tumor growth, but the combination of trabectedin with oncolytic herpes virotherapy significantly improved mouse survival and this effect was related to a reduction in the number of TAMs and of Myeloid Derived Suppressor Cells (MDSC) (34). In hematological malignancies, trabectedin not only had cytotoxic effects on neoplastic cells but also induced the apoptotic death of associated myeloid cells (35–37). In another mouse model of acute promyelocytic leukemia, a recent study showed that depletion of bone marrow inflammatory monocytes with trabectedin prevented disease relapse (38).

The macrophage-depleting activity *in vivo* of trabectedin raised the question whether this effect was responsible, at least in part, for its *in vivo* anti-tumor efficacy. Using a fibrosarcoma variant that was resistant to the anti-proliferative activity of trabectedin, *in vivo* treatment with the drug resulted in a significant tumor growth inhibition, as the tumor-supporting TAMs were depleted by trabectedin. This effect was abolished by the adoptive transfer of fresh macrophages that promptly re-instated tumor growth post-treatment (20). These results strongly supported the conclusion that the anti-tumor activity of trabectedin relies both on its effects on cancer cells as well as on its cytotoxic activity on monocytes-macrophages.

Results in cancer patients are scarce, in spite of the fact that trabectedin is a registered compound. Patients with STS receiving trabectedin as single treatment have been studied for monocyte counts from circulating blood over therapy cycles; a decrease in monocytes indeed occurred in some patients within few days after each injection of trabectedin. Furthermore, in selected patients undergoing neo-adjuvant therapy with trabectedin, where tumor biopsies were also available, immunohistochemistry of tumor sections collected before and after therapy revealed a dramatic decrease of macrophage infiltration, reinforcing the finding that this compound is able to kill *in vivo* macrophages in tumor tissues (20).

TRABECTEDIN INHIBITS THE PRODUCTION OF SELECTED INFLAMMATORY AND ANGIOGENIC MEDIATORS: IMPACT ON THE TUMOR MICROENVIRONMENT

The study of trabectedin on immune cells held other surprises. It is well-known that its mechanism of action is not limited to binding of and damaging DNA, but also includes the transcriptional inhibition of selected genes. At low (non-cytotoxic concentrations) trabectedin inhibited the mRNA levels and production of specific inflammatory mediators in LPS-stimulated monocytes and macrophages such as IL-6 and several chemokines including: CCL2, CCL3, CCL7, CCL14 and CXCL8 (19, 39).

Importantly, other inflammatory cytokines such as TNF and IL-1 were not significantly inhibited, raising the question if this selectivity could be mechanistically ascribed to the ability of trabectedin to interfere with specific transcription factors. Inflammatory cytokines and chemokines are under the control of the master regulator NF-kB, but many mediators can be also activated by other transcription factors, such as activator protein (AP-1), SP-1 and Smad3 for CCL2 (40), and AP-1, cFOS, and CCAAT/NF-IL-6 for IL-6 (41).

Of these, AP-1 activates also TNF, that was unaffected by trabectedin. So far, the search for transcription factors common to CCL2 and IL-6 and not to TNF, and specifically affected by trabectedin has been unsuccessful.

Inhibition of inflammatory and angiogenic mediators was observed also in tumor cells. *In vitro* treatment with trabectedin decreased the production of CCL2, CXCL8, IL-6, VEGF and PTX3 by myxoid liposarcoma (MLS) primary tumor cultures and/or cell lines, and freshly isolated ovarian cancer cells from ascites (19, 39). In *in vivo* experiments, using a xenograft mouse model of human MLS, a marked reduction of human CCL2, CXCL8 and PTX3 after trabectedin administration was observed, demonstrating that this effect on tumor cells occurs also *in vivo* (39). A recent paper by Casagrande et al. reported that trabectedin inhibited the release of cytokines by tumor cells in Hodgkin lymphoma, including M-CSF, IL-6, IL-13, CCL5 and CCL17. Furthermore, treatment of mice bearing xenografts of Hodgkin lymphoma confirmed the *in vitro* findings, and residual tumors had fewer TAMs and a reduced vessel network (36).

It has been previously reported that trabectedin downmodulates the expression of ECM-related genes produced by TAMs and fibroblasts, such as collagen type 1, fibronectin, osteopontin and the matrix-metalloprotease-2 (MMP2) (42). These findings are of interest because they indicate that trabectedin may have an impact on the high matrix remodeling, a key feature of the cancerous stroma. Matrix degradation in tumor tissues, as well as in regenerating tissues, is known to release growth factors that are bound to ECM in an inactive forms. For instance, several angiogenic factors such as VEGF become activated during matrix remodeling and are available in the local environment. TAMs are an important source of pro-angiogenic factors in the TME; trabectedin significantly reduced the production of VEGF and angiopoietin-2 in macrophages and, accordingly, in tumor-bearing mice treated with the drug a clear decrease of the vessel network was observed (19, 20, 43). This TAM-mediated effect on angiogenesis was not the only impact of trabectedin on tumor angiogenesis: when mice were treated with the macrophage depleting agent liposomal-clodronate, there was no relevant impact on the vessel network, in spite of a significant inhibition of tumor growth (20). This finding indicated that trabectedin might have additional effects on blood vessels. Indeed, Taraboletti's group demonstrated that trabectedin inhibited the matrix-invasion ability of endothelial cells and their morphogenetic branching (44). Mechanistically, trabectedin increased the expression of TIMP-1 and TIMP-2 that, by blocking the activity of the MMP enzymes, inhibited the proteolysis of ECM molecules, a required step in the process of matrix invasion (44). These anti-angiogenic effects of trabectedin were confirmed also in endothelial cells co-cultured with the conditioned medium of multiple myeloma cells, resulting in reduced capillary-like structures and fewer number of branching points (45). Other mechanisms of angiogenesis regulation acting *via* cancer cells were reported in mouse models of melanoma and myxoid liposarcoma, where trabectedin stimulated the tumoral expression of thrombospondin-1 (TSP-1), a major endogenous inhibitor of angiogenesis or of TIMP-1 (31, 44). Overall, the anti-angiogenic activity of trabectedin occurs *via* different mechanisms, involving both a direct inhibitory effect on endothelial cells, as well as a reduction of the angiogenic potential of cancer cells and macrophages.

THE ANALOGUE LURBINECTEDIN SHARES WITH TRABECTEDIN SIMILAR IMMUNOMODULATORY PROPERTIES

Among several synthetized analogues of trabectedin, the compound lurbinectedin showed very promising anti-tumor activity *in vitro* and further on good efficacy in a broad range of clinical trials. Lurbinectedin has been approved by the Food and Drug Administration (FDA) in 2020 for the treatment of small cell lung carcinoma (46, 47).

Lurbinectedin contains the same pentacyclic skeleton of the tetrahydroisoquinoline rings, but it is structurally different as a tetrahydro beta-carboline replaces the additional tetrahydroisoquinoline of trabectedin. The structural similarity of lurbinectedin and trabectedin explains the similarity of the mode of action of the two drugs. Both trabectedin and lurbinectedin bind guanines at the N2 position, in the minor groove. They are both more cytotoxic against cells that are deficient in Homologous recombination (e.g., cells with mutations of BRCA genes) and less toxic against cells deficient in Nucleotide Excision Repair (10–12, 48, 49). Both drugs modify transcription regulation by displacing some oncogenic transcription factors from their target promoters (16, 50), and at high concentrations they cause degradation of RNA-polymerase II (51).

Lurbinectedin presents some interesting clinical features with pharmacokinetic and pharmacodynamic differences compared to trabectedin (51–53). Early phase I/II clinical studies demonstrated that, at equivalent administration schedules, the maximal-tolerated dose of lurbinectedin was more than 3 times higher than that of trabectedin, and the plasmatic Area Under the Curve (AUC) was 5-10 higher (51, 52). This difference emerged from the clinical investigations and was not anticipated based on preclinical data. In fact both *in vitro* studies on different cancer cell lines and *in vivo* studies in tumor bearing mice suggested a similar cytotoxic potency of the two drugs. The difference appears to be due to the different volume of distribution of lurbinectedin that in humans is four times lower than that of trabectedin (53, 54). The different volume of distribution is not only related to the different degree of lipophilicity of the two molecules, but also to a different binding affinity for alpha 1-acid glycoprotein (AGP). In fact equilibrium dialysis experiments showed that both compounds bind AGP, but the affinity of binding of lurbinectedin was much greater than that of trabectedin, KD values being approximately 8 and 87 nM for lurbinectedin and trabectedin respectively (55). The finding could be clinically relevant as AGP can be very variable in patients with cancer, particularly when tumors are at advanced stage and some inflammatory mechanisms are activated.

In vivo studies in preclinical mouse models confirmed that the anti-tumor efficacy of lurbinectedin was similar to that of trabectedin (11, 43); it therefore seemed plausible that the mechanisms of action of lurbinectedin also included macrophages of the tumor stroma as targets, in addition to cancer cells. The modulatory effects of lurbinectedin on immune cells was studied in parallel experiments with trabectedin.

The results demonstrated that also lurbinectedin was able to significantly reduce monocyte viability at nM concentrations and to induce a caspase-dependent apoptotic cell death. Furthermore, similarly to trabectedin, this analogue inhibited selected inflammatory chemokines (CCL2 and CXCL8) and VEGF. In mouse tumor models, in addition to an excellent anti-tumor efficacy directed on cancer cells, lurbinectedin reduced the number of circulating monocytes, the tumor-infiltrating macrophages and the density of tumor vessels (43). To better analyze the comparison between trabectedin and lurbinectedin, a global gene expression analysis of drug-treated human monocytes was performed. Overall, the results indicated that the genes down- or up-modulated by trabectedin were also affected by lurbinectedin (43). As expected from previous results, several genes related to the inflammatory response, the DNA damage response and the apoptosis pathway were involved. Of interest several genes of the Rho GTPase family were significantly down-modulated by both compounds, a finding not appreciated in previous analyses (43).

RhoGTPases regulate intracellular actin dynamics and are involved in essential cell activities such as receptor signaling, cell adhesion, migration, and phagocytosis. Accordingly, monocytes treated with lurbinectedin or trabectedin showed a strongly impaired ability to migrate in response to chemo-attractants, such as the prototypical chemokine CCL2. This finding is important because the density of macrophages in tumors relies on the continuous migration of blood monocytes into tumor tissues (25, 27). Thus, not only trabectedin and lurbinectedin induce the apoptosis of monocytes/macrophages and inhibit their production of several biological mediators, the two drugs also have an impact on their mobilization and chemokine-induced attraction at tumor sites.

Overall, by comparing the two compounds for their activities on myeloid cells, and more in general on the TME, it can be concluded that lurbinectedin and trabectedin display very similar effects, *in vitro* and *in vivo*.

REPROGRAMMING THE IMMUNOSUPPRESSIVE MYELOID CELLS BY TRABECTEDIN: POTENTIAL FOR COMBINATION WITH IMMUNE CHECKPOINT THERAPY

Depletion of macrophages by trabectedin and lurbinectedin may alleviate the TAM-mediated immune-suppression of adaptive anti-tumor responses in the TME, as depicted in **Figure 2**. This effect may have an important impact on the clinical response to immunotherapy. In fact, it is well known that immunosuppressive macrophages and related myeloid cells may impair the response to checkpoint inhibitors (25, 56, 57).

The potential effects of trabectedin on adaptive immune cells have been studied in preclinical models. Early findings already pointed out that in murine treated tumors the number of infiltrating T cells was increased (20). Recent studies specifically investigated the potential of trabectedin to modulate T lymphocytes in mouse cancer models. Analyses of tumor tissue in trabectedin-treated mice revealed a greater number of CD3+ and CD8+ lymphocytes by flow cytometry and immunohistochemistry (58). mRNA expression of several T cell-associated genes were significantly up-regulated after trabectedin, including the cytotoxic molecules granzyme B and perforin, the anti-tumor cytokine IFN γ and IFN-responsive genes such as MX1, CXCL10 and the checkpoint molecule PD-1 (58). These findings strongly indicate an activation of the T cell-mediated immune response upon macrophage targeting by trabectedin. Similar findings were reported in other studies. Borgoni et al. investigated the effects of trabectedin on tumor-infiltrating leukocytes in a genetic model of pancreatic cancer, a highly immunosuppressive tumor; treatment with trabectedin significantly reduced the immunosuppression in the TME: T lymphocytes sorted from treated tumors, showed an increased percentage of IFNγ+ Eomes+ and PD-1+ T cells, compared to

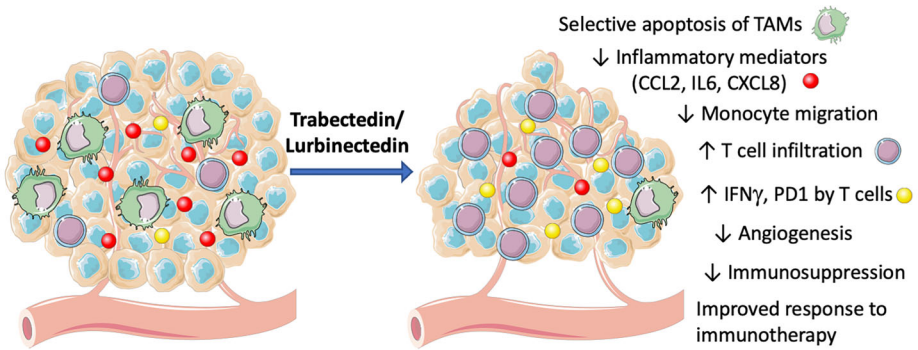

FIGURE 2 | Mechanisms of action of trabectedin and lurbinectedin on the TME. Trabectedin and lurbinectedin share complex mechanisms of action on immune cells of the TME. They induce a selective apoptosis of TAMs, decrease monocyte migration and specific inflammatory mediators (CCL2, IL6, CXCL8). Moreover, trabectedin and lurbinectedin decrease angiogenesis and immunosuppression; they increase T cell infiltration and their expression of IFNγ and PD1, therefore improving the response to immunotherapy. This figure was made with Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0. Unported License (https://smart.servier.com).

untreated tumors, that were characterized by a higher proportion of IL10-expressing T cells. This switch towards an effector phenotype ($IL10^{low}/IFN\gamma^{high}$) indicated an important immunomodulatory outcome mediated by trabectedin on adaptive immunity and possibly leading to an anti-tumor phenotype (30). In a mouse model of osteosarcoma, trabectedin significantly reduced tumor burden and enhanced the number of infiltrating CD8+ T lymphocytes. Interestingly, also in this case T cells showed higher expression of the inhibitory molecule PD-1 (33).

Based on this finding, it was of interest to investigate whether the combination of trabectedin with anti-PD-1 checkpoint inhibitors improved the response to immunotherapy. Combination of trabectedin and anti-PD-1 showed increased efficacy in osteosarcoma and ovarian cancer mouse models (33, 59); using a mouse fibrosarcoma poorly responding to anti-PD-1 alone, an improved anti-tumor response was achieved when mice were pre-treated with trabectedin (58); in another study, depletion of myeloid cells combined with chemotherapy and PD-1 blockade, synergistically inhibited the progression of a murine leukemia (38).

In the fibrosarcoma model (58), an important aspect that emerged was the correct timing of the combination trabectedin and checkpoint immunotherapy. It was found that the best protocol was a sequential treatment (trabectedin first, followed by anti-PD-1), rather than a simultaneous administration. This sequential protocol will prepare a reprogrammed TME - with depletion of immunosuppressive macrophages - but above all will preserve the T cell activation induced by anti-checkpoint antibodies. In fact, T cell expansion can be blocked by the anti-proliferative action of trabectedin. Therefore, a reasoned timing of administration would consider using trabectedin first, and the immunotherapeutic treatment after some days.

These preclinical studies demonstrated that trabectedin positively remodulates the TME, likely through mitigation of the TAM-mediated immunosuppression, and facilitates T cell reactivation by anti-PD-1 antibodies. These findings have provided a rational to test the combination of trabectedin and anti-PD-1 antibodies in the clinic.

Indeed, some clinical trials of combination immunotherapy with trabectedin or lurbinectedin are ongoing (**Table 1**). In most cases patients are advanced and refractory to previous therapies. In phase 1/2 or phase 2 studies, patients with soft tissue sarcoma and ovarian cancer have been treated with trabectedin and anti-PD-1, anti-PD-L1 or anti-CTLA-4 (nivolumab, durvalumab, ipilimumab), while patients with small cell lung cancer (SCLC) have been treated with lurbinectedin and anti-PD-L1 or anti-CTLA-4 (atezolizumab, ipilimumab). In the study NCT03138161 (ClinicalTrials.gov) previously untreated sarcoma patients received a combination of trabectedin and ipilimumab or nivolumab, as a first line therapy. Some patients achieved good clinical responses without serious toxicity (60). (**Table 1**).

In the study NCT03085225, trabectedin was given in combination with durvalumab to advanced STS patients and to patients with ovarian cancer. Also in this study clinical responses were observed. Of interest, the tumor infiltration of CD8+ T cells was associated with prolonged survival in patients with ovarian carcinoma (61).

Overall, these promising results suggest the combination of trabectedin or lurbinectedin with checkpoint inhibitors deserves further assessment in the clinic.

CONCLUSION

Trabectedin and lurbinectedin have multiple effects on immune cells of the tumor microenvironment and in particular on mononuclear phagocytes: at high concentrations they selectively induce a rapid caspase-dependent apoptosis in monocytes and TAM; at lower concentrations they inhibit the production of some inflammatory mediators with relevant activity for tumor biology; the two compounds also reduce monocyte adhesion and migration by inhibiting specific genes that organize the actin cytoskeleton. Furthermore, trabectedin and lurbinectedin hinder the production of angiogenic factors that are pivotal for tumor progression. Overall, in treated tumors there is a remarkable modulation of the TME with less immunosuppression and an increased presence of T lymphocytes. These conditions might be

TABLE 1 | Ongoing clinical studies using trabectedin or lurbinectedin in association with checkpoint blockade immunotherapy.

ClinicalTrials.gov Identifier	Clinical study	Combination therapy	Tumor type Published results
NCT03886311	Phase 2	trabectedin nivolumab talimogene laherparepvec*	Advanced sarcoma
NCT03138161	Phase 1 Expansion Phase 2	trabectedin nivolumab ipilimumab	Solid tumors (60)
NCT03085225	Phase 1b	trabectedin durvalumab	Advanced soft-tissue sarcoma Ovarian carcinoma (61)
NCT04253145	Phase 1/2	lurbinectedin atezolizumab	Small cell lung cancer
NCT04610658	Phase 1/2	lurbinectedin nivolumab ipilimumab	Small cell lung cancer

**Talimogene laherparepvec is an oncolytic herpes virus.*

ideal to better respond to immunostimulatory approaches, such as checkpoint blockade immunotherapies. Therefore, trabectedin and lurbinectedin are interesting compounds in oncology, both for their intrinsic anti-tumor activity and for their remodulating effects on immunity.

AUTHOR CONTRIBUTIONS

Writing the manuscript: PA, CB, ED, RF, and MD'I. Creation of image: ED. Reading and proofreading: PA, CB, ED, RF, and MD'I. The review has been approved by all authors.

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Antitumoral Effect of Plocabulin in High Grade Serous Ovarian Carcinoma Cell Line Models

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Ovarian cancer (OC) is a life-threatening tumor and the deadliest among gynecological cancers in developed countries. First line treatment with a carboplatin/paclitaxel regime is initially effective in the majority of patients, but most advanced OC will recur and develop drug resistance. Therefore, the identification of alternative therapies is needed. In this study, we employed a panel of high-grade serous ovarian cancer (HGSOC) cell lines, in monolayer and three-dimensional cell cultures. We evaluated the effects of a novel tubulin-binding agent, plocabulin, on proliferation, cell cycle, migration and invasion. We have also tested combinations of plocabulin with several drugs currently used in OC in clinical practice. Our results show a potent antitumor activity of plocabulin, inhibiting proliferation, disrupting microtubule network, and decreasing their migration and invasion capabilities. We did not observe any synergistic combination of plocabulin with cisplatin, doxorubicin, gemcitabine or trabectedin. In conclusion, plocabulin has a potent antitumoral effect in HGSOC cell lines that warrants further clinical investigation.

Keywords: plocabulin (PM060184), microtubule inhibitor, high-grade serous ovarian cancer (HGSOC), 3D cell culture, drug testing

INTRODUCTION

Ovarian cancer (OC) is the leading cause of death for patients with gynecological malignancies. It is an indolent disease, frequently diagnosed at advanced stages due to the lack of specific symptoms. For decades, treatment of OC has consisted of surgery and systemic adjuvant or neoadjuvant chemotherapy with a carboplatin/paclitaxel regimen. However, despite achieving initial complete remission, about 80% of patients with advanced disease will relapse and finally progress to a platinum-resistant OC (1).

Platinum response is one of the major prognostic factors in OC. The classical classification of recurrence in platinum-sensitive or platinum-resistant/refractory disease has been based on the cut-off of 6 months after completing chemotherapy, and no validated biomarkers, other than histological subtype, are known to predict likelihood of primary platinum-resistant or platinum-refractory disease (2). Few single agents have shown discrete activity in platinum-resistant OC, such as weekly paclitaxel, pegylated liposomal doxorubicin (PLD), gemcitabine, topotecan, cyclophosphamide or etoposide. The response to these single agents is usually less than 20%, with a median progression-free survival (PFS) less of 6 months and a median overall survival (OS) around 12 months (3).

In recent years some relevant progress has occurred in the treatment of high grade serous ovarian carcinoma (HGSOC), the most prevalent subtype of OC, with the introduction of poly-adenosine diphosphate ribose polymerase inhibitors (PARPi). These targeted therapies are now being administered as maintenance therapy after chemotherapy, achieving a relevant improvement in PFS, not only after first line chemotherapy (4–6), but also after platinum-sensitive relapse (7–9). However, the efficacy of current treatments remains limited, especially in platinum-resistant/refractory disease, and there is still a medical unmet need for testing and developing novel therapies for OC patients after progression to the current options.

Plocabulin (PM060184/PM184, PharmaMar) is a compound of marine origin derived from the Madagascan sponge *Lithoplocamia lithistoides*. Plocabulin belongs to a family of tubulin-binding agents that inhibits tubulin polymerization by binding to the dimer's end, with one of the highest known affinities among tubulin-binding agents. This mechanism alters the dynamic instability of microtubules and affects cells both in interphase and mitosis, inhibiting cell growth and migration (10–12). Recent studies have also demonstrated an antiangiogenic effect of plocabulin, which causes a reduction in vascular volume and induction of necrosis both *in vitro* and *in vivo* (13).

Phase I studies have demonstrated promising antitumor effects of plocabulin in patients with advanced tumors and, currently, it is being further assessed in clinical trials in advanced colorectal cancer, breast cancer and other solid tumors (11, 14).

In the current study, we explore for the first time the *in vitro* efficacy of plocabulin in a panel of 12 HGSOC cell lines with distinct sensitivities to cisplatin (CDDP), alone and in combination with other drugs currently applied in clinical practice. Studies have been done both in monolayer culture (2D) and three dimensional (3D) spheroids, a promising preclinical model for testing antitumor drugs.

MATERIAL AND METHODS

Cell Lines and Culture Conditions

PEA1, PEA2, PEO1, PEO4, PEO6, PEO14, PEO23, PEO16, OVCAR-3 and 59M cell lines were obtained from the European Collection of Authenticated Cell Cultures (ECACC), and cultured following the guidelines of the repository. OV866 (2) and TOV3041G were obtained from Centre Hospitalier de L´ Université de Montréal (CHUM), and kindly provided by Dr. Mes-Masson.

Some of these lines were established from the same patient during the course of disease and had received different treatment schemes prior to their establishment: PEA1/PEA2, PEO1/PEO4/PEO6 and PEO14/PEO23 (15). PEO1 and PEO16 harbor reported deleterious mutations in *BRCA2* (16). As previously reported by our group, four resistance groups were established according to their CDDP IC_{50} values (17). **Table 1** shows the treatment administered to the patient prior to the establishment of the cell line and the CDDP resistance group assigned.

Cells were maintained in the following culture media: PEA1, PEA2, PEO14, PEO23, PEO16 and OVCAR-3: Roswell Park Memorial Institute (RPMI); PEO1, PEO4, PEO6 and 59M: Dulbecco's Modified Eagle Medium (DMEM), both supplemented with 10% Fetal Bovine Serum (FBS) and 100 U/ml penicillin–streptomycin. OV866(2) and TOV3041G were grown in a combination of 199 and MCDB105 (1:1) media with 5% FBS and 50 µg/ml gentamicin (Merck, MA, USA). All cells were incubated at 37°C in a 5% CO_2 incubator.

All cell lines were tested periodically for mycoplasma infection and authenticated by genetic profiling using polymorphic short tandem repeat loci with the Geneprint 10 kit (Promega, WI, USA).

Drug Treatment Assays

For monolayer culture experiments, cells were seeded in flat bottom 96-well plates (Corning, NY, USA) 24 hours before drug exposure (cell density was previously calculated for each cell line to avoid confluence at the final time point). Then, cells were exposed to different drug concentrations for 72 hours. After this time, cellular confluence was measured with sulforhodamine B (SRB) colorimetric assay.

In the case of 3D culture, spheroids were cultured using ultra-low attachment (ULA) plates (Corning) as previously described (17, 18). Cell density was previously calculated so that the spheroids had a diameter of 300–400 µm at day 4, optimal to mimic the diffusion state in the tumor, which is

TABLE 1 | Cell line characteristics. Previous treatments received by the patients and CDDP sensitivity group based on our previous report (17).

Cell line	Previous treatments	CDDP sensitivity
PEA1	NO	VR
PEA2	CDDP, PREDNIMUSTIN	VR
PEO1	CDDP, 5-FU, CHLORAMBUCIL	PR
PEO4	CDDP, 5-FU, CHLORAMBUCIL	VR
PEO6	CDDP, 5-FU, CHLORAMBUCIL	R
PEO14	NO	S
PEO23	CDDP, CHLORAMBUCIL	R
PEO16	RADIOTHERAPY	PR
OVCAR-3	CYCLOPHOSPHAMIDE, ADRIAMYCIN, CDDP	PR
OV866(2)	CARBOPLATIN, TAXOL	VR
TOV3041 G	CDDP, CARBOPLATIN, TAXOL	PR
59M	NO	R

CDDP, cisplatin, 5-FU, 5-Fluorouracil; S, sensitive; PS, partially resistant; R, resistant; VR, very resistant.

about 100 µm in depth for nutrients and oxygen, avoiding excessive necrotic areas. In these experiments, after 4 days of culture, spheroids were exposed to plocabulin for 72 hours. Cell viability was then measured using CellTiter-Glo (CTG) Luminescent assay (Promega).

Colorimetry (SRB) and luminescence (CTG) were measured using a Synergy 4 microplate reader (BioTek, VT, USA), and in both cases half maximal inhibitory concentration (IC_{50}) values were calculated using linear regression with GraphPad Prism 7 software (GraphPad Software, CA, USA).

Possible synergisms were assayed, in 2D conditions, between plocabulin and other chemotherapeutic agents currently administered to patients with HGSOC in routine clinical practice: CDDP, doxorubicin, gemcitabine and trabectedin. For these assays, we selected 7 cell lines with different sensitivities to CDDP, with or without a previous treatment. These cells were: PEA1, PEA2, PEO4, PEO14, OV866(2) and TOV3041G, which covered all CDDP possible scenarios.

The experimental design was based on the evaluation of two drugs on a 6x6 matrix (**Figure 1A**). One of the drugs is dosed by increasing concentrations by row, and the other one by column. With this design, the bottom left well corresponds to the control without drugs, and the top right well corresponds to the maximum combined concentration of both drugs. First row and the first column correspond to single drugs, and the remaining wells contain increasing drug combinations, each well with a different dose.

We used the SynergyFinder Plus web tool (https://synergyfinder.org) to explore the synergistic effects of plocabulin with the other drugs (19). This tool applies four different algorithms: ZIP (zero interaction potency), HSA (highest single agent), Bliss (Bliss independence) and Loewe (Loewe additivity) (20, 21). To increase the robustness of the analysis we decided that only if the four algorithms showed global positive results (synergy score > 10), we could confirm the existence of synergy between the two drugs (**Figure 1**) (22).

CDDP, doxorubicin and gemcitabine were provided by the pharmacy of the Hospital La Paz. Plocabulin and trabectedin were kindly provided by PharmaMar (Madrid, Spain).

Invasion and Migration Assays

Invasion and migration capacity was evaluated in 2D and 3D conditions.

For monolayer culture experiments, cells were treated with plocabulin for 72 hours and then transferred into transwell inserts in low serum conditions (1% in the top chamber and basal FBS conditions in the bottom chamber to induce cell mobility). 8 µm pore transwell inserts were employed for migration assays, and pre-coated inserts with Matrigel® were used for invasion experiments (Corning). Twenty-four hours later, cells were removed from the top chamber using a cotton swab and the inserts were fixed by the Diff-Quick method (QCA, Tarragona, Spain). Pictures of the inserts were taken and cells were counted manually with ImageJ (NIH, MD, USA) using a representative area of each well.

In 3D conditions cells were plated in ULA plates and spheroids let to grow for four days. At day 4, plocabulin was added at the correspondent IC_{50} dose for 72 hours. After this time, for invasion assays, drugs were removed and Matrigel® (1:3 with culture media) was added to the spheroids. Pictures were taken every day. For migration assays, spheroids were transferred onto a Matrigel® layer, and pictures were taken as done for invasion assays.

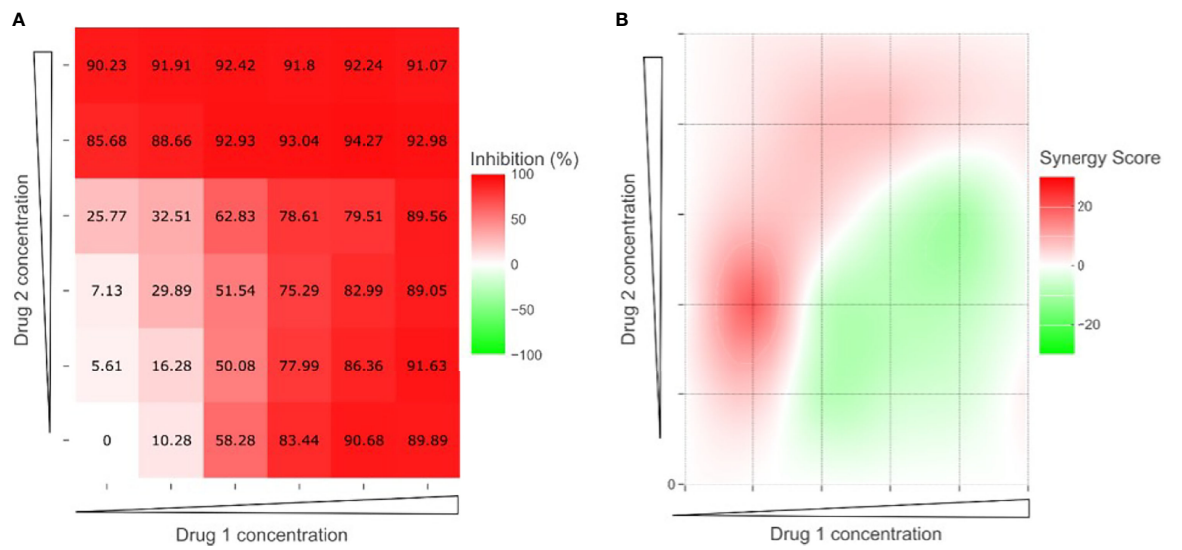

FIGURE 1 | SynergyFinder Plus analysis for an example of two-drug combinations. **(A)** Dose-response matrix calculated as percentage of growth inhibition. **(B)** Synergy distribution map; red indicates synergy (synergy score > 0) and green indicates antagonism (synergy score < 0).

Microtubule Network and Mitotic Spindle Staining by Immunofluorescence

For the study of microtubules, immunofluorescence staining was performed on OV866(2) cell line for α- and γ-tubulin. Briefly, cells were seeded on glass coverslips and 24 hours later were exposed to different concentrations of plocabulin (control, 0.1 nM, and 1 nM) for 48 hours. After this time, two different IF approaches were done:

- Detection of alterations in microtubules and mitotic spindle: Cells were fixed with methanol for 10 minutes at -20°C and incubated with a blocking solution (5% bovine serum albumin in PBS) for 30 minutes and incubated with the corresponding primary and secondary antibodies, as previously described (11).

Pictures were taken with a Leica DM IRM fluorescence microscope equipped with a 100X oil immersion objective and a DFC 340 FX digital camera (Leica, Wetzlar, Germany). Micronuclei were scored in a minimum of 5 fields for each treatment condition.

Antibody and Hoechst details are listed on **Supplementary Table 1**.

Cell Cycle Assays

For cell cycle studies, OV866(2) cells were exposed to 0.1 or 1 nM concentrations of plocabulin for 24h. After this time, cells were fixed with 70% ice-cold ethanol for 15 minutes at 4°C and then stained with a propidium iodide solution (Merck) for 30 minutes, in the dark, at room temperature. After washing with phosphate buffered saline, cells were analyzed for cell cycle on a Celigo S plate cytometer (Nexcelom, MA, USA).

Statistical Analysis

Statistical analysis of all experiments was carried out by means of the Student's T-test using Microsoft Excel (Microsoft, WA, USA). Statistical significance is reported when p-value ≤ 0.05.

All experiments were performed at least in duplicate.

RESULTS

IC_{50} Determination

Table 2 shows the IC_{50} values determined for each cell line and drug in 2D and 3D conditions.

When cultured in monolayer, plocabulin was effective in 11/12 cell lines, at doses in low nanomolar/picomolar range (< 1.2 nM), as we can see in the IC_{50} values obtained. Only PEO14 was resistant to a concentration of 10 nM. Besides PEO14, 59M showed the highest IC_{50} value for plocabulin; they are both chemo naïve cell lines but sensitive (PEO14) or resistant (59M) to CDDP, according to our sensitivity stratification. We could not correlate plocabulin response with previous treatments or *BRCA* status, but it showed antitumoral activity in all CDDP sensitivity groups.

However, in 3D spheroids only PEO4, PEO6 and PEO16 showed a response to plocabulin in a low nanomolar range. Of these cells, as we can see in the ratio PM 3D/2D column, PEO4 was almost 3 times more resistant to plocabulin in 3D conditions, whilst PEO6 and PEO16 were sensitized to plocabulin in these culture conditions. All the other cell lines had IC_{50} values over 10 nM, which is 100–1000 times more resistance than in monolayer culture (except for 59M, where the increase was only 10 times).

TABLE 2 | IC_{50} values for plocabulin (PM060184) in 2D and 3D conditions.

Cell Line	PM060184 (nM)				Ratio PM 3D/2D
	IC_{50} 2D	Std. Dev.	IC_{50} 3D	Std. Dev.	
PEA1	0.07	0.04	> 10	N/D	N/D
PEA2	0.23	0.04	> 10	N/D	N/D
PEO1	0.03	0.01	> 10	N/D	N/D
PEO4	0.05	0.02	0.16	0.05	2.95
PEO6	0.37	0.05	0.24	0.13	0.65
PEO14	> 10	N/D	> 10	N/D	N/D
PEO23	0.35	0.08	> 10	N/D	N/D
PEO16	0.30	0.36	0.05	0.02	0.17
OVCAR-3	0.03	0.01	> 10	N/D	N/D
OV866(2)	0.08	0.05	> 10	N/D	N/D
TOV3041 G	0.07	0.02	> 10	N/D	N/D
59M	1.15	0.09	> 10	N/D	N/D

Std. Dev., Standard deviation; N/D, not determined. N/D values were not calculated since an IC_{50} value was not reached, therefore standard deviations or ratios cannot be performed. Data are represented as mean and standard deviation. 3D/2D ratio has been calculated for cell lines when both IC_{50} values were available.

Invasion and Migration Assays

Cell mobility assays were performed in 7 cell lines, chosen by their different sensitivity to CDDP: PEA1, PEA2, PEO1, PEO4, PEO14, PEO16 and OV866(2).

We observed a reduction of both transwell migration (3 cell lines) and invasion (4 cell lines) when treating cells with plocabulin (**Figure 2**). The highest inhibition of migration was achieved in PEO14 (93.3% inhibition, p value = 0.02), followed by OV866(2) (48.1% inhibition, p value = 0.07) and PEA2 (42.6% inhibition, p value = 0.04), with the exception of PEA1, where we saw a non significant increase of migration. Regarding invasion through a Matrigel® layer, again, we saw the highest inhibition in PEO14 (91.6% inhibition, p value = 0.04), followed by OV866(2) (50.9% inhibition, p value = 0.04), PEA2 (41.1% inhibition, p value = 0.37) and PEA1 (27.3% inhibition, p value = 0.43). The latter, although not significant, show a similar trend towards inhibition of migration and invasion (**Figure 2B**). PEO1, PEO4 and PEO16 were not evaluable, since they did not invade or migrate through the transwell inserts.

In 3D experiments, only cell lines that make either compact aggregates (PEO1, PEO4 and PEO14) or tight spheroids (OV866 (2) and PEO16) were assayed, since loose aggregates (PEA1 and PEA2) cannot be transferred to Matrigel® without disintegration. Of all these selected cell lines, only OV866(2) migrated (**Figure 3A**) and invaded (**Figure 3B**) in basal conditions when transferred to Matrigel®, and this behavior was partially inhibited when cells were treated with plocabulin. Plocabulin reduces spheroid volume and spread both in invasion and migration experiments. Migratory spread was reduced by 22.7%, while invasion was reduced by 56.6%, although these results did not

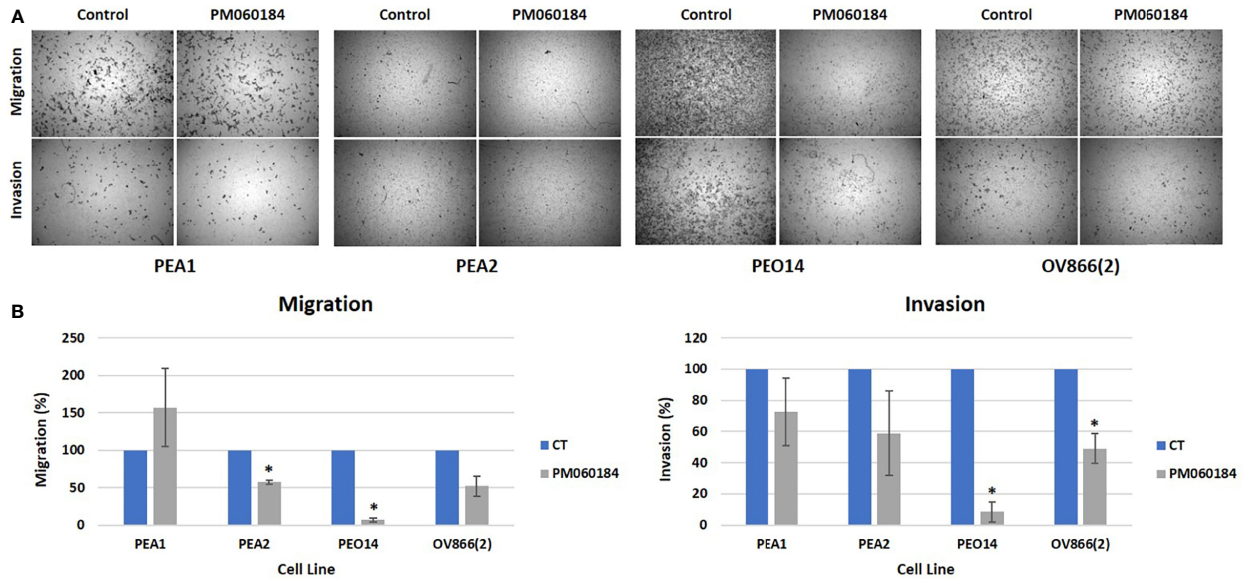

FIGURE 2 | Plocabulin effect on 2D migration and invasion. **(A)** Transwell migration and invasion images of a representative experiment of PEA1, PEA2, PEO14 and OV866(2) cells. **(B)** Bar plots represent the quantification of the data calculated with the mean values of at least two experiments. CT: untreated control; *: p value < 0.05.

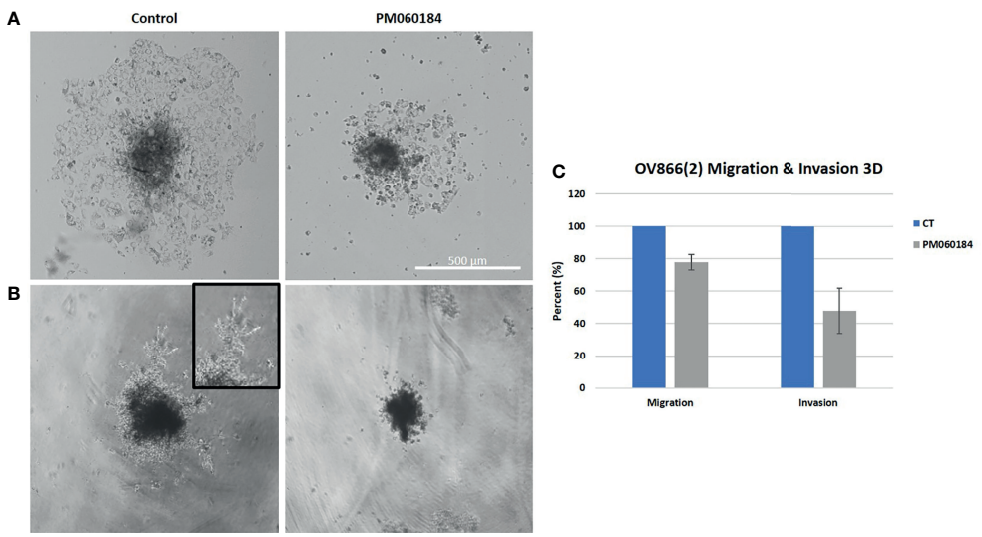

FIGURE 3 | Plocabulin effect on OV866(2) spheroids migration **(A)** and invasion **(B)**. Pictures are of a representative experiment. **(C)** Bar plots represent the quantification of the data calculated with the mean values of at least two experiments. CT: untreated control.

reach statistical significance (p values = 0.14 and 0.17, respectively) (**Figure 3C**).

Immunofluorescence

The effect of plocabulin treatment on the microtubule network of OV866(2) cells was analyzed by immunofluorescence staining of α- and γ-tubulin.

Plocabulin treatment induced microtubule depolymerization in a concentration-dependent manner. At IC_{50} value doses (0.1 nM), microtubule distribution was slightly disorganized, and this effect was accentuated at 1 nM (**Figure 4A**). Treatment with plocabulin also caused the appearance of aberrant mitoses, chromosome missegregation and multinucleated cells in a concentration-dependent manner. In untreated cells, mitoses showed a bipolar spindle and chromosome alignment at the metaphase plate. 24h treatment with plocabulin produced an increase of multinucleated cells; at 0.1 nM, 43.0% presented micronuclei, versus 5.2% in control cells (p value < 0.001) while

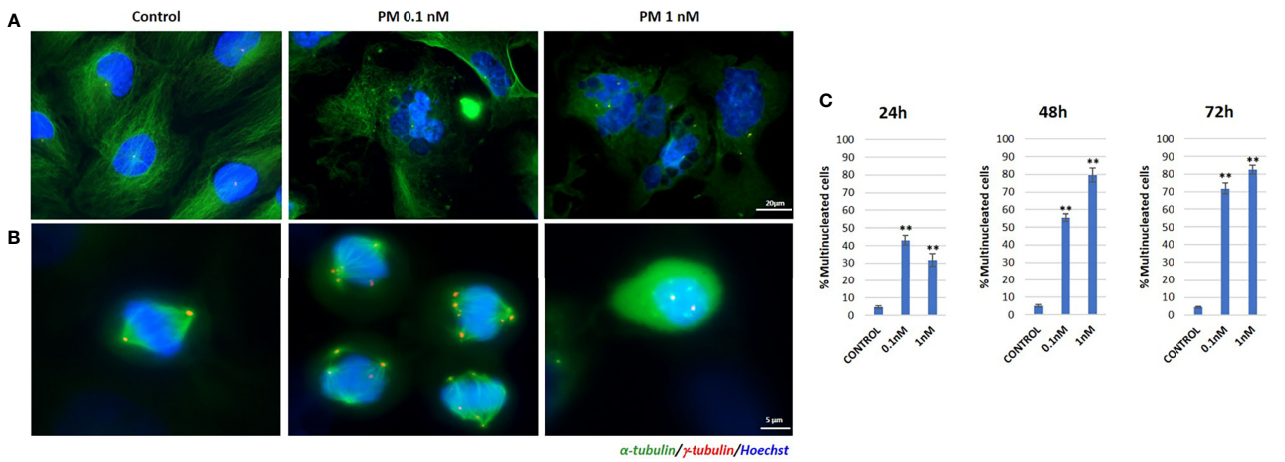

FIGURE 4 | Immunofluorescence staining of α- and γ-tubulin in plocabulin treated OV866(2) cells. **(A)** Effects of 48h treatment with plocabulin on microtubule network and appearance of multinucleated cells. **(B)** Aberrant mitotic spindle polarization and chromosome missegregation after treatment with plocabulin. **(C)** Bar plots represent percentage of multinucleated cells after treatment with plocabulin (0.1 and 1 nM) at 24, 48 and 72h. PM: PM060184; **: p value < 0.01.

31.8% (p value < 0.001) were multinucleated at 1 nM of plocabulin (**Figures 4A, C**). The percentage of multinucleated cells increased in a statistically significant manner (p value < 0.001) with time and drug concentration (**Figure 4C**) data that suggest an apoptotic death of these cells. Cell cycle disruption and an increase of apoptosis were also seen in cell cycle experiments, in conjunction with a decrease of G_0/G_1 phase (**Figure 5**).

Synergisms

We did not find any clear synergism in the four combinations tested in any of the cell lines (PEA1, PEA2, PEO4, PEO14, OV866(2) and TOV3041G). Although some combinations showed punctual synergy at individual dose combinations, overall, none of the experiments showed a positive synergy score for all four algorithms analyzed in SynergyFinder Plus (**Supplementary Table 2**). Nor did we observe any additive effect reducing the effective dose of the other agent tested. **Figure 6** shows an example of these drug response curves in OV866(2) cell line.

DISCUSSION

Plocabulin is a novel tubulin-binding agent of marine origin that has been proven to potently disrupt cellular microtubules and mitosis and thus inhibit the proliferation of tumor cell lines (12). In the present study we have investigated the antiproliferative effect of plocabulin in a panel of HGSOC cell lines, including CDDP resistant scenario, and our results demonstrate that plocabulin has a dose-dependent potent cytotoxic activity, with IC_{50} values within low nanomolar range. Other preclinical *in vitro* and *in vivo* studies have been done with plocabulin in endothelial cells (13), patient-derived colorectal cancer organoids, with dose responses very similar to ours (23), or

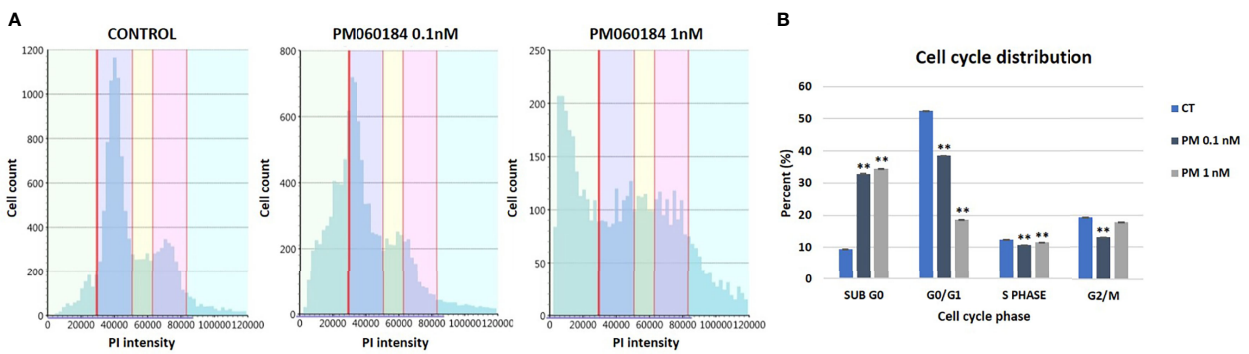

FIGURE 5 | Cell cycle experiments in OV866(2) cells. **(A)** Cell cycle diagrams obtained by Celigo S plate cytometer of OV866(2) cells treated for 24h with plocabulin at 0.1nM and 1nM, and the untreated control. **(B)** Bar plots represent the percentage of cells at each phase of the cell cycle. CT: untreated control; PM: PM060184; PI: propidium iodide; **: p value < 0.01.

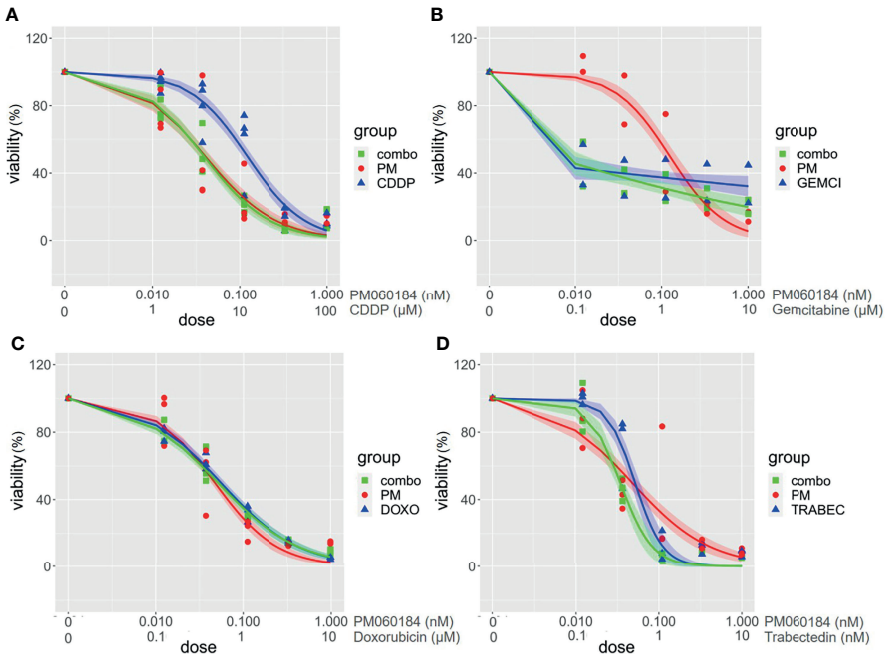

FIGURE 6 | Drug combination assays in OV866(2) cell line. Graphs represent viability curves for individual drugs (PM060184: red; cisplatin (CDDP), doxorubicin, gemcitabine or trabectedin: blue, and for combinations: green). Range concentration of each drug are described in the X-axis. PM060184 was combined with **(A)** CDDP, **(B)** Gemcitabine, **(C)** Doxorubicin, and **(D)** Trabectedin.

gastrointestinal stromal tumor (GIST) patient-derived xenograft (PDX) mice (14). All of them show promising results enhancing the antitumor effect of plocabulin in different solid tumors, supporting the development of clinical trials to explore the activity of plocabulin in patients. In a first-in-human phase I clinical trial the main dose-limiting toxicity was peripheral sensory neuropathy, similarly to other tubulin-binding agents. Although an encouraging clinical benefit was observed, tolerability should be improved. Therefore, the recommended dose and schedule is not well defined yet (24).

We have not been able to establish a possible association between the *BRCA* status of the cell lines and the response to plocabulin. Only two of the cell lines tested (PEO1 and PEO16) have mutations in *BRCA2* (16), and the IC_{50} values obtained for plocabulin do not show a correlation with a *BRCA* mutated genotype. Nevertheless, future experiments with a wider series of *BRCA* mutated cell lines and the use of other drugs as PARPi could be interesting to understand this matter.

3D *in vitro* models are used in cancer research as a bridge model between *in vitro* cancer cell line cultures and *in vivo* tumor. Our data show that, when cultured as spheroids, only three cell lines remain sensitive to plocabulin in a low nanomolar range, and PEO4 is still almost three times more resistant in 3D vs. 2D culture. These results are expected, since 3D spheroids are more complex models than 2D. Their spatial architecture promotes the establishment of diffusion gradients that could modify what it is seen in monolayer, where all cells are equally exposed to drugs (25). Our group has already published a work that supports the theory that OC cells tend to be more resistant to CDDP treatment when growing on 3D (17), and similar results have also been recently described for different drugs in 3D models of colorectal cancer (26), hepatocarcinoma (27), glioblastoma (28), breast cancer (29), melanoma (30), and also in OC (25).

Cell migration plays an important role in many physiological and pathophysiological processes such as wound healing, tissue development, angiogenesis, inflammation and cancer, where the process of tumor metastasis involves invasion and migration of cancer cells (31, 32). OC predominantly metastasizes by shedding away from primary tumors and moving through the abdominal cavity in ascites fluid towards the mesothelium, where molecules such as fibronectin, laminin, type IV collagen and mesothelin promote adhesion and migration to the basement membrane/extracellular matrix (ECM) (33). Recurrent disease is very difficult to treat since it often becomes resistant to chemotherapy. Anti-migratory agents could significantly improve cancer treatment, decreasing the dependency on therapeutics and the associated side-effects by delaying the formation of metastases. Furthermore, they have been shown to sensitize migrating cells to antiapoptotic drugs (31, 34, 35).

Our results show that plocabulin can inhibit invasion of PEA1, PEA2, PEO14 and OV866(2) and migration of PEA2, PEO14 and OV866(2) HGSOC cell lines in monoculture, and in 3D spheroids of OV866(2) cells, reducing spheroid volume and cell sprouting area, even though the latter become more resistant to plocabulin in 3D. We and others have previously reported that 3D tumor spheroid-based migration assays reflect better the solid tumor microenvironment and represent both cell-cell and cell-ECM interactions. Our technique is highly reproducible and

therefore appropriate for the evaluation of therapeutic drugs with anti-migratory properties (18, 31).

We and others have previously published the importance of the angiogenic process in OC and its relation to poor prognosis (36). Moreover, antiangiogenic treatment with bevacizumab is approved in OC for first line and relapse settings. Preclinical studies have reported an antiangiogenic effect of plocabulin in GIST PDX (14), and also in endothelial cells, where it inhibits the migration and invasion abilities at picomolar concentrations that suppress microtubule dynamics but do not affect cell survival (13). To our knowledge, this is the first study to evaluate the effects of plocabulin on tumor cell invasion and migration, and together with the aforementioned studies, it demonstrates an important effect in the global process of metastasis, since it can inhibit the migration of tumor and endothelial cells. All these results suggest that this secondary mechanism of action could also be beneficial in OC and should be further investigated in a combined model that includes tumor and endothelial cells.

As previously reported by Martínez-Díez et al. in a lung cancer cell line, we have observed in OV866(2) HGSOC cell line that plocabulin has a potent depolymerizing effect on microtubules, which affects cells in interphase and mitosis. In this cell line, plocabulin treatment causes the appearance of multipolar mitoses, chromosome missegregation and multinucleated cells that do not undergo anaphase/cytokinesis, forcing cells to enter senescence or apoptotic death, as seen in cell cycle experiments. Martínez-Díez et al. also reported that plocabulin-induced disorganization and fragmentation of the microtubule network could be related to the inhibition of cell migration in cells where the antiproliferative effects of this drug were not evident (11).

In this work we have also explored combinations of plocabulin with various drugs currently administered to patients with HGSOC in routine clinical practice, but none of them showed a synergistic or additive effect. Part of the reasons for the lack of interaction between the drugs is that plocabulin as a single drug is already effective at low concentrations. Combination with paclitaxel was not tested because paclitaxel and plocabulin share a similar mechanism of action (microtubule inhibitors) and dose-limiting toxicity (neurotoxicity) (24). The combinations were evaluated using SynergyFinder Plus software, a very robust and restrictive tool, since to ensure a positive synergy, all the four algorithms had to produce consistent results. To date, only one phase I clinical trial has been developed using plocabulin in combination with another drug, gemcitabine, but results are still under analysis (NCT02533674). Our results do not support the use of plocabulin in combination with other drugs, based on our combination approach. However, we do believe that it could have an interesting antitumoral activity when used in monotherapy in the treatment of OC, especially in the case of platinum-resistant relapses, where there is an unmet medical need. Our data reflect that plocabulin is effective in OC cell lines that exert different sensitivities to CDDP, but further studies are needed to confirm these findings.

As mentioned throughout the discussion, this work presents a series of strengths, such as the use of a large panel of HGSOC cell lines, the use of 3D models that better represent tumor architecture, and the analyses of drug effects in less studied processes such as migration and invasion in 2D culture and in spheroids. Moreover, we have employed a robust and restrictive tool for the exploration of drug combinations. This method did not reveal any synergies in our hands, but they cannot be discarded by complementary approaches, like animal model experiments. Nevertheless, we recognize a series of limitations. First of all, we have only been able to demonstrate migration and invasion inhibition in four cell lines in 2D and only one in 3D. Confirmation in other cell lines that migrate and invade would be desirable. Also, 3D spheroids were exposed to a maximum concentration of plocabulin of 10 nM, 100-1000 times stronger than IC_{50} values obtained in monolayer culture, but still very low, and may not be enough when scaled to *in vivo* models.

To our knowledge, this is the first work to describe the *in vitro* effects of plocabulin, a novel tubulin-binding agent, in OC. Our results show that plocabulin has potent cytotoxic activity in a panel of HGSOC cell lines, including CDDP resistance scenario, and that it inhibits migration and invasion of tumor cells and spheroids. Further clinical evaluation of this drug in OC would be warranted.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Concept, VH-S, AR, and MMe. Methodology, all authors. Formal analysis, VH-S, SZ, AR, and MMe. Supervision, JT, AR, and MMe. Project administration, MMe and AR. Writing and original draft preparation, VH-S, AR, and MMe. Writing, review and editing, all authors. 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/fonc.2022.862321/full#supplementary-material

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REVIEW
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Efficacy of Eribulin in Soft Tissue Sarcomas

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Soft tissue sarcomas are a highly heterogenous group of tumors with limited systemic therapy options. Eribulin, a synthetic analogue of halichondrin B, is a potent mitotic inhibitor. A phase 3 trial of previously treated advanced Liposarcoma and Leiomyosarcoma demonstrated superiority of eribulin to dacarbazine. Eribulin appears to be particularly effective for liposarcomas. It has also been shown to be a safe and effective treatment alternative to doxorubicin in patients where doxorubicin is contraindicated. From retrospective studies, eribulin has demonstrated efficacy in patients with angiosarcoma, pleomorphic sarcomas, synovial sarcomas, rhabdomyosarcomas, angiosarcomas, and myxofibrosarcomas. Future areas of development include liposomal eribulin, which may provide increased efficacy and lower toxicity, and delineation of biomarkers of response and resistance, allowing better selection of patients for treatment.

Keywords: sarcoma, eribulin and related compounds, eribulin, STS, liposarcoma, leiomyosarcoma, review

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INTRODUCTION

STS make up approximately 80% of all sarcomas. There are over 100 different subtypes (WHO Classification of Tumours Editorial Board, 2020). Liposarcoma (LPS) and Leiomyosarcoma (LMS) are two of the most common subtypes, with an annual incidence of approximately 0.9 and 0.7 per 100,000 respectively (Ducimetière et al., 2011). The mainstay of management for localized disease is complete surgical resection, with or without perioperative radiation and chemotherapy. Approximately 50% of patients with high grade tumors develop metastatic disease. The prognosis for patients with advanced disease is poor, with a median overall survival of approximately 19 months (Tap et al., 2020).

Doxorubicin, with or without ifosfamide, is the first line treatment in the majority of patients with advanced STS. There are limited second line treatments and the choice depends on STS subtype and patient performance status. Second line treatments include pazopanib, trabectedin, eribulin, and gemcitabine, with or without docetaxel or dacarbazine. Pazopanib, a tyrosine kinase inhibitor of angiogenic growth receptors, has shown superiority to placebo in a randomized placebo-controlled phase 3 trial in STS (van der Graaf et al., 2012). Trabectedin has shown superiority to dacarbazine for treatment of LPS and LMS in a phase 3 randomized clinical trial (Demetri et al., 2016). Both pazopanib and trabectedin have U.S. Food and Drug Administration (FDA) approval for the treatment of STS (National Cancer Institute, 2020). Evidence from phase 2 trials suggests efficacy of gemcitabine in STS, either alone or in combination with docetaxel, dacarbazine (Ducoulombier et al., 2016), or, more recently, nab-paclitaxel (Digklia et al., 2021). Gemcitabine does not currently have FDA approval for use in STS.

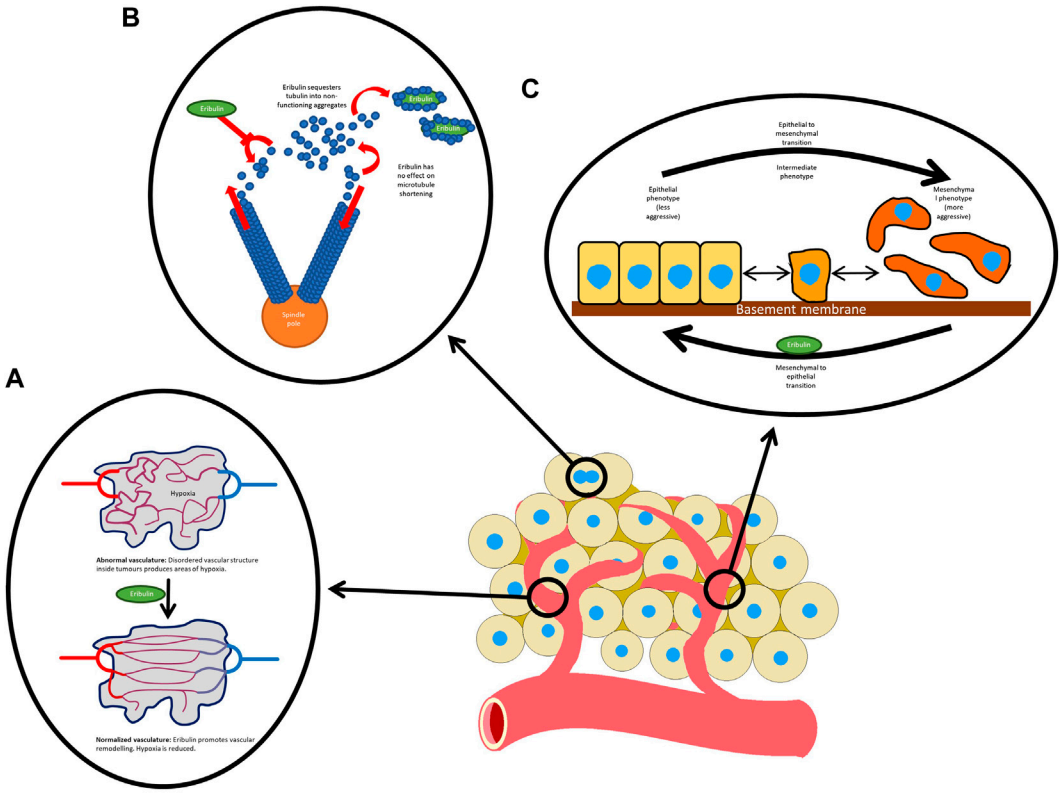

FIGURE 1 | Mechanisms of action of eribulin: **(A)** normalizes the tumor vasculature; **(B)** inhibits microtubule growth without having any effect on microtubule shortening. Eribulin also sequesters tubulin, reducing the supply available to microtubules; and **(C)** reverses the mesenchymal to epithelial transition.

Eribulin is an inhibitor of microtubule polymerisation and is a synthetic analogue of the naturally occurring anticancer agent halichondrin B found in marine sponges (Shetty and Gupta, 2014). As well as its use in STS, it is also used in metastatic breast cancer in patients who have progressed on first and second line treatment.

In this review, we summarize preclinical and clinical data showing efficacy of eribulin in STS. We compare the efficacy across different STS subtypes. We also review potential predictive biomarkers of eribulin response as well as possible combination regimes and other future perspectives.

Mechanism of Action of Eribulin

There are several mechanisms of action of eribulin and these are detailed in **Figure 1**. The predominant mechanism involves binding to the positive end of microtubules and inhibiting the growth phase (Smith et al., 2010). It displays a distinct mechanism of action from other tubulin targeting agents, including taxanes (McBride and Butler, 2012). Several other anticancer mechanisms have been suggested. In one study, vascular remodeling was demonstrated by affecting gene expression in pericytes (Agoulnik et al., 2014) and another showed improved oxygenation of tumors after treatment with eribulin (Ueda et al., 2016). Eribulin has also been shown to suppress transforming growth factor beta 1 (TGF-β1), an important growth factor that promotes cell proliferation, differentiation and metastasis (Ueda et al., 2016).

Although the predominant mechanism of action of eribulin in STS and breast cancer is likely similar, there is evidence that eribulin induces distinct differentiation patterns depending on the cell of origin. In breast cancer cells, eribulin reverses the epithelial to mesenchymal transition (Yoshida et al., 2014). Epithelial to mesenchymal transition produces a more invasive cellular phenotype and therefore is believed to underlie metastatic spread. Markers involved in the transition such as the matrix modifying enzyme MMP, the mesenchymal marker vimentin and laptm4a, a protein in volved in transport across the enodosomal and lysosomal membranes have been shown to be upregulated in STS (De Vita et al., 2017). In LPS, eribulin has been observed to promote expression of adipocytic markers and, in LMS, to promote expression of smooth muscle markers. Therefore, eribulin promotes differentiation to adipocytic and smooth muscle lineage respectively (Cortes et al., 2018).

Eribulin has also been shown to have effects on cell motility. In one study LPS cells were treated with Eribulin and compared with untreated cells. Eribulin was found to stop migratory activity in the treated cells and it was shown that Rho proteins, which are believed to be instrumental in cell migratory activity, was downregulated (De Vita et al., 2016).

Preclinical Efficacy in Soft Tissue Sarcomas

Robust *in vitro* activity of eribulin has been shown against a wide range of STS, including fibrosarcoma, LMS, LPS and synovial sarcoma by induction of G2–M cell-cycle arrest and apoptosis (Asano et al., 2018). Interestingly, eribulin has shown improvement of vascular perfusion in LMS and clear cell sarcoma xenografts (Nakai et al., 2020). In a number of non-STS cell lines, including breast cancer and non-small cell lung cancer, combination activity of eribulin with other anticancer agents such as bevacizumab, capecitabine, carboplatin, cisplatin, doxorubicin, everolimus, gemcitabine, and palbociclib has been shown. The combination of eribulin and pazopanib has also shown a synergistic effect in myxoid, pleomorphic LPS, and LMS cell lines (Escudero et al., 2021). Combination therapies of eribulin have also shown activity in mouse xenograft models. In one study, a combination of eribulin plus an AKT inhibitor led to increased tumor suppression in a mouse xenograft STS model (Hayasaka et al., 2019). In another, combing eribulin with irinotecan resulted in tumor regression of rhabdomyosarcoma xenografts (Robles et al., 2020).

Phase 1 Data

In a phase 1 dose finding study of 40 patients, the dose limiting toxicities of grade 3 and 4 febrile neutropenia was found at a dose of 2.0 mg/m^2. The maximum tolerated dose was set at 1.4 mg/m^2. No non-hematological dose limiting toxicities were seen (Morgan et al., 2015). A schedule of 1.4 mg/m^2 of eribulin on days 1, 8, and 15 of a four weekly cycle was found to cause grade 3 or 4 neutropenia in 64% of patients (Vahdat et al., 2009). Therefore, the standard dose of eribulin is 1.4 mg/m^2 on days 1 and 8 of a three-weekly cycle (Vahdat et al., 2009; Schöffski et al., 2011; Morgan et al., 2015). Eribulin displays linear pharmacokinetics with a rapid distribution phase followed by a slow elimination phase. Mean terminal half-life is approximately 40 h. The majority of the drug (82%) is excreted faecally (Kawai et al., 2017).

In early clinical data, eribulin has shown activity against a number of tumor types, including non-small cell lung cancer, head and neck cancer (Mukohara et al., 2012), cervical (Goel et al., 2009), urothelial and melanoma. Only one phase 1 study included a sarcoma patient. 12 patients experience stable disease, one of which was an endometrial stromal sarcoma. The mean duration of stable disease was 86 days (Tan et al., 2009).

Non-Randomized Phase 2 Trials of Eribulin in Soft Tissue Sarcomas

In a non-randomized phase 2 trial by Schöffski et al., response to eribulin was assessed in 128 patients with STS (Schöffski et al., 2011). Eligible patients had histologically proven metastatic STS of high or intermediate grade, had received no more than one previous chemotherapeutic regime or two single chemotherapeutic drugs, and had disease progression in the last 6 months. One hundred and fifteen patients in total were assessable for the primary endpoint, which was made up of 38 LMS, 32 LPS, 19 synovial sarcomas and 26 with "other" STS. No patients with embryonal rhabdomyosarcomas, chondrosarcomas, osteosarcomas, Ewing sarcoma, gastrointestinal stromal tumors, dermatofibrosarcoma protuberans or inflammatory myofibroblastic sarcomas were included. The primary endpoint was PFS at 12 weeks.

The best results were found in the LPS group, with 15 patients (46.9%) being progression free at 12 weeks. This was followed by 12 (31.6%) in the LMS group, 4 (21.1%) in the synovial sarcoma group and 5 (19.2%) in "other" sarcomas. The five patients in the other histological subtypes were two fibroblastic sarcomas, two epithelioid sarcomas and one solitary fibrous tumor. The most common grade 3–4 adverse events (AEs) included neutropenia (52%), leukopenia (35%), anemia (7%), fatigue (7%) and raised alanine aminotransferase (5%).

Another phase 2 study, by Kawai et al., included 51 Japanese patients with STS who had received one or more prior chemotherapies for advanced disease. In that study, 16 patients had LPS, 19 had LMS and the remaining 16 consisted of synovial sarcoma, undifferentiated pleomorphic sarcoma, rhabdomyosarcoma, endometrial sarcoma, fibrosarcoma, solitary fibrous tumor, alveolar soft part sarcoma and malignant peripheral nerve sheath sarcoma. The LPS group had a median PFS of 6.8 months (95% CI 5.1–8.4) and the LMS group 2.9 months (95% CI 1.3–8.2). In the "other" sarcoma group, median PFS was 4.1 months (95% CI 2.6–5.6). The most common grade 3–4 AEs were neutropenia (86%), leukopenia (38%) and lymphopenia (33%) (Kawai et al., 2017).

Randomized Phase 3 Trial of Eribulin Versus Dacarbazine in Previously Treated Patients With Advanced Liposarcoma or Leiomyosarcoma

To further assess the efficacy of eribulin in STS, a phase 3 randomized open label trial was undertaken (Schöffski et al., 2016). Patients with intermediate or high grade advanced LPS or LMS, who had received at least two previous systemic regimens for advanced disease and had measurable disease with RECIST 1.1, were randomized to either eribulin (1.4 mg/m^2 on days 1 and 8) or dacarbazine (850 mg/m^2, 1,000 mg/m^2, or 1,200 mg/m^2 depending on the center on day 1) in a 21-day cycle. The primary endpoint was median OS. Secondary endpoints were PFS, PFS at 12 weeks, and safety and tolerability as assessed with CTCAE v4.02. 351 patients of the 452 randomized patients were anthracycline pre-treated (77.7%). The LMS group made up 297 (67%) patients and 131 (45%) were of uterine origin.

The primary endpoint of median OS was met. The median OS was 13.5 months (95% CI 10.9–15.6) in the eribulin arm compared to 11.5 months (95% CI 9.6–13.0) in the dacarbazine arm, with a HR of 0.77 (95% CI 0.62–0.95). There was no statistically significant difference in median PFS, with 2.6 months (95% CI 1.9–2.8) PFS in both arms (95% CI 1.8–2.7). Likewise, the proportion of patients who had not progressed at 12 weeks was also similar, with 76 patients (33%, 95% CI 27.2–39.9) having not progressed after 12 weeks in the eribulin arm versus 64 patients (29%, 22.8–35.0) in the dacarbazine group. Furthermore, the response rates were low, with a non-significant difference (ORR 3.9 vs. 4.9%).

Treatment related AEs were common, with 224 (99%) and 218 (97%) patients experiencing AEs in the eribulin and dacarbazine arms respectively. Grade 3 AEs were higher in the eribulin (152, 67%) versus the dacarbazine arm (126, 56%). There was study drug withdrawal in 17 (8%) patients in the eribulin arm versus 11 (5%) in the dacarbazine arm, and dose reduction in 58 (26%) patients in the eribulin arm versus 32 (14%) in the dacarbazine arm.

Subgroup Analysis

As a highly heterogenous group of tumors, it is unsurprising that a variety of responses would be seen in different subtypes of STS. In the preplanned OS subgroup analysis, performed in the previously described phase 3 randomized trial, LPS patients benefited from eribulin (15.6 vs. 8.4 months; HR: 0.511; 95% CI: 0.346–0.753) compared to LMS patients (12.7 vs. 13.0 months; HR: 0.927; 95% CI: 0.714–1.203). Although the small numbers involved in different LPS subtypes make it difficult to make any firm conclusions on the relative responsiveness of different LPS subtypes, the benefit from eribulin was observed across all LPS subtypes. The analysis showed more robust benefit for pleomorphic LPS, with a median OS of 22.2 months in the eribulin arm versus 6.7 months in the dacarbazine arm (HR 0.18 95% CI 0.04–0.85). The dedifferentiated subtypes had an extended median OS in the eribulin arm of 18.0 versus 9.6 months (HR 0.43 95% CI 0.23–0.79) in the dacarbazine arm. The myxoid subtype showed a more modest 13.5 months OS in the eribulin arm, versus 8.1 months in the dacarbazine arm (HR 0.79 95% CI 0.42–1.49) (Demetri et al., 2017).

Furthermore, performance status (PS) is important when selecting patients most likely to benefit from eribulin. Patients with a PS of 0 showed greater benefit when given eribulin compared to dacarbazine, with an OS of 19.9 vs. 13.1 months (HR: 0.579; 95% CI: 0.407–0.823). However, in patients with a PS of 1–2 there was no statistically significant difference in OS between the eribulin group (9.2 months) and the dacarbazine group at (9.9 months, HR: 1.09; 95% CI: 0.82–1.44).

Although there was no statistically significant benefit in the LMS cohort many patients did achieve objective responses. A retrospective analysis of archival samples of 77 LMS patients who participated in the trial were reviewed. It was found that patients with TP53 mutations were more likely, and patients with ATRX mutations less likely, to achieve disease control with eribulin. A positive correlation between TP53 mutation and PFS was shown [$p = 0.036$; HR 0.51 (95% CI 0.26–0.93)] but no impact on OS was seen. ATRX mutations were shown to have a negative impact on both PFS and OS (Wozniak et al., 2021).

Based on these data, eribulin was approved by the FDA for the treatment of unresectable or metastatic LPS in patients who had received prior anthracycline-based chemotherapy (The U.S. Food and Drug Administration, 2016) and for the treatment of inoperable STS in patients who have received previous chemotherapy for advanced or metastatic disease (Committee for Medicinal Products for Human Use and European Medicines Agency, 2016).

Retrospective Studies in Leiomyosarcoma and Liposarcoma

After the successful phase 3 trial, there have been several retrospective real-world studies in Japanese patients who received eribulin for advanced STS. In one study, eribulin was given to 256 patients with STS of which 73 were LMS and 70 LPS (Kobayashi E. et al., 2019). Patients had received a median of two previous chemotherapy regimen prior to eribulin. It found a partial response in 5 out of 72 LMS and 2 out of 70 LPS. Eribulin has also been shown to be an effective first line treatment for STS. In six patients where doxorubicin was contraindicated due to cardiac co-morbidities or advanced age, median progression-free survival (PFS) was 9.7 months (confidence interval not reached) (Tsuchihashi et al., 2020). In a recent retrospective study of 23 patients with advanced STS (predominantly LPS and LMS), body composition has been shown to be a predictor of eribulin toxicity. Grade 4 hematological toxicities were significantly higher in those with low skeletal muscle gauge ($p = 0.02$). Grade 3 and 4 non-hematological toxicities were also associated with low skeletal muscle gauge ($p = 0.04$) as well as low serum albumin level ($p = 0.02$) (Kobayashi H. et al., 2019).

Angiosarcomas

Angiosarcomas are a highly aggressive tumor of endothelial tissue. They represent 1–2% of all sarcomas. They can develop throughout the body but about 60% are cutaneous (Cao et al., 2019). Anthracyclines, such as doxorubicin, and taxanes can be used to treat advanced angiosarcomas. Taxanes are usually preferred in older patients with more comorbidities (Fujisawa et al., 2020).

In a single-arm prospective observational study of 25 patients who had previous treatment with a taxane, eribulin was given for cutaneous angiosarcoma (Fujisawa et al., 2020). The age of enrolled patients ranged from 62 to 88 (median age 74) years, and 88% had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Median OS was 8.6 months and PFS 3.0 months. The best overall response rate (ORR) was 20% (5 out of 25). A total of 16 grade 3 or 4 Serious Adverse Events (SAEs) were seen. 56% (14 out of 25) underwent dose reductions and 44% (11 out of 25) had their treatments postponed due to AEs. Excellent responses to eribulin in scalp cutaneous angiosarcomas have also been reported in two cases reports. In the first case, a patient with previous scalp angiosarcoma presented with lung metastases. The patient was treated with eribulin and his disease remained well controlled after nine cycles of treatment (Wada et al., 2018). In the second case, a very good partial response was seen in a local recurrence of a scalp angiosarcoma when treated with eribulin (Iwamoto et al., 2018). In another case report, eribulin was given as an eighth line treatment for metastatic cardiac angiosarcoma with a partial response maintained for 4 months (Inagaki et al., 2018).

Retrospective Studies in Other Subtypes

There are many other subtypes of STS that have limited treatment options. Small numbers of non LPS and LMS STS subtypes were included in phase 1 and 2 studies. These data are summarized in,

TABLE 1 | Phase 2 and 3 clinical trials for eribulin in STS.

Author	Study type	ECOG Performance Status	Soft tissue sarcoma subtype	Number of patients receiving eribulin	Median overall survival (months)	Median progression free survival (months)
Schöffski et al. (2016)	Randomised phase 3 trial versus dacarbazine	0 (49%) 1 (50%) 2 (1%)	LPS and LMS	228	15.6	2.6
Schöffski et al. (2011)	Non-randomised, single arm phase 2 trial	0 (64%) 1 (36%)	LPS LMS Synovial Other sarcoma	32 38 19 26	Not reported Not reported Not reported Not reported	2.6 2.9 2.6 2.1
Kawai et al. (2017)	Non-randomised, single arm phase 2 trial	0 (53%) 1 (47%)	LPS and LMS Other sarcoma	35 16	17.0 7.6	5.5 2.0

TABLE 2 | Phase 1, 2, and 3 data for non LPS and non LMS STS subtypes.

Author	Phase	Total number of non LPS or LMS STS	STS subtypes with stable disease or better
Tan et al. (2009)	1	1	Endometrial stromal sarcoma
Schöffski et al. (2011)	2	45	4 synovial sarcomas 2 fibroblastic sarcomas 2 epithelioid sarcomas 1 solitary fibrous tumor
Kawai et al. (2017)	2	16	Endometrial stromal sarcoma (2/2 patients) Synovial sarcoma (1/3 patients) Solitary fibrous tumor (1/2 patients) Fibrosarcoma (1/2 patients)
Schöffski et al. (2016)	3	0	

Table 2. In a real-world observational study of 256 Japanese patients, eribulin was shown to have antitumor activity against multiple subtypes (Kobayashi E. et al., 2019).

Median age in the study was 62 (range 17–87) years and 84% (214 out of 256) had an ECOG performance status of 0 or 1. Median time from diagnosis to initiation of eribulin was 2.5 years (range 0.2–29.2). Target lesions were most commonly retroperitoneal or intraperitoneal (40.4%). The most common number of prior chemotherapy regimens was one (31.8%) followed by two (29.0%). Only 7.1% received eribulin first line. The most common prior chemotherapeutic regimen was doxorubicin monotherapy (36.9%), followed by pazopanib (32.2%), gemcitabine and docetaxel (26.7%) and doxorubicin and ifosfamide (22.7%). A total of 174 grade 3 or 4 SAEs were seen. The most AE was neutropenia, which was seen in 52.5%. Fifty-five patients (21.6%) underwent dose reduction.

A partial response was seen in 17 patients. Excluding LPS and LMS, 10 out of 143 had partial responses. This was seen in 2 out of 19 undifferentiated pleomorphic sarcomas, 3 out of 15 synovial sarcomas and 2 out of 12 rhabdomyosarcomas. A partial response was also seen in one patient, each in the angiosarcomas (14 patients in total), myxofibrosarcoma (5 patients in total) and undifferentiated round cell sarcoma (1 patient in total).

Another retrospective Japanese study of 82 STS patients treated with eribulin included 45 patients that had neither LPS nor LMS STS. Overall, 72% had received prior anthracycline based chemotherapy and 75% had a PS of 0 or 1. The 45 patients that had neither LPS nor LMS STS consisted of 13 undifferentiated pleomorphic sarcomas, six synovial sarcomas, five malignant peripheral nerve sheath tumors and 21 unspecified subtypes. A partial response was seen in one myxofibrosarcoma. Stable disease for at least 6 months was seen in one undifferentiated pleomorphic sarcoma, one synovial sarcoma and one sclerosing epithelioid fibrosarcoma (Nakamura et al., 2019). Eribulin has shown a clinically meaningful level of activity in several STS subtypes in the Japanese population. This has led to approval in Japan of eribulin for all pre-treated STS patients in 2016. (Eisai Global, 2016).

FUTURE PERSPECTIVES

Liposomal preparations of several chemotherapeutic agents have been developed and have the advantage of improved targeting of tumor sites and decreased toxicity. This has been shown to produce improved efficacy in several cases (Fanciullino and Ciccolini, 2009). A liposomal formulation of eribulin has been developed, which aims to replicate some of these successes. In pre-clinical studies, changes to the liposome formulation reduced the release rate of the liposome, reducing Cmax and increasing the half-life (Yu et al., 2013). This could allow higher doses to be

used while reducing associated toxicities. A phase 1 study, which did not include any patients with STS, has shown a good side effect profile and response rates that compared favorably to non-liposomal eribulin (Evans et al., 2019). Pending results from further trials in other tumor types, liposomal eribulin is a promising future therapy for STS.

Patient selection is key in determining response to eribulin in STS and identification of biomarker signatures is key (Emambux and Italiano, 2017). In one study of 52 patients with triple negative breast cancer, lack of the transcription co-repressor transducin-like enhancer of split 3 (TLE3) was associated with poorer outcomes when treated with eribulin (Kashiwagi et al., 2017a). In another study, mutations in the Phosphoinositide 3-kinase and AKT pathway in HER-2 negative breast cancer xenografts was also linked to a poorer response to eribulin (Gris-Oliver et al., 2021). In osteosarcomas, increased expression of the drug efflux pump P-glycoprotein and the tubulin isotype βIII-tubulin was associated with lower responsiveness to eribulin (Sampson et al., 2016). It is unclear whether similar mechanisms are involved in STS or whether other markers of response and resistance are important. Future drug targets, such as P-glycoprotein, may increase the efficacy of eribulin.

Correlation of microRNA expression levels with oncological outcomes in various cancer types has also been investigated. A panel of a total 26 miRNAs that correlate with eribulin response ($p < 0.05$) have been identified by using archival tumor tissue from patients treated in the non-randomized phase 2 trial of eribulin. However, this hypothesis should be validated by prospective trials (Wiemer et al., 2017).

Eribulin has been shown to have important effects on the tumor immune microenvironment. In one study in breast cancer, patients with higher levels of tumor infiltrating lymphocytes (TILs) receiving eribulin had a better disease-free survival than those with lower levels of TILs (Kashiwagi et al., 2017b). The epithelial to mesenchymal transition is believed to be detrimental to the immune microenvironment. Eribulin has been shown to reverse this process (De Vita et al., 2017). Therefore, reversal of this may promote TIL cytotoxic activity. In a retrospective cohort study in breast cancer, tissue samples were obtained before and after treatment in ten patients. Five patients were deemed responders and five non-responders. PD-L1 expression became negative in six patients. This was significantly associated with response to eribulin ($p = 0.024$) (Goto et al., 2018). A recent phase I/II trial of eribulin in combination with pembrolizumab showed promising antitumor activity in metastatic triple negative breast cancer. In the subgroup analysis both PD-L1 positivity and being treated in the first line setting was associated with a greater ORR (Tolaney et al., 2021). This suggests immunotherapies may have a synergistic effect in combination with eribulin. However, a phase 2 trial of 19 LMS patients treated with eribulin in combination with pembrolizumab found the PFS at 12 weeks to be only 42.1%. This failed to reach the primary endpoint of a 60% PFS at 12 weeks (Nathenson et al., 2020). Recently, data from the LPS cohort was presented showing a PFS rate at 12 weeks of 67% and a median PFS of 27 weeks (Nathenson et al., 2021). Furthermore, the authors reported that three patients with angiosarcoma showed significant responses in addition to one patient with SMARCA4 deficient thoracic sarcoma.

Eribulin in combination with other anticancer therapies may produce synergistic anticancer activity. Cyclin dependent kinase (CDK) 4/6 inhibitors restrict phosphorylation of the retinoblastoma protein stopping cells from exiting G1 and proceeding through the cell cycle. They have found widespread use in advanced hormone receptor positive breast cancer. A phase 2 trial of palbociclib in well differentiated or dedifferentiated LPS showed a favorable PFS of 17.9 weeks (Dickson et al., 2016). A combination schedule of CDK 4/6 inhibitors with eribulin may have a synergistic effect due to their distinct actions on cell division. The ERIGE trial was a phase 2 trial of eribulin in combination with gemcitabine for advanced triple negative breast cancer. This found an overall response rate of 37.3%. In a recent proof of concept phase 2 trial, the combination of eribulin with gemcitabine has shown encouraging results in advanced liposarcoma and leiomyosarcoma pretreated patients with a 3 months PFS rate of 73% (Kim et al., 2021). In another study, the combination of eribulin and the AKT inhibitor MK-2206 was associated with synergistic activity in both sarcoma cell lines and in STS murine xenograft mouse models (Hayasaka et al., 2019). The combination of lenvatinib, a multiple kinase inhibitor with anti-angiogenic activity, and eribulin may also show synergistic anticancer activity. In a single arm phase 1b/II study of lenvatinib and eribulin in 14 LMS and 6 LPS the overall response rate by RECIST 1.1 was found to be 27% (5/18). 15 patients experienced at least one grade 3 or 4 AE with hypertension (4 patients, 27%), hand-foot-syndrome (4 patients, 27%) and proteinuria (3 patients, 20%) being the most common. These studies suggest possible roles of CDK4/6 inhibitors, gemcitabine, AKT inhibitors and lenvatinib as combination therapies with eribulin.

Retroperitoneal sarcomas (RPS) make up about 16% of all sarcomas (Carbone et al., 2021). Local recurrence is more common post-resection than at other sites and a large subset of patients have unresectable disease at diagnosis. The use of neoadjuvant chemotherapies would aim to shrink RPS and allow successful resection of previously unresectable tumors, and to improve margin status thereby reducing the chance of recurrence. Ifosfomide and doxorubicin can be given for RPS as a neoadjuvant therapy. However, due to poor response rates and high levels of toxicities, they are not always suitable (Almond et al., 2018). As eribulin has shown efficacy in metastatic LPS, it may be an appropriate neoadjuvant therapy in locally advanced retroperitoneal LPS. An ongoing phase 1b clinical trial is using neoadjuvant eribulin and radiotherapy in RPS. The primary endpoint is determination of the recommended phase 2 dose. Secondary endpoints include the assessment of anti-tumor activity of combined eribulin and radiotherapy, and surgical outcomes of retroperitoneal LPS after neoadjuvant chemoradiation. Estimated completion date of this trial is February 2022 (U. S. National Library of Medicine, 2021).

It is still unclear why eribulin is more effective at extending OS than PFS. To date, this was replicated with similar results in

the EMBRACE trial, a phase 3 study of eribulin in advanced breast cancer (Cortes et al., 2011). One possible explanation is that eribulin sensitizes tumor cells to later lines of chemotherapy. In one pre-clinical study, eribulin promoted vascular remodeling in tumors and improved perfusion to tumor cells. This was shown to improve the anti-tumor activity of capecitabine (Funahashi et al., 2014). Another explanation is that eribulin may promote immune system mediated anticancer activity which may continue after eribulin has been stopped.

CONCLUSION

Eribulin is licensed by the FDA for the treatment of unresectable and metastatic liposarcoma for patients who have received prior chemotherapy with an anthracycline. It is also useful off-label as a first line treatment, particularly in patients at risk of doxorubicin toxicity. Responses have also been demonstrated in LMS, however it failed to show superiority to dacarbazine in a phase 3 trial so any use in LMS would be off-label. Responses have also been demonstrated in angiosarcomas, undifferentiated pleomorphic sarcomas, synovial sarcomas and rhabdomyosarcomas. Low numbers of patients in these cohorts make comparisons with other chemotherapeutic regimes difficult.

In the future, biomarkers such as P-glycoprotein and miRNAs may improve patient selection. The development of a liposomal formulation of eribulin may allow for higher doses to reach tumor cells while reducing the side effect profile. Clinical trials for this in breast cancer are ongoing. Eribulin may have synergistic effects when combined with other therapies such as CDK 4/6 inhibitors, AKT inhibitors and immunotherapies. There may be a role for eribulin as a neoadjuvant treatment for RPS and a clinical trial is ongoing. It is uncertain why eribulin extends OS but not PFS. However, the most likely explanation is that eribulin sensitizes tumor cells to later lines of chemotherapy.

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

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Determination of Extravasation Effects of Nal-Iri and Trabectedin and Evaluation of Treatment Options for Trabectedin Extravasation in a Preclinical Animal Model

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Background: Extravasation during chemotherapy administration can lead to dangerous adverse effects ranging from pain to tissue necrosis. Evidence-based data about prevention and treatment of extravasation injuries of some clinically used compounds still remains elusive. This work aimed to investigate, in a preclinical mouse model, the effects of extravasation of two chemotherapeutic agents, nanoliposomal irinotecan (nal-Iri) and trabectedin. In addition, we aimed to study treatment options for injuries induced by extravasation of these substances.

Methods: Mice were subcutaneously injected with nal-Iri or trabectedin applied in clinically used concentration. Doxorubicin was used as a positive control. In subsequently performed experiments, hyaluronidase, DMSO and tacrolimus were tested as potential treatments against extravasation-induced injuries by trabectedin. Systemic effects were analyzed by observation and documentation of the health status of mice and local reactions were measured and graded. In addition, hematoxylin-eosin stained histological sections of the treated skin areas were analyzed.

Results: Of the two tested substances, only trabectedin showed vesicant effects. Subcutaneous injection of trabectedin caused erythema formation in mice by day two that was progressing to skin ulcerations by day five. Furthermore, we found that topical treatment of mice with tacrolimus or DMSO reduced the vesicant effects of trabectedin. The results observed *in vivo* were supported microscopically by the analysis of histological sections.

Conclusions: We recommend classifying trabectedin as a vesicant agent and nal-Iri as a non-vesicant agent. Furthermore, our results obtained in a preclinical model suggest that tacrolimus and DMSO might be suitable treatment options of trabectedin extravasations, a finding that might be further utilized in clinical studies.

Keywords: extravasation, nanoliposomal irinotecan, trabectedin, hyaluronidase, DMSO, tacrolimus

INTRODUCTION

The term "extravasation" describes the escape of drugs, injected into blood vessels, into the surrounding tissue. In case of extravasation during cytostatic or cytotoxic therapy, serious tissue damage can occur, which can progress to tissue necrosis. Such extravasation can therefore—especially in the field of oncology—present an emergency with an acute need for action. The incidence of symptomatic extravasation varies between 0.5% and 6% (Barlock et al., 1979; Kassner, 2000; Langstein et al., 2002; Goolsby and Lombardo, 2006).

Different risk factors for extravasation are to be considered. They can be summed up into four categories: patient-specific (e.g., cardiovascular disease, obesity), health care-specific (e.g., time pressure, establishment of intravenous access), procedure-specific (e.g., volume and duration of infusion, quality of equipment) and substance-specific (e.g., chemical properties, time of exposure) (Larson, 1982; Boschi and Rostagno, 2012; Pérez Fidalgo et al., 2012; de Wit et al., 2013; Reynolds et al., 2014). Timely identification of these potential risk factors is crucial to successfully prevent or minimize the risk for extravasation injuries (Goolsby and Lombardo, 2006; Schulmeister, 2011).

Possible early symptoms of extravasation injuries can include pruritus, paraesthesia, burning pain, induration, edema, erythema, epidermolysis, or blistering. Late symptoms can be ulcerations or necrosis with chronic pain symptoms. As an example, the analysis of the incidence of clinical symptoms in a cohort of 545 patients that had been treated with chemotherapeutic drugs from January 1994 to December 2015, showed that 18% of patients with chemotherapy drug extravasation had clinical symptoms without visible lesions, 73% developed cutaneous superficial lesions, and 9% ulcerated lesions (Onesti et al., 2017). The clinical course of tissue damage induced by a cytostatic agent depends very strongly on the toxicity of the used substance. In the context of extravasation, chemotherapeutic agents are divided into three categories: vesicants, irritants and non-vesicants (Ener et al., 2004). Vesicant substances can have serious tissue-damaging effects, which can range from diffuse tissue damage to blistering, epidermolysis, ulceration, or necrosis. Vesicants can be divided into two groups: DNA binders and DNA non-binders. Adverse effects of DNA binders are usually more dangerous than those caused by DNA non-binders, as binding to the DNA allows these substances to persist over a prolonged time in the tissue. It is assumed, that DNA binders, after their release from necrotic cells might be endocytosed by healthy cells around the extravasation site. This way lesions become larger, deeper, and more painful, and the extravasation damage may become chronic (Ener et al., 2004). DNA non-binders are metabolized and neutralized quicker and this way they may cause only mild- to moderate lesions that heal in a significantly shorter time (Schulmeister, 2011; Onesti et al., 2017). Irritant substances may cause local pain, with or without inflammation and, e.g., through irritation of the venous vessels may cause vasospasm leading to a venous flow obstruction, which may raise the risk of extravasation due to the resulting increased hydrostatic pressure. Chemically-induced phlebitis is often associated with this mechanism (Onesti et al., 2017). Non-vesicants, on the contrary, cause no local or systemic damage (Ener et al., 2004).

Treatment options depend on the stage of extravasation and the type of the administrated substance. Intervention should be carried out as early as possible to prevent comorbidities and delays in primary therapy (Scuderi and Onesti, 1994; Shenaq et al., 1996). Treatment protocols proposed in the literature vary widely ranging from conservative to aggressive methods (Brown et al., 1979; Zenk et al., 1981; Siwy and Sadove, 1987; Falcone et al., 1989; Bertelli, 1995; Friedman, 1998; Harris et al., 2001; Wilkins and Emmerson, 2004). Unfortunately, data on antidotes that can be used to neutralize effects of a specific vesicant are available only in a limited number of cases.

In our study we aimed to investigate the damaging effects of the nanoliposomal form of the topoisomerase-inhibitor irinotecan (nal-Iri) and of the tetrahydroisoquinoline alkaloid trabectedin. Nal-Iri is used for treatment of refractory pancreatic cancer (Bien et al., 2016) and trabectedin is therapeutically utilized in patients with soft tissue sarcomas and ovarian tumours (Brodowicz, 2014; Ventriglia et al., 2018). While irinotecan has been classified as a non-vesicant (Jordan et al., 2005), trabectedin exhibited severe adverse effects in human oncological patients, as shown in case reports (Theman et al., 2009; Yoshimi et al., 2017). Yet, the adverse effects of extravasation of neither of these substances have been directly assessed in a preclinical mouse model. After characterising effects of these compounds, we aimed to study potential treatment options using hyaluronidase injection or topical treatment with DMSO or tacrolimus ointment (Bertelli et al., 1994; Bertelli, 1995; Ohtsuki et al., 2018) to prevent or minimize injuries induced by extravasation of these chemotherapeutics.

MATERIALS AND METHODS

Animals

BALB/c mice derived from Division of Laboratory Animal Science and Genetics (Himberg, Austria) were bred at the Medical University of Vienna under specific pathogen-free conditions. For the experiments, mice aged eight to 12 weeks were used. All experimental procedures of this study were carried out under the approval of the Animal Experimental Ethics Committee of the Medical University of Vienna and the Austrian Federal Ministry for Education, Science and Research (Permission Nr. BMBWF-66.009/0196-V/3b/2019).

Subcutaneous treatment of mice with trabectedin, nal-Iri and doxorubicin within a 2 day period

Trabectedin (Yondelis®) was obtained from Pharma Mar, S.A. Madrid, Spain, nal-Iri (Nal-Irinotecan, Onivyde®) from Les Laboratoires Servier Industrie, Gidy, France) and doxorubicin (Adriblastin®), used as a positive control, from Pfizer Corporation Austria Ges.m.b.H., Vienna. The quantities of the used substances were determined in a preliminary test in which

we intended to inject them subcutaneously in ascending order (150 µl > 200 µl > 250 µl > 300 µl) in order to determine which of these amounts would suffice to cause a visible effect. We applied these substances in concentrations used for treatment of human patients, as specified in result section. As a negative control, to eliminate any bias caused by mechanically triggered effects, we administered 0.9% NaCl.

After removing the hair in the vicinity of the scapula (clean shave), the above substances were subcutaneously injected with plastic syringes and 30-gauge needles. During the observation period, treated mice were kept in separate cages, and tramadol (10–20 mg/kg) was added to the drinking water to relieve the animals from pain.

In the pilot experiment, the observation period was 48 h. The health status of the animals was continuously assessed, both with regard to their general condition and with regard to local reactions. For documentation purposes, the animals were photographed daily under anaesthesia, i.e., at the time of treatment, 24 h after treatment and at the end of the experiment when they were sacrificed by cervical dislocation.

Investigation of Antidotes Against Extravasation Injuries of Trabectedin

Following the examination of adverse effects caused by nal-Iri or trabectedin within a 2 day period, we aimed to determine possible therapeutic interventions against trabectedin induced injuries. To this end, hyaluronidase (Hylase®, Dessau, Riemser Pharma GmbH, Berlin), 0.1% tacrolimus monohydrate ointment (Protopic®, Leo Pharma A/S, Ballerup, Denmark) and dimethyl sulfoxide (DMSO, Cat. Nr. 76855, Sigma Aldrich, Saint Louis, MO, United States), were tested as possible antidotes in an experiment lasting 5 days. To this end, animals were divided into positive control group (trabectedin), hyaluronidase group (hyaluronidase treatment after trabectedin injection), tacrolimus group (tacrolimus ointment treatment after trabectedin injection) and DMSO group (DMSO treatment after trabectedin administration).

Briefly, animals were injected with trabectedin as described in the previous section and in addition, hyaluronidase, tacrolimus or DMSO were administered subsequently. We injected overall 20 µl of hyaluronidase (150 IU/ml) subcutaneously, with a Hamilton syringe, around the injection site of trabectedin, immediately after the administration of trabectedin. Tacrolimus ointment and DMSO were applied topically at the site of trabectedin injection three times daily until the end of day five. The experimental animals were monitored/treated for 5 days, and the injection site was macroscopically inspected and photographed daily.

In Vivo Assessment

Assessment schemes for the severity of the local and systemic reactions were derived from the grading systems of the "Common Terminology Criteria for Adverse Events" (National Cancer Institute, N. I. o. H., U.S. Department of Health and Human Services, 2010), the "Infusion Nurse Society" (Infusion Nursing Standards of Practice, 2006) and based works of Amjad et al. (2011) and Alexander (2020). Skin damage was graded using the following scheme: 0° = no damage, 1° = skin pallor, 2° = erythema, 3° = ulcer/necrosis (assigned when the area of ulceration/necrosis comprised at least 15% of the total afflicted area). The area of the affected skin was measured.

Processing of Skin Samples and Staining

After cervical dislocation, skin samples around the injection sites were cut out and histological sections were prepared from areas of the greatest macroscopically visible effects. Briefly, samples were kept in 4% paraformaldehyde (PFA) solution at 4°C overnight and transferred into 0.1% PFA solution the next day. They were embedded in paraffin using the TBS88 Paraffin Embedding System (Medite Medical GmbH, Burgdorf, Germany) and after storing at +4°C used for preparing series of sections with thickness of 5 µM using the Leica microtome (Wetzlar, Germany).

Before hematoxylin/eosin (H&E) staining, sections were deparaffinized with xylene for 1 hour. The Continuous Linear Stainer COT20 (Medite) was then used for the H&E staining. Briefly, a sequence of following steps, each taking two and a half minutes, was executed: Histolab-Clear, ethanol (100%–70%), H_2O distilled, hematoxylin (hematoxylin acidic according to MAYER), flowing water (×5), eosin, running water, ethanol (70%–100%), Histolab-Clear and xylene exposure.

Microscopy

The Olympus BX61VS slide scanner (Olympus, Tokyo, Japan) was used to image the stained histological H&E sections using ×10 objective lens and analyzed using the Olympus OlyVIA Ver.2.9.1 software.

Statistics

IBM SPSS Statistics 27.0 and GraphPad Prism 5 software were used for statistical analysis. Nominal data were analyzed using $\chi 2$ test, ordinal data by Kruskal-Wallis analysis of variance and metric data by simple analysis of variance (ANOVA, ANalysis Of VAriance) and subsequent post-hoc tests with a Tukey-B correction. A bilateral significance level of 5% and a statistical power of 90% were applied. Graphs were created using GraphPad Prism 5 software.

RESULTS

Extravasated Trabectedin but not Nanoliposomal Irinotecan Caused Local Adverse Effects Within a 2-Day Treatment

To assess the extent of skin damage caused by the tested chemotherapeutics nal-Iri and trabectedin and by the positive control substance doxorubicin, we used four mice in each group. We observed these mice several times daily and recorded images of the injected skin area at 24 and 48 h when the animals were sacrificed and the skin around the injection site was excised and used for histological assessment.

Originally, we intended to inject increasing amounts of these compounds by applying 150 µl, 200 µl, 250 µl and 300 µl in ascending order. However, subcutaneous injection of doxorubicin (applied at 2 mg/ml) and of trabectedin (applied

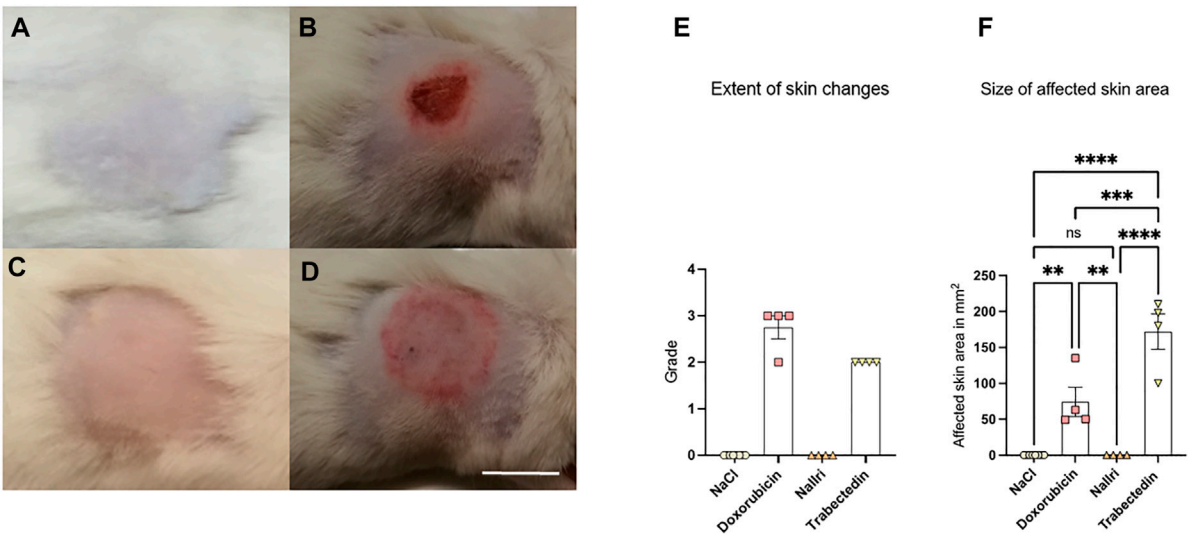

FIGURE 1 | Representative images of treated area of mice at day two after subcutaneous injection of **(A)** 150 µl or 300 µl NaCl (grade 0), **(B)** 150 µl doxorubicin (grade 3), **(C)** 300 µl nal-Iri (grade 0), **(D)** 150 µl trabectedin (grade 2). **(E)** Grading of skin changes where grade 0 was assigned to all mice treated with NaCl or nal-Iri. **(F)** Size of affected skin area with at least grade 2 statistically analyzed using ANOVA followed by a post-hoc test with a Tukey-B correction. $^{**}p < 0.01$, $^{***}p < 0.005$, $^{****}p < 0.0001$, ns = not significant. Scale bar = 1 cm.

at 0.05 mg/ml) induced already at the lowest volume of 150 µl substantial skin changes (**Figures 1B,D**). In case of nal-Iri (applied at 4.3 mg/ml concentration, **Figure 1C**), and 0.9% NaCl (used as a negative control where we injected to four mice in each group either 150 µl or 300 µl, respectively, **Figure 1A**), neither significant changes in the skin color nor presence of ulceration/edemas in the skin of such treated animals were found even at the highest applied volume of 300 µl. Therefore, in case of all mice treated with nal-Iri or NaCl, their lesions were classified with grade 0 (**Figure 1E**). The average area of the skin discoloration in both these experimental groups was assigned to 0 ± 0 mm^2 (**Figure 1F**).

In case of doxorubicin treatment, one mouse developed erythema without ulceration (classified as grade 2) and three mice developed skin ulceration (classified as grade 3, **Figure 1E**). In case of trabectedin, all four treated animals developed erythema and edema (**Figure 1E**). The average area of the skin discoloration in doxorubicin group was 74 ± 41 mm^2 and in trabectedin group 172 ± 50 mm^2 (**Figure 1F**).

Treatment of mice with any of the above substances (doxorubicin, trabectedin or nal-Iri) did not cause an impairment in the general health- or nutritional status or their appearance. Furthermore, no obvious changes in digestion or abnormal behavior were observed in any of treated animals.

We further assessed morphological changes in the skin around the injection site by H&E staining. For detailed assessment of sections, we took into consideration different factors including dermal remodeling with granulation, loss of epidermal continuity, thickening of epidermis, presence of loosened connective tissue and the size of the edema (maximal thickness observed in tissue sections). The most typical phenotypic skin changes from each group of animals are presented on **Figures 2A–D**. The analysis of histological sections revealed that three of four of doxorubicin-treated mice and one of four of the trabectedin-treated mice showed dermal remodeling with granulation and a loss of epidermal continuity (**Figures 2B,D**, respectively), while the mice of the nal-Iri and NaCl groups did not show such changes (**Figures 2A,C**, respectively). Furthermore, three of four of doxorubicin-treated mice and two of four of trabectedin-treated mice (**Figures 2B,D**, respectively), but none of the mice in the NaCl- or nal-Iri group (**Figures 2A,C**, respectively) showed a segmentally thickened epidermal layer.

We found that the thickness of the afflicted tissue possibly reflecting the degree of edema formation was 1000 ± 327 µM in doxorubicin group, 663 ± 170 µM in trabectedin group, 138 ± 75 µM in nal-Iri group and 88 ± 25 µM in NaCl control group (**Figure 2E**). Statistical analysis using ANOVA followed by a post-hoc test with a Tukey-B correction revealed no significant difference between the NaCl groups and the nal-Iri group, but a significant difference between the two mentioned groups and the trabectedin and doxorubicin groups (**Figure 2E**).

Altogether, the data obtained within this 2-day experiment showed the non-vesicant properties of nal-Iri and revealed significant vesicant properties of trabectedin. Furthermore, these experiments confirmed the widely-known vesicant-properties of doxorubicin (Barlock et al., 1979; Kassner, 2000).

DMSO and Tacrolimus Reduced Adverse Effects of Trabectedin Extravasation

Following the investigation of the adverse effects caused by nal-Iri and trabectedin, we aimed to determine possible therapeutic interventions that might lead to minimizing adverse effects of

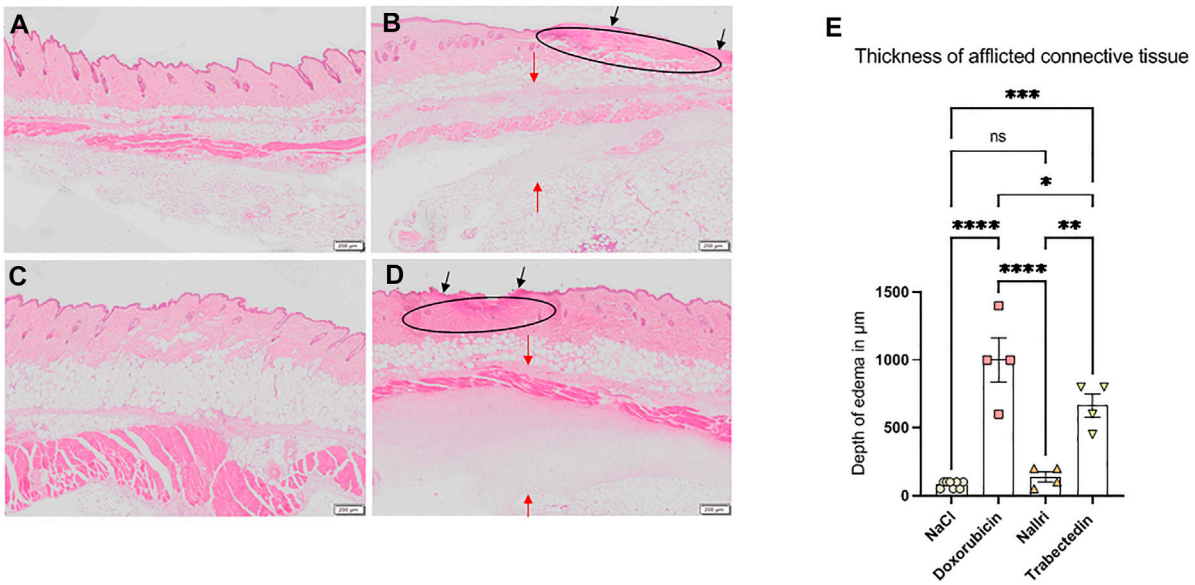

FIGURE 2 | Images of H&E stained sections of affected skin area at day two of treatment with **(A)** 300 µl NaCl, **(B)** 150 µl doxorubicin, **(C)** 300 µl nal-Iri, **(D)** 150 µl trabectedin. Histological sections in B and D show the loss of epidermal continuity (black arrows), replacement of the dermis with granulation tissue (ellipse) more pronounced in B than in **(D)**. Due to edematous changes, the loosening of the connective tissue occurs (red arrows in B and D). **(E)** Thickening of afflicted connective tissue layer statistically analyzed using ANOVA followed by a post-hoc test with a Tukey-B correction. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$, ns = not significant.

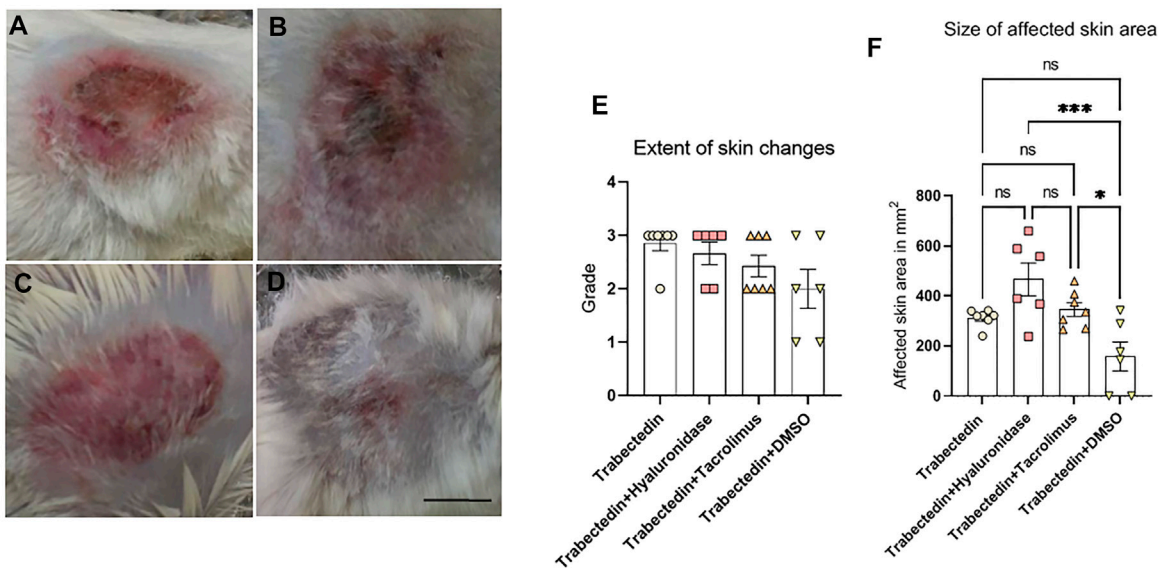

FIGURE 3 | Representative images of treated area in mice at day five after treatment with **(A)** trabectedin (grade 3), **(B)** trabectedin and hyaluronidase (grade 3), **(C)** trabectedin and tacrolimus (grade 2), **(D)** trabectedin and DMSO (grade 2). **(E)** Extent of skin changes assessed by grading. **(F)** Size of affected skin area in lesions graded at least as grade 2, statistically analyzed using ANOVA followed by a post-hoc test with a Tukey-B correction *$p < 0.05$, ***$p < 0.005$, ns = not significant. Scale bar is 1 cm.

trabectedin extravasation. To this end, we injected trabectedin subcutaneously and such treatment was followed by subcutaneous injection of hyaluronidase around the injection site of trabectedin or by topical application - three times daily around the injection site - of tacrolimus ointment or DMSO. Trabectedin group was comprised of seven mice, hyaluronidase

group of six mice, tacrolimus group of seven mice and DMSO group of six mice. We followed the mice daily for a period of 5 days.

Among these animals, two of six mice treated with DMSO started to show signs of lethargy on day five, otherwise no impairment of activity or any changes in the nutritional status or digestion were found. Representative figures of the skin area around the injection site observed at day five of mice injected with 150 µl of 0.05 mg/ml trabectedin only, or with additional subsequent treatment with hyaluronidase, tacrolimus ointment or DMSO are shown on **Figures 3A–D**.

The assessment of the macroscopically discernable skin changes at day five revealed that six of seven mice of trabectedin group developed ulceration and necrosis (grade 3) while one mouse exposed to trabectedin developed only erythema and edema classified as grade 2 (**Figure 3E**). In the hyaluronidase group, grade 3 was observed in five of six experimental mice and the affected skin area of one mouse was characterized as grade 2 (this difference in the extent of macroscopically observed pathological skin changes compared to the positive control as analyzed by Kruskal-Wallis analysis of variance, was not statistically significant, $p = 0.435$). The pathological skin changes in mice treated with trabectedin and tacrolimus were less pronounced compared to the control trabectedin group ($p = 0.037$), as only three of seven mice developed grading three. Significant improvement in comparison to the control trabectedin group was also seen in the trabectedin/DMSO group ($p = 0.017$), as only two of six mice exhibited signs of ulcerations at day five (**Figure 3E**).

Analysis of the size differences of affected areas (**Figure 3F**) revealed that macroscopically visible damages were significantly more spread out in the hyaluronidase group ($467 \pm 160\ mm^2$) than in the trabectedin control group ($313 \pm 35\ mm^2$). In case of mice exposed to trabectedin and treated for 5 days with tacrolimus, the affected area ($346 \pm 73\ mm^2$) did not significantly differ from the control group. In contrast, a 5-day treatment with DMSO resulted in a smaller affected area ($159 \pm 143\ mm^2$), yet this difference was not statistically significant. Altogether, these findings on the size of the affected area concur with the above presented macroscopic classification of pathological skin changes in the control and tested experimental groups.

Next we evaluated obtained histological skin sections and found that the microscopic appearance corresponded to macroscopic findings presented above. Representative images of the H&E stained histological sections of the skin areas around the injection sites are shown in **Figures 4A–D**.

In the positive control group (trabectedin), sections of all seven mice showed dermal remodeling/granulation as well as loosened connective tissue and loss of epidermal continuity. Thickening of the epidermis was found in samples of five mice of this group. In the hyaluronidase group consisting of six mice, sections of five mice exhibited dermal remodeling/granulation and loosened connective tissue while all six mice had thickened epidermis. Overall, the differences in these parameters in the hyaluronidase group in comparison to the positive control group, as determined using $\chi 2$ test, were not statistically significant. In the tacrolimus group, no dermal remodeling/granulation was found in any of the analyzed samples ($p < 0.001$) and no loss of epidermal continuity was observable in samples from five of seven of these mice ($p = 0.005$). A better outcome, as assessed by the above parameters, was observed also in the DMSO group where only two of six mice showed dermal remodeling/granulation and thickening of epidermis ($p = 0.009$). In contrast, loosened connective tissue and loss of epidermal continuity was found in four of six mice from this group ($p > 0.05$).

We also quantified edema formation by assessing the thickness of the affected tissue layer. In the positive control group edema thickness was 817 ± 349 µM, in the hyaluronidase group 633 ± 281 µM, in the tacrolimus group 650 ± 152 µM and finally, 517 ± 343 µM in the DMSO group. Due to insufficient integrity of the edema region in the respective paraffin tissue samples, this evaluation did not include the assessment of one mouse of the trabectedin group and one mouse of the tacrolimus group. Overall, the differences between the test groups and the positive control trabectedin group were not statistically significant.

DISCUSSION

A clinical investigation of the effects of the extravasation of chemotherapeutic agents in the context of a prospective clinical study is often associated with many hurdles and obstacles and might not be easily ethically justifiable. To avoid this problem, in our study of local and systemic effects of extravasation of the oncological therapeutics nanoliposomal irinotecan (nal-Iri) and trabectedin, we used a preclinical mouse model. To the best of our knowledge, neither the effects of nal-Iri nor of trabectedin extravasation have been systematically tested in a preclinical mouse model.

Briefly, subcutaneous injection of nal-Iri did not cause any systemic reaction in the observed period, that is, no pathological changes in the general condition, nutritional condition or in behaviour were observed. Furthermore, such injection did not result in any harmful effects on the skin, as the appearance of the treated skin areas of these mice in the nal-Iri group was indistinguishable from that of the mice in the NaCl groups. Overall, neither macroscopic nor histological differences regarding the local reaction between the two groups could be detected. In summary, these results agree with the previous literature, according to which damage induced by nal-Iri was, due to the non-vesicant nature of irinotecan (Jordan et al., 2005), not to be expected, and since no adverse effects of extravasation of its nanoliposomal form administrated to metastatic pancreatic cancer patients were reported (Wang-Gillam et al., 2016).

After demonstrating vesicant effects of extravasated trabectedin in a preliminary experiment lasting for 2 days, we aimed to investigate possible treatment options. We treated mice that have been challenged with trabectedin injection with either hyaluronidase, tacrolimus or DMSO within a 5 day period.

Hyaluronidase, an enzyme that temporarily disintegrates tissue due to the degradation of hyaluronic acid, has been in

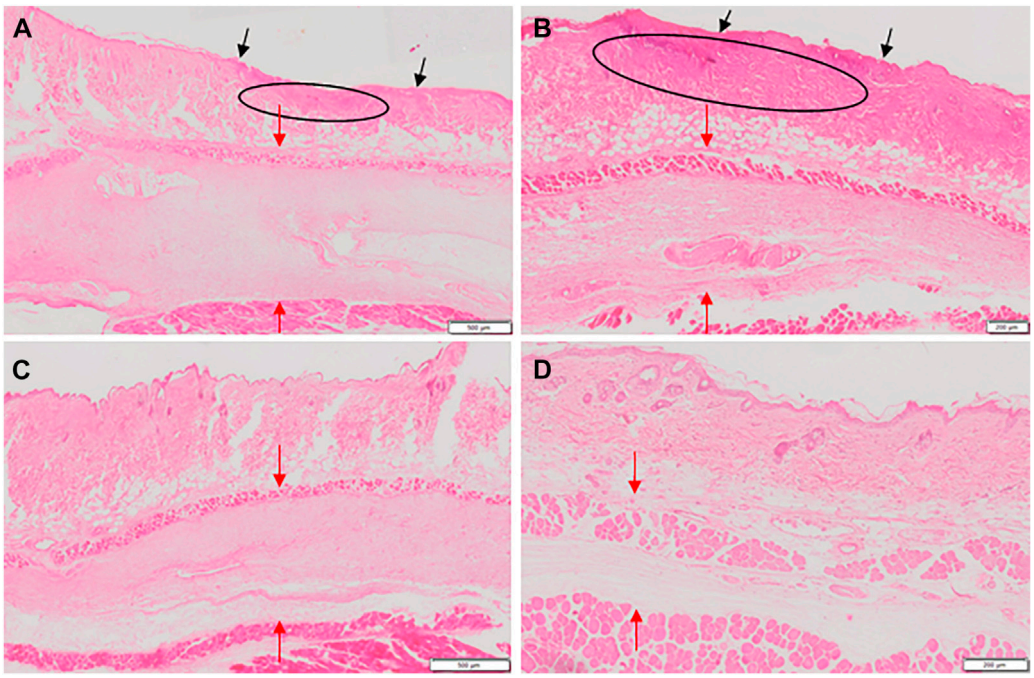

FIGURE 4 | Images of H&E stained sections of affected skin area at day five of treatment with **(A)** trabectedin, **(B)** trabectedin and hyaluronidase, **(C)** trabectedin and tacrolimus, **(D)** trabectedin and DMSO. Histological sections in A and B show a loss of epidermal continuity (black arrows in A and B) and a replacement of the physiologic structure of the dermis by the granulation tissue (ellipse), more pronounced in B than in A. Due to the edematous changes, the connective tissue was severely loosened (red arrows) in A, B, C and D.

earlier studies suggested as antidote for treating of nafcillin extravasation (Zenk et al., 1981) and later on for treating of plant vinca alkaloids extravasation (Bertelli et al., 1994; Jordan et al., 2005; Goolsby and Lombardo, 2006; Reynolds et al., 2014). Yet, in our study, the use of hyaluronidase did not result in successful prevention or minimization of extravasation injuries. To the contrary, its use tended to strengthen the adverse effects of extravasated trabectedin. The area of macroscopically visible changes reaching at least grade 2 at day five of treatment, in fact, was the largest among all four experimental groups. Histological parameters were comparable to the control trabectedin group.

Tacrolimus is a substance with anti-inflammatory- and immunosuppressive properties used for treatment of patients after organ transplantation (Plosker and Foster, 2000; Bush and Lin, 2006) and applied topically as an ointment for treatment of atopic dermatitis patients (Ohtsuki et al., 2018). In our study, topical application of tacrolimus ointment attenuated the extent of macroscopic skin damage, as revealed by grading of the observed changes at day 5 of the treatment, as only three of seven mice developed grade 3. Although the affected area of macroscopically visible changes in mice exposed for 5 days to tacrolimus did not significantly differ from the control trabectedin group, histological findings revealed that the use of tacrolimus decreased dermal remodeling/tissue granulation and reduced the loss of epidermal continuity in comparison to the positive control group.

Topical application of dimethylsulfoxide (DMSO) has been recommended for treating of anthracyclines-, mitomycin C- or cis-platin extravasation induced tissue necrosis in clinical studies, probably due to its effect as a radical scavenger, anti-inflammatory and vasodilatory activity (Bertelli, 1995; Rosenstein, 1999; Jordan et al., 2005; de Wit et al., 2013). The above mentioned drugs, similarly as trabectedin, possess DNA-binding properties and therefore persist longer in the tissue, promoting tissue damage and necrosis (Zewail-Foote and Hurley, 1999; Ener et al., 2004; de Wit et al., 2013). Mechanistically, DMSO penetrating deeply into the tissue might foster dilution of these substances (Olver et al., 1988; Bertelli, 1995) and consequently counteract these adverse effects. Use of DMSO in our study diminished formation of ulceration/necrosis around the injection site as only two of six mice exhibited signs of ulcerations at day five, two mice presented grade 2 and two mice developed grade 1. In addition, suppression of dermal remodeling/granulation was seen in mice of the DMSO-treated group. However, two of the six mice presented signs of apathy on day five of the treatment and had to be sacrificed shortly thereafter. Although we did not investigate the cause of a sudden worsening of their health status, we think that it might be linked to a release of the trabectedin into systemic circulation.

In summary, the local effects observed in our study suggest that between the two tested substances nal-Iri and trabectedin, only trabectedin has the damage potential of a vesicant. Altogether, obtained data does not support the use of hyaluronidase in the

treatment of trabectedin extravasation injuries. Although more thorough investigation of adverse effects of trabecetedin extravasation is needed for better comprehension of its damaging impact, the presented data suggest that tacrolimus but mainly DMSO might be suitable antidotes for extravasation injuries caused by trabectedin.

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 animal study was reviewed and approved by the Animal Experimental Ethics Committee of the Medical University of Vienna and the Austrian Federal Ministry for Education, Science and Research (Permission Nr. BMBWF-66.009/0196-V/3b/2019).

AUTHOR CONTRIBUTIONS

Conception and design: MU, PU, and JB. Acquisition of data: VJ and OK. Statistical analysis: OK and VJ. Analysis and interpretation of data: MU, PU, JB, VJ, and OK. Drafting the article: MU, OK, VJ, JB, and PU. Reviewed submitted version of manuscript: all authors. Administrative/technical/material/intellectual support: MU, CS, CT, M-BA, and GS.

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DNA damage response and repair genes in advanced bone and soft tissue sarcomas: An 8-gene signature as a candidate predictive biomarker of response to trabectedin and olaparib combination

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Background: Advanced and unresectable bone and soft tissue sarcomas (BSTS) still represent an unmet medical need. We demonstrated that the alkylating agent trabectedin and the PARP1-inhibitor olaparib display antitumor activity in BSTS preclinical models. Moreover, in a phase Ib clinical trial (NCT02398058), feasibility, tolerability and encouraging results have been observed and the treatment combination is currently under study in a phase II trial (NCT03838744).

Methods: Differential expression of genes involved in DNA Damage Response and Repair was evaluated by Nanostring® technology, extracting RNA from pre-treatment tumor samples of 16 responder (≥6-month progression free survival) and 16 non-responder patients. Data validation was performed by quantitative real-time PCR, RNA *in situ* hybridization, and immunohistochemistry. The correlation between the identified candidate genes and both progression-free survival and overall survival was investigated in the publicly available dataset "Sarcoma (TCGA, The Cancer Genome Atlas)".

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Results: Differential RNA expression analysis revealed an 8-gene signature (CDKN2A, PIK3R1, SLFN11, ATM, APEX2, BLM, XRCC2, MAD2L2) defining patients with better outcome upon trabectedin+olaparib treatment. In responder *vs.* non-responder patients, a significant differential expression of these genes was further confirmed by RNA *in situ* hybridization and by qRT-PCR and immunohistochemistry in selected experiments. Correlation between survival outcomes and genetic alterations in the identified genes was shown in the TCGA sarcoma dataset.

Conclusions: This work identified an 8-gene expression signature to improve prediction of response to trabectedin+olaparib combination in BSTS. The predictive role of these potential biomarkers warrants further investigation.

KEYWORDS

bone and soft tissue sarcomas, predictive biomarkers, DNA damage response and repair genes, trabectedin, olaparib

Introduction

Bone and soft tissue sarcomas (BSTS) are a wide and heterogeneous family of rare tumors sharing features of mesenchymal origin (1). In advanced stages, when the disease is unresectable or metastatic, prognosis is dismal. Medical treatment may delay progression, with marginal improvement in overall survival (OS) (2–5). This scenario is further complicated by the relative poorness of predictive factors to improve sarcoma patient selection for patient-tailored treatments, with the noteworthy exception of gastrointestinal stromal tumors (6). Hence, in the sarcoma field, there is a huge need to explore innovative therapies, focusing on combinations of cytotoxic compounds, target therapies and immunotherapeutic strategies, to optimize treatment personalization and increase tumor control in terms of mass shrinkage or - at least - sarcoma growth arrest. In recent years, several combinations have been tested (7–10) but, once again, these studies demonstrated promising results only in small subsets of patients. Hence, there is an urgent need to identify predictive biomarkers of tumor response to refine patient selection, according to the concept of precision medicine (11). In this perspective, we focused on the combination of trabectedin, an isoquinoline alkylating agent of marine origin, and the inhibitor of the enzyme poly-(ADP-ribose) polymerase 1 (PARP1) olaparib. Preclinical and clinical data confirmed feasibility and suggested hints of activity in a fraction of the enrolled patients, emphasizing the need to improve patient selection through the identification of specific predictive factors (12, 13). Both drugs under study – trabectedin and olaparib – had already been studied as single agents, looking for predictive factors among the tightly intertwined mechanisms ruled by DNA Damage Response and Repair (DDRR) genes (14–24). However, potential predictive factors of combined trabectedin +olaparib treatment response have not been investigated, so far.

Trabectedin creates DNA adducts by interfering with active transcription, wherein its activity is dependent on transcription-coupled nucleotide excision repair (TC-NER) (25–27). NER defects make tumor cells less sensitive to trabectedin damage and high expression levels of ERCC1 and XPG/ERCC5 ("signs" of a proficient NER machinery) have been described as predictive of better response to trabectedin treatment (14–16, 18). Tumor cells bearing homologous repair deficiency (HRD) are more sensitive to trabectedin-induced cell death, as they cannot recruit the proper machinery to repair the double-strand breaks (DSBs) generated upon trabectedin treatment. Non-homologous end joining (NHEJ) defects, instead, seem to have only a minor effect on trabectedin efficacy (14–16, 28). At present, none of these potential predictive factors of response upon trabectedin treatment has received approval for clinical use.

Differently, PARP1-inhibitors (PARP1i) have been marketed with specific indications with respect to homologous recombination (HR) status, which is considered a predictive biomarker of response to PARP1i (24, 29, 30). The European Medicine Agency has approved olaparib use in ovarian cancer with HRD, while for pancreatic, prostate and breast cancers the indication more strictly refers to *BRCA1/2*-mutated patients. Hence, *BRCA* mutational status and HRD are clinical-grade approved predictive biomarkers, to better select patients who might benefit more from PARP1i treatment. Indeed, in cells showing HRD, NHEJ takes action upon DSBs formation, but with respect to HR, considered "error-free", NHEJ provides DSBs repair with practically no consideration for sequence

homology. The error-prone NHEJ promotes accumulation of DNA damage, and its activity is a major driver for PARP1i synthetic lethality in HR-defective cells (31).

The different mechanisms of action of trabectedin and olaparib with respect to DDRR genes imply that finding predictive factors of response to their combined treatment cannot be assumed to be a simple summation. The objective of the present translational, exploratory study, was to look for a potential predictive gene expression signature of response to trabectedin and olaparib in sarcoma patients, taking advantage of tumor specimens derived from patients treated with this combination in the phase Ib TOMAS trial (13).

Materials and methods

Patient-derived samples

Patient samples were all derived from the Phase Ib TOMAS study patient cohort. Only patients treated at or above the third dose level were selected (trabectedin 0.920 mg/m^2 q21d, olaparib 200 mg BID). The available material included pre-treatment biopsies or surgical specimens (formalin-fixed, paraffin-embedded; FFPE). All enrolled patients gave written, signed informed consent for the use of tumor samples for biomarker and exploratory analyses. The clinical study protocol was approved by the Institutional Review Board (IRB) and Ethics Committee of each participating center. All study procedures were performed in accordance to the Declaration of Helsinki.

DNA and RNA extraction; DNA data analysis

DNA was extracted from patients' specimens as previously described (13). DNA purity was checked by NanoDrop™ (Thermo Fisher Scientific, Life Technology, Monza, Italy). DNA concentration was determined by the Qubit dsDNA BR (broad range) and HS (high sensitivity) assay kits (Thermo Fisher Scientific) and the Qubit® 3.0 Fluorometer (Thermo Fisher Scientific). DNA fragmentation was assessed by gel electrophoresis and by 2100 Bioanalyzer Instrument, with High Sensitivity DNA assay Kit (Agilent Technologies, Agilent Technologies, Inc., Santa Clara, California).

Good quality DNA samples underwent whole exome sequencing (WES) using the Twist Bioscience® Human Core Exome (Consensus CDS) + IntegraGen content, for a genomic target of 37 Mb by IntegraGen SA (IntegraGen SA, Evry, France), and Novaseq 6000 sequencer (Illumina) with an average sequencing depth of 135X depth per exome and a coverage >98% at >50X. Genetic discovery analysis was performed by an in-house NGS pipeline (32, 33), constructed for WES analyses of paired cancer genomes in order to call somatic variations, indels and copy number alterations (CNA).

By means of publicly available databases (ClinVar) and PolyPhen-2 prediction tool (34), all benign variants were filtered out from the genetic analysis. Mutations were first looked into in the ClinVar database; if pathogenic, no further analysis was performed. If one mutation was found as being of unknown significance in the ClinVar database, or not described at all, PolyPhen-2 prediction tool was used to inquire its potential detrimental effect on protein function. Lower-quality DNA samples were analyzed with Oncomine-Comprehensive cancer panel v3 (Thermo Fisher Scientific) and meaningful alterations were filtered in with Ion ReporterTM 5.18.2.0 (Thermo Fisher Scientific).

RNA was extracted from FFPE samples with Maxwell® RSC FFPE Kit (Promega Corporation, Madison, WI, USA) and Maxwell® RSC Instrument. RNA purity, concentration and fragmentation were determined using DeNovix DS-11+ Spectrophotometer (DeNovix Inc., Wilmington, DE, USA), Qubit®3.0 Fluorometer (Invitrogen by Life Technologies, Eugene, Oregon, USA) and the Agilent 2100 Bioanalyzer System (Agilent Technologies, Wilmington, DE, USA), respectively.

Nanostring® nCounter assay

Expression of DDRR genes in tumor samples, was determined by NanoString® nCounter Technology (NanoString Technologies, Seattle, WA, USA) by means of the nCounter®Vantage 3D™ RNA DNA Damage and Response Panel. Following manufacturer's instructions, samples were prepared for hybridization, processed in the Prep Station, counted by the nCounter® Analysis System.

Quantitative real-time polymerase chain reaction and droplet digital absolute qPCR

For Real-time PCR analysis, 1 µg of total RNA was reverse-transcribed into cDNA using SuperScript IV VILO Master Mix (Thermo Fisher Scientific). TaqMan PCR analysis was performed with TaqMan Gene Expression Master Mix by means of ABI PRISM 7900HT System (Applied Biosystems, Monza, Italy). Taqman probes (Thermo Fisher Scientific) were as following: CDKN2A (Hs00923894_m1), APEX2 (Hs00205565_M1). Fluorescence data were automatically converted into Ct (cycle threshold) values. Data export (threshold 0.20) and analysis was performed by Microsoft Office Excel. Expression data were normalized to the geometric mean of housekeeping genes. For housekeeping genes, the Taqman probes (Thermo Fisher Scientific) were as

following: B2M (Hs00984230_m1), UBC (Hs00824723_m1), GAPDH (Hs99999905_m1), ACTB (Hs99999903_m1).

The search for *CDKN2A* gene copy number was carried out by droplet digital PCR (ddPCR) as follows: DNA isolated from FFPE tumor tissues (as described above) was amplified using ddPCR Supermix for Probes (Bio-Rad, Hercules, CA, USA), using *CDKN2A* and housekeeping genes (*EIF2C1*, *AP3B1*, *RPP30*) probes (Bio-Rad, Segrate, Italy), according to manufacturer's protocols as described in (35).

In situ hybridization

The RNAscope® Assay was used for *in situ* hybridization on FFPE tissue following standard protocol procedures (36). Specific preparation and pre-treatment included target retrieval lasting 10-15 minutes and Protease Plus incubation for 30 minutes.

Immunohistochemistry

CDKN2A/p16 immunohistochemistry (IHC), on FFPE tumor tissues, was performed with a BOND-MAX automated staining platform (Leica Biosystems, Buccinasco, Italy), according to standard procedure. The specimens were sectioned at a thickness of 3 µm and stained on glass slides baked at 60°C for 30 minutes. Deparaffinization, rehydration and antigen retrieval were performed by Bond Dewax Solution, Bond Wash Solution, ethanol and Bond ER Solution 1 (prediluted; pH 6.0) antigen retrieval solution (Leica), performed on the BOND-MAX automated slide stainer (Leica) for 30 minutes at 95°C. The ready-to-use primary CDKN2A/p16 primary anti-human antibody (6H12; Leica), was incubated for 20 minutes at room temperature, followed by visualization with the Bond Polymer Refine Red Kit (Leica). The specimens were counter-stained with hematoxylin. Slide fixation was performed with mounting medium and observation under optical microscope (Leica DM750) equipped with Leica ICC50W camera (Leica).

Statistical analyses

The nSolver software v3.0 was used to normalize the number of transcript copies with the geometric mean of 12 housekeeping genes. Log2-fold changes in gene expression were calculated comparing gene expression of samples from non-responder with responder patients. A patient was defined as responder in presence of a progression-free survival (PFS) ≥6 months. Differential expression analysis was performed using nSolver Advanced Analysis software (version 4.0, NanoString Technologies, Seattle, Washington, US), using the Differential Expression module (DE) in default settings. A gene was defined differentially expressed in a significant way, if associated with a p-value <0.05. Volcano plot was generated using the EnhancedVolcano R package v1.8. Volcano plot displays each gene's -log10(p-value) and log2 fold change with the selected covariate. Highly statistically significant genes fall at the top of the plot above the horizontal lines, and highly differentially expressed genes fall to either side. Horizontal lines indicate various False Discovery Rate (FDR) thresholds or p-value thresholds if there is no adjustment to the p-values. Genes are colored if the resulting p-value is below the given FDR or p-value threshold.

Concerning the analysis of RNA ISH and IHC data, to compare expression levels between the two patient groups, an expert pathologist blinded to the treatment groups evaluated the staining intensity and the percentage of positive cells. Score 1: <25% positive cells, mild intensity staining; score 2: 25%< positive cells<50%, mild intensity staining; score 3: 50% <positive cells<75% strong intensity staining; score 4: positive cells >75%, strong intensity staining. Chi square Test was applied to calculate p value for ISH analysis: Wilcoxon rank-sum test was performed to compare the percentage of p16 positive IHC expression in responder *vs.* non-responder patients and calculate p-value.

Bioinformatic analyses

We analyzed the genomic data that had been generated for the TOMAS study, to match the RNA expression data, looking for any genetic alteration that could affect DDRR gene function. We broadened our analysis to all known DDRR and related genes and looked both into point mutations and copy number variation alterations.

Analysis of genomic and transcriptomic data of 255 primary sarcoma samples from The Cancer Genome Atlas (TCGA) was performed using CBioPortal v3.6.17 (37), considering the dataset named "Sarcoma (TCGA, PanCancer Atlas)". 249 soft tissue sarcoma samples were considered for survival analysis, excluding samples lacking complete genomic and expression data, and desmoid tumor samples. A gene was considered altered in a tumor sample if associated with a somatic mutation, a gene copy number alteration, or associated with an expression level higher/lower than two standard deviations (|z-score| > 2) with respect to the mean expression measured in diploid samples. CBioPortal was used also to visualize data of the TCGA sarcoma cohort, for retrieving patient clinical information, gene expression and CNV data. Survival analysis was performed with *survival* v3.2.13 and *survminer* v0.4.9 R packages.

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Results

Patients' demographics and analysis of DDRR gene mutations

The characteristics of patients eligible for the analyses are described in Table 1. 32 patients were included in the analysis. Bone sarcomas were a small fraction of the cohort, with only two cases of Ewing's sarcoma (6%) and one osteoblastic osteosarcoma (3%). Concerning STS, the most prevalent histology was leiomyosarcoma (LMS; n=11), followed by synovial sarcoma (SS; n=5), liposarcomas (LPS; n=5), malignant peripheral nerve sheath tumors (MPNST; n=2) and undifferentiated pleomorphic sarcomas (UPS; n=2). The four remaining histotypes (grouped under the term "other") included one malignant phyllodes tumor of the breast (MPT), one malignant myoepithelioma of the upper limb, one pleural solitary fibrous tumor and one myxofibrosarcoma of the limb.

TABLE 1 Patients' demographics and tumor characteristics.

Gender	**N (%)**
Male	16 (50)
Female	16 (50)
Age at protocol start	
Median age, years (range)	61 (21-80)
Histotype	**N (%)**
Ewing's sarcoma (ES)	2 (6)
Osteoblastic osteosarcoma (OS)	1 (3)
Leiomyosarcoma (LMS)	11 (34)
Synovial sarcoma (SS)	5 (16)
Liposarcoma (LPS)	5 (16)
Dedifferentiated Liposarcoma (DDLPS)	3
Myxoid liposarcoma (MLPS)	1
Pleomorphic Liposarcoma (PLPS)	1
Malignant peripheral nerve sheath tumor (MPNST)	2(6)
Undifferentiated pleomorphic sarcoma (UPS)	2 (6)
Other	4(13)
Anatomic location of primary tumor	**N (%)**
limb	18 (56)
uterus	7 (22)
retroperitoneum	4 (13)
pleural	1 (3)
breast	1 (3)
spine	1 (3)
Grade	**N (%)**
G2	3 (9)
G3	29 (91)
Disease stage at protocol start	**N (%)**
Locally advanced inoperable	3 (9)
Metastatic	29 (91)
Metastases – anatomic location	
Lung	29 (100)
Liver	9 (31)
Bone	12 (41)
Lymph nodes	3 (10)
Soft tissues	3 (10)

Among both responder and non-responder patients, we filtered out all benign or uncertain variants and found a few damaging, pathogenic mutations in DDRR genes. Considering the responder patient cohort (Table 2), patient 10 (TOMAS-10), affected by metastatic uterine LMS, had one *TP53* (pathogenic; ClinVar) and one (probably damaging; PolyPhen-2) *ERCC2* mutation. Other two *TP53* variants were detected in two LMS patients (one uterine and one retroperitoneal LMS); S215N being likely pathogenic/of unknown significance, and I195T being pathogenic, as described in ClinVar for both missense mutations. One "probably damaging" *ERCC6* mutation (as predicted by PolyPhen2 tool, being of unknown significance in the ClinVar database) was present in the tumor sample of one uterine LMS patient, and one MLPS patient's tumor harbored both one pathogenic (ClinVar) *PTEN* mutation, and one *PIK3CA* mutation (possibly damaging, according to PolyPhen-2). One probably damaging (PolyPhen-2) *PIK3CA* mutation was observed also in TOMAS-39 patient, affected by malignant phyllodes tumor (MPT).

Among non-responder patients (Table 3), *TP53* mutations were found in two non-responder patients affected by metastatic uterine LMS (C242S and Y205D), both of uncertain pathogenicity according to ClinVar, but probably damaging according to PolyPhen-2. One mutation predicted as "damaging" on BRCA1 protein function (by Polyphen2; of uncertain significance according to ClinVar) was detected in patient TOMAS-29 (metastatic synovial sarcoma of the lower limb). Another patient affected by metastatic synovial sarcoma had a *RAD51C* missense mutation (pathogenic/likely pathogenic in ClinVar). Patient TOMAS-25 had three deleterious *ARID1A* indels, while patient TOMAS-26 tumor sample harbored a gain-of-function mutation in the *ERBB2* gene, predicted as "possibly damaging" on protein function.

Gene copy number analysis was performed to identify differences among the two groups (Table 4). Dedifferentiated liposarcomas showed *MDM2/CDK4* gene amplifications, as already detected by diagnostic cytogenetics (TOMAS-43, TOMAS-48; both responder patients). Significant differences in copy number gain in *CDKN2A* gene (evaluated in comparison to housekeeping genes) was reported for responder in comparison to non-responder patients (paired Student's t-test; p=0.038). *MYC* gene amplification was detected in one responder patient affected by malignant myoepithelioma, and *MYC* amplification associated with *HEY1* amplification (possibly due to close chromosomal location) was identified in patient TOMAS-34 (non-responder patient affected by uterine LMS). Finally, *ERBB2* amplification was observed in one case of non-responder UPS of the limb.

TABLE 2 Likely pathogenic mutations in DDRR and related genes among responder patients.

Responders	*TP53*	*ERCC2*	*ERCC6*
	G245S (TOMAS-10; LMS_UT) G245S (TOMAS-10; LMS_UT) I195T (TOMAS-44; LMS_RP)	G615W (TOMAS-10; LMS_UT)	E272K (TOMAS-41; LMS_UT)
	PIK3CA R93W (TOMAS-38; MLPS_LIMB) P104S (TOMAS-39; MPT)	***PTEN*** R173C (TOMAS-38; MLPS_LIMB)	

UT, uterus; RP, retroperitoneum.

Differential expression of DDRR genes among responder and non-responder patients

Thirty-two RNA samples were extracted from FFPE archival tumor tissue from patients subsequently treated with trabectedin and olaparib combination. Significant differential expression levels of DDRR genes were found between the group of 16 responders (PFS ≥ 6 months), and that of 16 non-responders (PFS < 6 months). In detail, the expression of *CDKN2A, PIK3R1, SLFN11, ATM*, (and *POLK*) were significantly higher in responders; whilst *APEX2, BLM, XRCC2, MAD2L2*, and *KRAS* were significantly higher in non-responders (Figures 1A, B).

Validation of biomarker expression by RNA-ISH, qRT-PCR and IHC

Differential expression of selected candidate biomarkers and their exact subcellular and tissue localizations were analyzed by RNA-ISH. Specific probes for *CDKN2A*, *PIK3R1, SLFN11*, and *ATM* were more hybridized in tissue slices from tumors of responder patients than in those samples derived from non-responder patients (Figure 2A). *APEX2, BLM*, *XRCC2*, and *MAD2L2* were less hybridized in tissue slices from responder patient-derived tumors than in those ones from non-responder patients (Figure 2B). A heatmap was generated based on the ISH scores, displaying the differential expression of the eight identified genes among the two patient groups (Figure 2C).

The expression levels were further confirmed by qRT-PCR (Figure 3A) and also at the protein level in terms of IHC expression for CDKN2A/p16, where a significant difference was detected between responders and non-responders (p=0.041; Figures 3B, C).

Correlation of candidate biomarker gene expression levels and overall survival in TCGA sarcoma cohort

The sarcoma dataset was derived from genomic and expression analysis of 255 sarcoma samples from the TCGA sarcoma cohort. 249 samples were selected, being the ones with all data of interest available, and excluding desmoid tumors from the dataset, given their peculiar clinical-pathological behavior (37). The gene characterized by the highest number of genomic or transcriptomic alterations (Figure 4) was *CDKN2A* (altered in 19% of patients), followed by *BLM* (altered in 13% of patients), and *MAD2L2* (altered in 12% of patients). The most frequent *CDKN2A* alteration was homo-deletion (n=38, 15% of patients).

We subsequently focused on differences in expression levels of the eight identified candidate genes in the sarcoma cohort of TCGA dataset, to look for any correlation with survival outcomes. We found a significant relation between *MAD2L2* (Log-rank; p=0.0017) and *BLM* (Log-rank; p=0.025) expression levels and OS (Figure 5). The expression levels of the other six genes were not significantly related to OS (Supplementary Figure 1).

Discussion

Our work has focused on a relevant translational research question stemming from the clinical results of the phase Ib TOMAS trial (13), asking whether there might be any way to predict response to trabectedin+olaparib treatment in BSTS patients. Of course, the answer to this question is a multi-factorial, poly-genic one, especially considering the low prevalence of BRCA1/2 defects in BSTS (38). Indeed, we identified few differentially expressed

TABLE 3 Likely pathogenic mutations in DDRR and related genes in non-responder patients.

Responders	*TP53*	*ERCC2*	*RAD51C*
	C242S (TOMAS-32; LMS_UT) Y205D (TOMAS-34; LMS_UT)	T231M (TOMAS-29; SS_LIMB)	L138F (TOMAS-21;SS_LIMB)
	ERBB2 R678W (TOMAS-26; MPNST_LIMB)	***ARID1A*** Three deleterious indels (TOMAS-25; UPS_LIMB)	

UT, uterus; RP, retroperitoneum.

TABLE 4 Copy number differences in DDRR and related genes among responder and non-responder patients.

Responders	***CDKN2A***	***MDM2***	***CDK4***	***MYC***
	2.45 (TOMAS-10; LMS_UT) 1.44 (TOMAS-18; SS_LIMB) 1.9 (TOMAS-30; LMS_UT) 2.64 (TOMAS-33; SS_LIMB) 2.4 (TOMAS-48; DDLPS_RP)	38 (TOMAS-43; DDLPS_RP) 20 (TOMAS-48; DDLPS_RP)	24 (TOMAS-43; DDLPS_RP) 23 (TOMAS-48; DDLPS_RP)	5.08 (TOMAS-45; malignant myoepithelioma)
Non-responders	***CDKN2A***	***ERBB2***	***HEY1***	***MYC***
	1.3 (TOMAS -9; MPNST_LIMB) 1.84 (TOMAS-17; SS_LIMB) 1.42 (TOMAS-21; SS_LIMB) 1.34 (TOMAS-34; LMS_UT)	5.56 (TOMAS-35; UPS_LIM	5 (TOMAS-34; LMS_UT)	5 (TOMAS-34; LMS_UT)

UT, uterus; RP, retroperitoneum.

DDRR genes, which could provide the basis for a "personalized-medicine" approach to sarcoma treatment with this combination.

We analyzed WES and targeted-panel NGS data of patients from the TOMAS study, for whom RNA expression data were also available (32 patients), looking for any mutation, indel or CNV in DDRR genes, to integrate the expression signature with any mutational input which would not modify expression levels, but could alter gene function as well. Indeed, loss of function gene mutations might have the same effect of reduced gene expression for a given gene, while gain of function could correspond to gene overexpression. Indeed, we observed some relevant DDRR genes mutations, indels and CNVs in both responder and non-responder patients.

We then moved to expression profile analysis and identified a difference in terms of DDRR gene expression between 16 responder

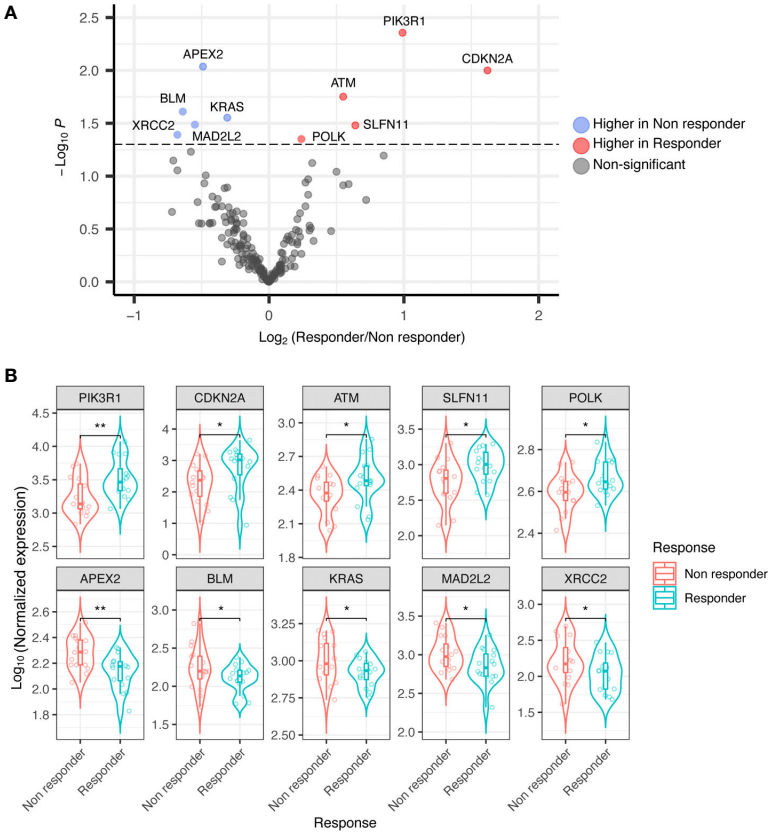

FIGURE 1
(A) Volcano plot showing differential expression of DDRR genes in responder *vs.* non-responder patients. **(B)** Boxplot showing differential expression of DDRR genes in responder *vs.* non-responder patients, with normalized expression. P-value by Wilcoxon Rank-Sum test. *p<0.05; **p<0.01.

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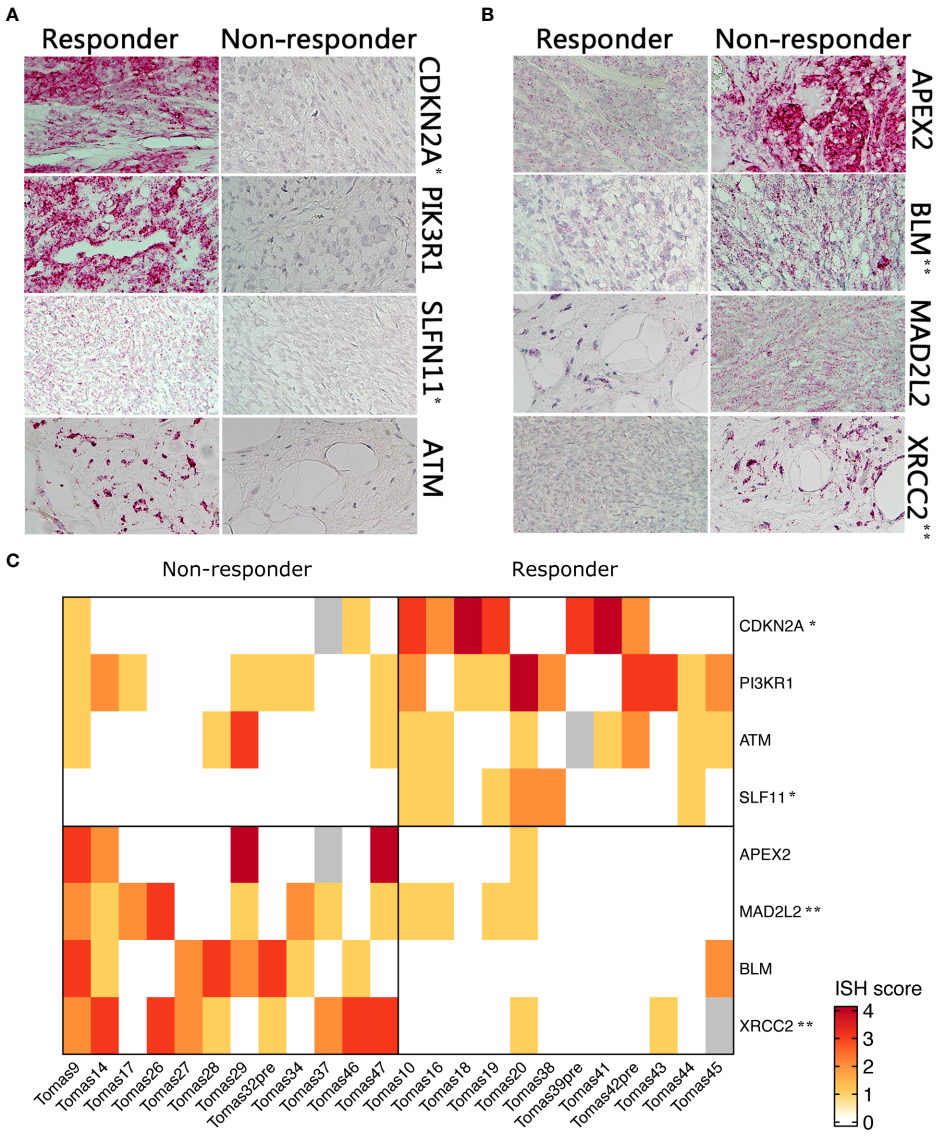

FIGURE 2
RNA ISH of selected genes in responder *vs.* non-responder patients. **(A)** Higher expressed genes in responder patients **(B)** higher expressed genes in non-responders **(C)**. Heatmap showing differential RNA ISH staining between responder and non-responder patients. ISH score was assigned by an expert pathologist on the basis of staining intensity and percentage of positive cells. P-value was calculated by Chi-square test. *p<0.05; **p<0.01.

and 16 non-responder patients from the phase Ib TOMAS trial. We found an 8-gene signature of differentially expressed DDRR genes which significantly correlates with better outcome in patients treated with trabectedin+olaparib combination, separating our patient population in two groups according to PFS (longer or shorter than 6 months). The differential gene expression data were corroborated with ISH data, confirming expression at the sub-cellular level with RNA *in situ* hybridization technique, and also at the protein level (*e.g.* CDKN2A/p16). Our signature emerged from a broad DDRR panel, including 180 genes.

CDKN2A, PIK3R1, SLFN11, ATM were characterized by significantly higher expression levels in responder patients; *APEX2, BLM, XRCC2, MAD2L2* displayed instead significantly higher expression levels in non-responder patients. Each gene deserves a separate discussion, being implicated in different aspects of DNA damage response and repair cellular machinery.

CDKN2A is a well-known tumor suppressor gene with a pivotal role in cell cycle control, slowing down G1 to S phase progression. It is involved in DDRR, and its low expression is also a negative prognostic factor across several tumor types

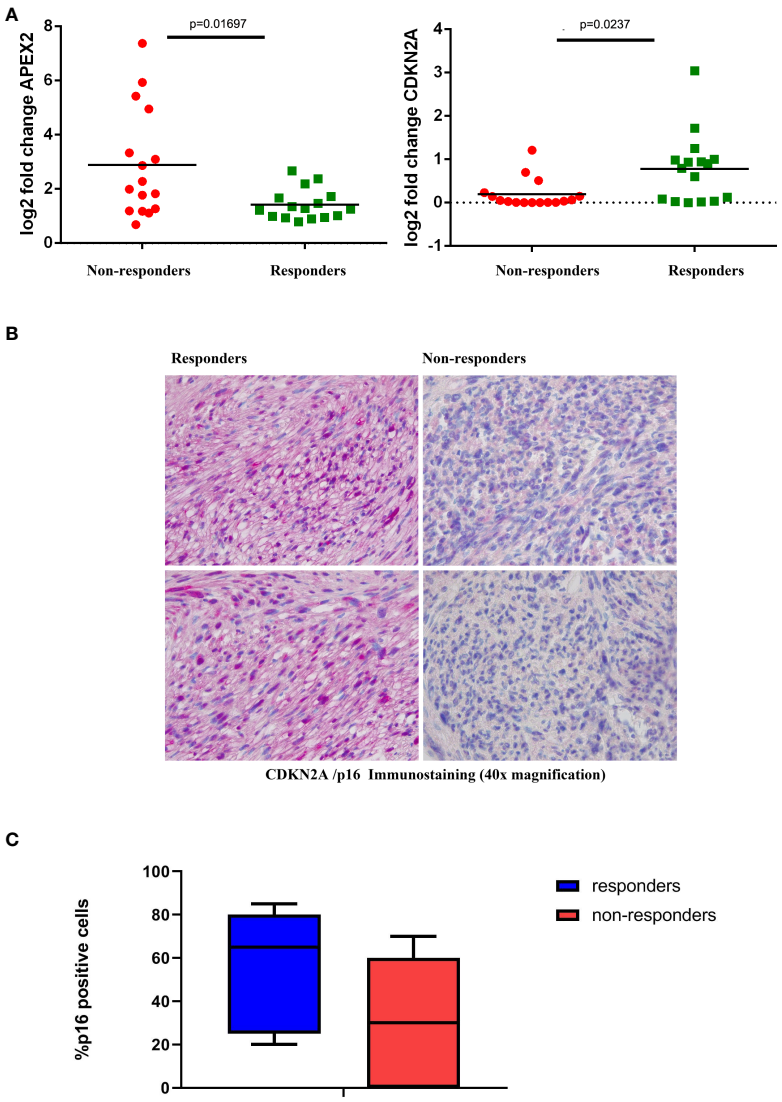

FIGURE 3
Validation Assays **(A)** Expression levels of representative genes (CDKN2A, left; and APEX2, right) among responder and non-responder patients. Statistically significant differential expression was shown between the two groups (Wilcoxon rank-sum test). **(B)** Representative IHC staining of CDKN2A/p16 in tumor samples from responder and non-responder patients. **(C)** Box plot distribution of CDKN2a/p16 expression level (percentage of IHC positive cells) in responders and non-responders patients.

(39–43). Similarly, low expression levels of *PIK3R1*, the gene encoding the regulatory subunit of *PIK3CA* (p85α), have been associated to poor prognosis, in particular in breast cancer (44). In the TCGA sarcoma cohort dataset, our analyses did not show any significant relationship between *CDKN2A* and *PIK3R1* expression and survival, suggesting that these two genes are unlikely prognostic factors in STS and potentially might be involved in response to the treatment in this case series.

Considering *SLFN11, ATM, APEX2, BLM, XRCC2* and *MAD2L2* expression, a functional role in response to trabectedin+olaparib treatment might be hypothesized based on their biological roles. *SLFN11* and *ATM* showed higher expression levels in responder patients. *SLFN11* had already been associated to PARPi response (45). SLFN11 enhances cancer cell sensitivity to DNA-damaging agents (46), through a peculiar mechanism. Indeed, *SLFN11* prevents the synthesis of proteins, which are crucial for cell survival upon significant extents of DNA damage. Namely, *SLFN11* downregulates type II RNAs, inducing reduced translation of DDRR genes such as *ATM* and *ATR* (47). In this view, *ATM* higher expression in responder patients seems controversial, because it would lead to a more HR-proficient tumor cell profile in terms of DDRR response. *APEX2* is a base-excision repair apurinic/apyrimidinic endonuclease (48). In multiple myeloma cells, it has been

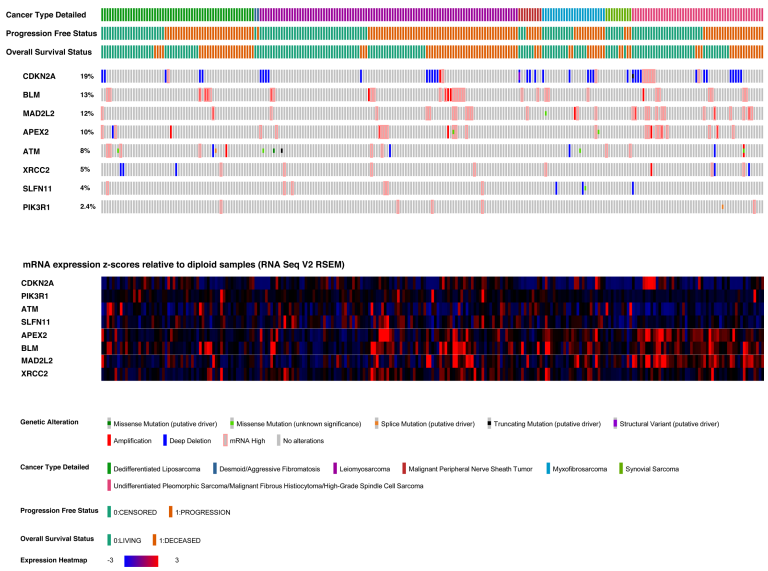

FIGURE 4
Oncoprint and heatmap of candidate biomarkers in the TCGA sarcoma cohort.

described as a key regulator of HR (49). Hence, lower *APEX2* expression in responder patients is consistent with HR impairment and better response to trabectedin+olaparib response. What is more, *APEX2* has been described as synthetic lethal in cells bearing BRCA2 defects (50). Considering *BLM*, its role in HR is well-known, both for initiation of HR upon DSBs and for Holliday junction dissolution at the end of the repair process (51). Hence, its lower expression in responder patients could have a direct implication driving sarcoma cells towards a HR-deficient phenotype. *XRCC2* is also involved in DSBs repair by HR (52). In our patient population, expression was consistently higher in non-responder patients compared to responders. Given the relevance of HR deficiency for both olaparib and trabectedin mechanism of action, *XRCC2* role in resistance to trabectedin +olaparib treatment could be at least partially explained.

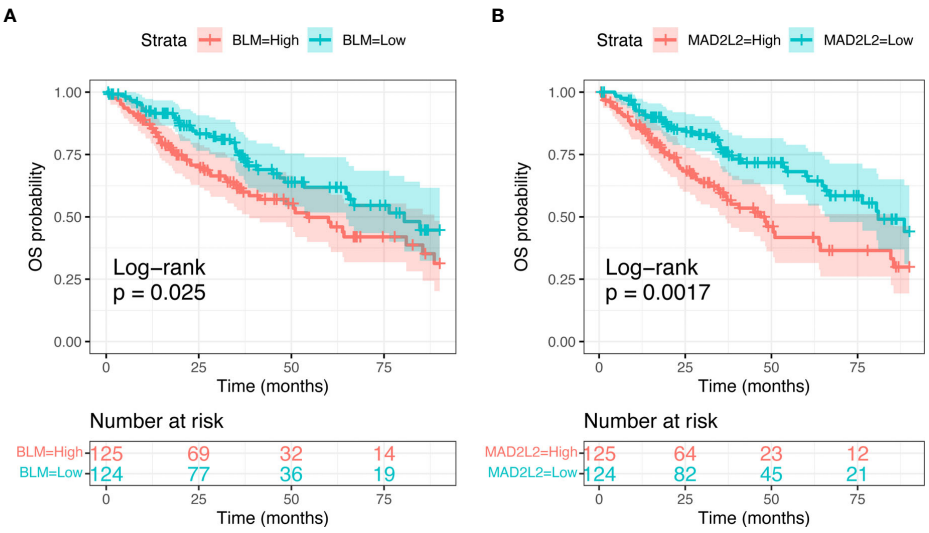

FIGURE 5
Kaplan-Meier curves showing Overall Sirvival in the sarcoma TCGA cohort, according to selected candidate biomarker genes (BLM, **A**, and MAD2L2, **B**).

MAD2L2, instead, has a more prominent role in NHEJ (53). As anticipated, NHEJ does not affect trabectedin efficacy in a relevant way. Theoretically, proficient NHEJ might influence PARPi action, supporting our observation that responder patients show lower levels of *MAD2L2* expression. Both *XRCC2* and *MAD2L2* higher expression was associated with worse survival in the TCGA sarcoma cohort.

Looking for potential DDRR gene function differences, which might not be reflected in expression levels, a few noteworthy mutations have emerged from analysis of WES and targeted-panel NGS data from the TOMAS phase Ib study. Indeed, apart from the expected *TP53* mutations, which we detected in our LMS patients, we observed one *ERCC2* mutation in one responder patient (Tables 2, 3), who displayed a mutation resulting in a G615W amino acid substitution, predicted as "probably damaging" (PolyPhen2 score of 1). *ERCC2* is involved in TC-NER, so a loss of function mutation could represent a "resistance mechanism" to trabectedin. This mutation might have represented our patient's Achilles' heel to maintain a sustained response (PFS=10 months). One responder affected by a metastatic myxoid liposarcoma of the lower limb carried a mutation resulting in the R173C *PTEN* amino acid substitution (TOMAS-38), which is a loss of function mutation. Indeed, *PTEN* mutations have been described as synthetic lethal with PARPi (54). Among DDRR genes, we also included *ERBB2* for its potential effects in DNA damage and repair pathways. We found *ERBB2* gene amplification in one patient affected by metastatic UPS of the lower limb, and one gain of function point mutation resulting in the amino acid substitution R678W in a patient affected by metastatic MPNST of the lower limb. *ERBB2* amplification has already been reported in UPS (55), as well as *ERBB2* gain of function in MPNST (56). The specific R678W substitution, falling into *ERBB2* transmembrane domain, confers significant cell survival advantage with respect to wild-type *ERBB2* (57). Intriguingly, it has been found that *ERBB2* expression affects the repair of specific DNA lesions produced by chemotherapy, linking *ERBB2* to the DNA damage and repair response (58).

In conclusion, the response of BSTS patients to trabectedin and olaparib combination correlates with the expression of DDRR genes. *CDKN2A, PIK3R1, SLFN11, ATM* and *APEX2, BLM, XRCC2, MAD2L2* differential expression discriminates responder and non-responder patients. The predictive role of these potential biomarkers warrants further investigation; we will explore this gene signature within data derived from our ongoing TOMAS-2 multicentric, randomized, phase II study.

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 Institutional review board - Candiolo Cancer Institute, str. prov 142 km 3.95 Candiolo, Italy. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: GGr, YP; Methodology: MLC, GF, UM, EB, YP, GCr; Formal Analysis: MLC, UM, EB, GF, YP, CI, CL, AP, IS; Investigation: AM, MLC, AP, GCh, EM, GGi, SB, LDA, MS, APDT; Visualization: CL, UM, EB, GF, YP; Writing - Original Draft Preparation, AM, YP; Writing - Review & Editing, AM, DS, GGr, GF, YP, MA, DS; Supervision, YP, GGr; Project Administration, YP, GGr; Funding Acquisition, GGr, DS, YP, AB, LDA. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

GGr has received fees for consulting/advisory roles from PharmaMar, Lilly, Novartis, Bayer, and Eisai. LDA received travel grant from PharmaMar and Lilly. MA has received fees for consulting/advisory roles from Bristol- Myers Squibb, Merck, and Roche. AB served in a consulting/advisory role for Illumina and Inivata. AB is cofounder and shareholder of NeoPhore. AB is a member of the NeoPhore scientific advisory board.

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/fonc.2022.844250/full#supplementary-material

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From seaside to bedside: Current evidence and future perspectives in the treatment of breast cancer using marine compounds

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To date, only few marine natural compounds have been proved to be active in breast cancer (BC). The main marine-derived drugs that have been studied for the treatment of BC are tubulin-binding agents (eribulin and plocabulin), DNA-targeting agents (cytarabine and minor groove binders—trabectedin and lurbinectedin) and Antibody-Drug Conjugates (ADCs). Notably, eribulin is the only approved cytotoxic drug for the treatment of advanced BC (ABC), while cytarabine has a limited indication in case of leptomeningeal diffusion of the disease. Also plocabulin showed limited activity in ABC but further research is needed to define its ultimate potential role. The available clinical data for both trabectedin and lurbinectedin are of particular interest in the treatment of BRCA-mutated tumours and HR deficient disease, probably due to a possible immune-mediated mechanism of action. One of the most innovative therapeutic options for the treatment of BC, particularly in TNBC and HER2-positive BC, are ADCs. Some of the ADCs were developed using a specific marine-derived cytotoxic molecule as payload called auristatin. Among these, clinical data are available on ladiratuzumab vedotin and glembatumumab vedotin in TNBC, and on disitamab vedotin and ALT-P7 in HER2-positive patients. A deeper knowledge of the mechanism of action and of the potential predictive factors for response to marine-derived drugs is important for their rational and effective use, alone or in combination. In this narrative review, we discuss the role of marine-derived drugs for the treatment of BC, although most of them are not approved, and the opportunities that could arise from the potential treasure trove of the sea for novel BC therapeutics.

KEYWORDS

breast cancer, eribulin, trabectedin, lurbinectedin, antibody drug conjugate (ADC), cytarabine, plocabulin

1 Introduction

More than 50 years ago, the very first drug extracted from the sea, cytarabine, arrived in clinics. Cytarabine (also known as Ara-C, Cytosar-U®) was isolated from a marine sponge and demonstrated activity against cancer cells *via* blocking DNA polymerase (Dyshlovoy and Honecker, 2018). Due to its biological activity, the drug was approved by the Food and Drug Administration (FDA) for the treatment of leukaemia in 1969, and since then Cytarabine has remained a relevant player in the therapeutic strategy for haematological malignancies.

Inexplicably, shortly after this initial success, the history of marine-drug development suffered a prolonged setback and appeared to have ended. In fact, no further compounds were approved by health authorities for almost 40 years and the search for novel anticancer drugs from natural sources had also declined in favour of computational and high-throughput screening approaches to rational drug design.

At the beginning of the 21st century, however, the development of medicines from the sea experienced a renaissance and gained a new momentum (Stonik, 2009; Brönstrup and Sasse, 2020). Supported by modern biochemical approaches, the renewed interest in marine anticancer derivatives has led to the identification of novel marine molecules that were undetectable in the past and have a specific mechanisms of action.

The role of marine natural products as candidates anticancer drugs is now widely recognised and represents an important field of research and development. In the last two decades, the number of the available marine-drugs has almost doubled (Dyshlovoy and Honecker, 2015) and as of March 2022, the list of the marine-derived drugs officially approved by the regulatory agencies for cancer treatment encompasses 12 compounds, with Eribulin the only one approved for breast cancer (BC), including in a chronological list: 1) the spongian nucleoside Cytarabine, 2) the spongian macrolide Eribulin mesylate, 3) the Brentuximab vedotin, 4) the ascidian alkaloid Trabectedin, 5) the marine-derived HDAC inhibitor Panobinostat, 6) the ascidian depsipeptide Plitidepsin, 7) the Polatuzumab vedotin, 8) the Enfortumab Vedotin, 9) the ascidian alkaloid-derived Lurbinectedin, 10) the Belantamab Mafodotin, 11) the Disitamab Vedotin, and 12) the Tisotumab vedotin.

Besides, more than 30 additional candidate anticancer marine-derived molecules are currently in various stages of development (Ha et al., 2019), most of which are antibody-drug conjugates (ADC) and some of which have a promising activity against BC.

In this narrative review, we discuss the potential role of marine-derived drugs in the treatment of BC (Figure 1) and the opportunities offered by the potential treasure trove of the sea for novel BC therapeutics.

2 Tubulin-binding agents

Eribulin mesylate and Plocabulin are two of the major anti-microtubule cytotoxic agents isolated from marine sources (Figure 2).

2.1 Eribulin

Eribulin mesylate is one of the most important marine compounds studied for its anticancer activity. It was isolated for the first time in 1986 from a natural product, the black sea sponge off the coast of Japan, *Halichondria okadai*. Despite its interesting mechanism of action with promising antitumor effects, its complex structure and the presence of contaminants made it difficult to use until 1992 when the total synthesis of halicondrin B was completed and many synthetic analogues were successfully developed, including eribulin.

Eribulin has a unique mechanism of action: unlike other compounds such as taxanes and vinca alkaloids, it exerts its cytotoxic effect by suppressing microtubules polymerization without affecting depolymerization, thereby preventing spindle formation, which ultimately leads to mitotic arrest and subsequent cell apoptosis (Gourmelon et al., 2011). Because of this mechanism of action, eribulin may show activity also in case of taxane-resistant tumour cell lines.

Moreover, some preclinical studies of human BC models have suggested that eribulin may also have a non-mitotic activity. Indeed, eribulin can affect tumour microenvironment and restore its vasculature and perfusion, downregulating the expression of vascular endothelial growth factor (VEGF) and TGFbeta genes. These alterations could be responsible for the potential enhancement of the subsequently administered chemotherapy by both reducing hypoxia-driven chemoresistance and increasing the intratumoral delivery of the drug. As a consequence, eribulin might contribute to modify the advanced BC (ABC) disease-trajectory and the post-progression survival outcome, as observed in phase III trials. Besides, preclinical studies have also shown that eribulin can affect the epithelial-mesenchymal transition process (EMT) (Ueda et al., 2016) and can favourably impact on immunological tumour microenvironment (De Sanctis et al., 2018). Notably, in triple-negative BC (TNBC) patients, the high level of tumour-infiltrating lymphocytes (TILs) has been shown to predict the efficacy of eribulin, with a significant DFS improvement (Kashiwagi et al., 2017), supporting a drug-related synergistic engagement of the anticancer immune-response. Following these suggestions, eribulin was tested in association with PD-1 inhibitors. Interesting preliminary results were reported in the phase Ib/II ENHANCE1 study, which evaluated the combination of eribulin with pembrolizumab in PD-L1-positive advanced TNBC patients. An ORR of 26% was observed, which was

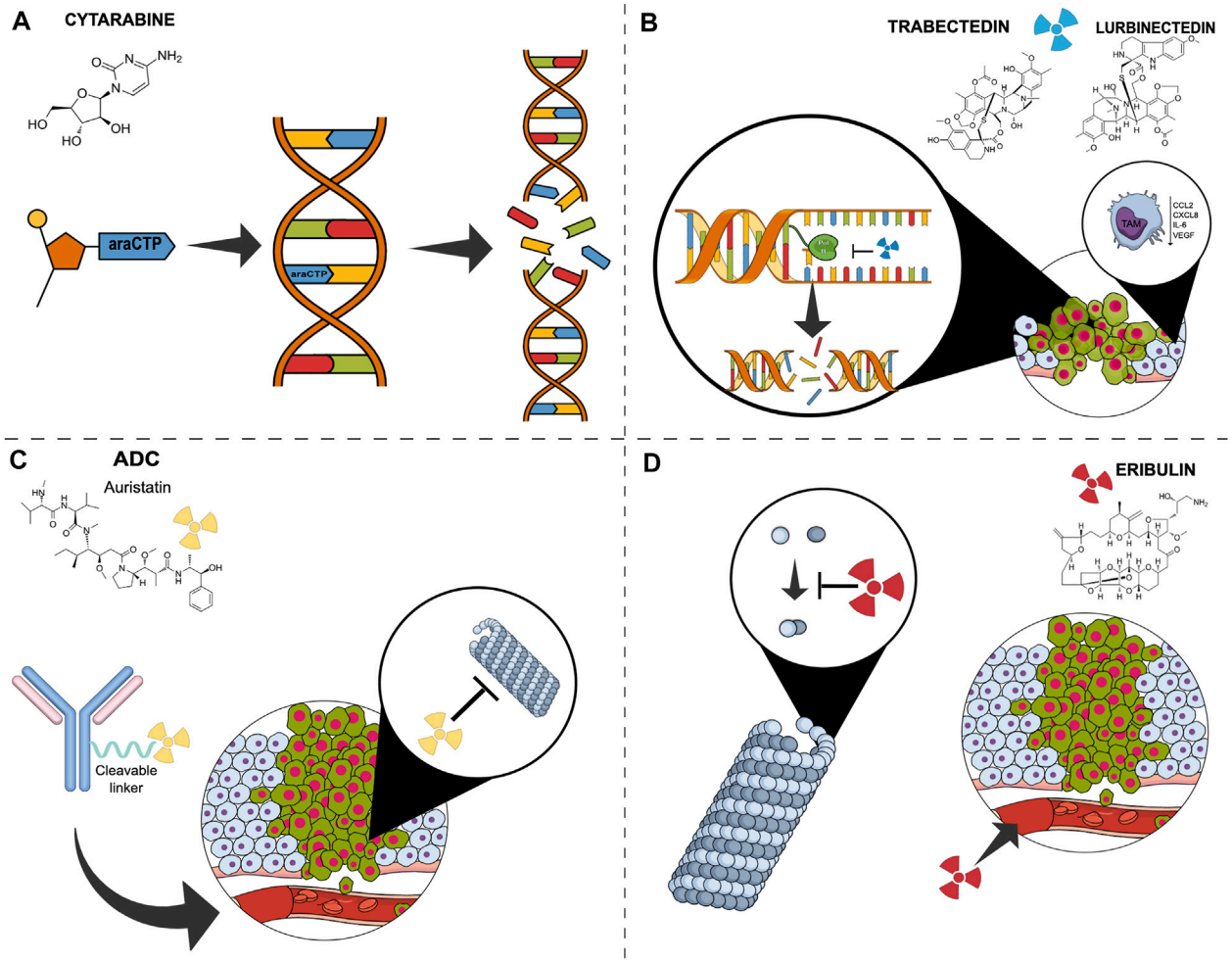

FIGURE 1
Simplified mechanisms of action of marine derivate compound in breast cancer. **(A)** Cytarabine is incorporated into DNA as its activated form, ara-cytidine 5′-triphosphate, and promotes abnormal fragment binding of newly synthesised DNA, leading to apoptosis. **(B)** Trabectedin and Lurbinectedin bind to the minor groove of DNA inhibiting transcription, resulting in double-strand DNA breaks and cell death. They also affect the tumour microenvironment by negatively modulating tumour-associated macrophages' activity. **(C)** Antibody-drug conjugates consist of a monoclonal antibody bound to a payload, generally monomethylauristatin E, a cytotoxic compound that binds to tubulin, leading to both disruption of mitotic spindle assembly and arrest of tumour cells in the mitotic phase of the cell cycle. **(D)** Eribulin inhibits the microtubule growth phase forming non-productive tubulin aggregates. It also acts on angiogenesis. Abbreviations: ADC, antibody drug conjugate; ara-CTP, ara-Cytidine-5′-triphosphate; CCL2, C-C Motif Chemokine Ligand 2; CXCL8, C-X-C Motif Chemokine Ligand 8; DAR, Drug-antibody Ratio; EGFR2, epidermal growth factor receptor; IL6, Interleukin 6; mgDNA, minor groove DNA; MMAE, monomethylauristatin E; RNApol, RNA polymerase; TAM, tumor-associated macrophages; tc-NER, Transcription-coupled nucleotide excision repair; VEGF, Vascular-Endothelial Growth Factor.

higher than the ORR observed in the past with either eribulin or pembrolizumab as monotherapy, in the same setting of patients (ORR 10 and 21%, respectively) (Tolaney et al., 2021).

Eribulin was approved by the regulatory agencies in the United States (November 2010) and in Europe (March 2011) for the treatment of ABC patients who had received at least one or two lines of prior chemotherapy, respectively. In Europe, the recommended dose of eribulin refers to the active substance (eribulin, 1.23 mg/m2) whereas in the United States to the salt form (eribulin mesylate, 1.4 mg/m2) and it should be administered intravenously over 2–5 min on Days 1 and 8 of every 21-days cycle (Goel et al., 2009).

The eribulin approval derived from the results of the EMBRACE trial, a randomized, open-label, phase III study, which demonstrated for the first-time ever in heavily pre-treated ABC the benefit of a cytotoxic single-agent in terms of statistically significant overall survival (OS) improvement, compared to the best treatment physician's choice (TPC) (13.1 vs. 10.6 months, $p = 0.041$) (Cortes et al., 2011). In a subsequent phase III trial (study E-301), pre-treated ABC patients were randomized to receive eribulin or capecitabine

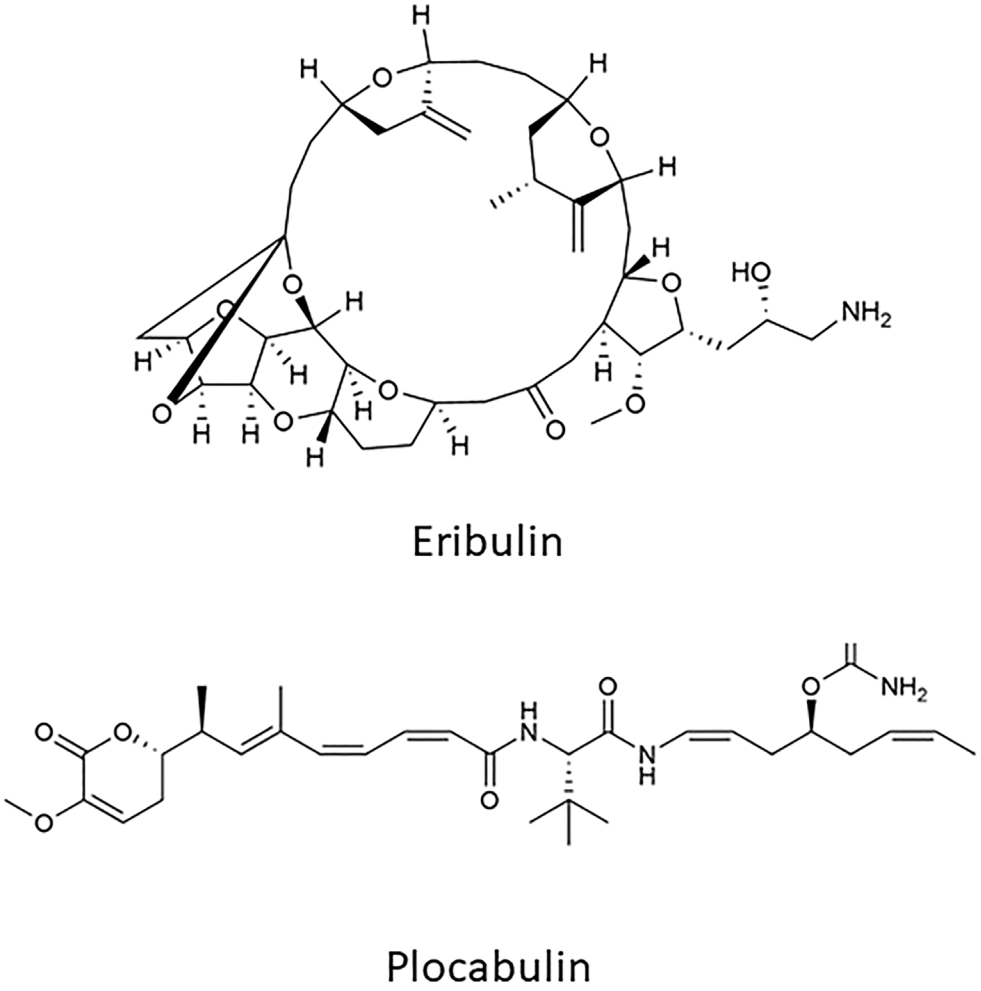

FIGURE 2
Chemical structure of Eribulin and Plocabulin.

as first, second or third-line therapy (Kaufman et al., 2015). Eribulin failed to demonstrate superiority over capecitabine, showing similar results in terms of OS (15.9 vs. 14.5) and no differences in progression free survival (PFS) and ORR. Notwithstanding this finding, however, a subsequent post-hoc pooled analysis of the EMBRACE and E-301 trials showed that eribulin prolonged OS in the entire patient population and in all patient subgroups (Twelves et al., 2014). Although real-world evidence supports the efficacy of eribulin in chemo-pretreated ABC regardless of cancer subtypes (Pedersini et al., 2018), a greater clinical benefit was observed in the case of TNBC (Twelves et al., 2014).

Besides TNBC, emerging data suggest that eribulin is also effective and tolerable in patients with HER2-positive ABC. Given the promising results of clinical trials with anti-HER2 agents in combination with conventional chemotherapies, the use of eribulin with trastuzumab was investigated in several studies. This combination was tested in a phase II trial for the first-line treatment of HE2-positive ABC and showed an ORR of 71.2% with a median PFS of 11.6 months (Sakaguchi et al., 2018). Another phase II trial assessed the combination of eribulin with the dual antiHER2 block, trastuzumab and pertuzumab, in taxane-pretreated HER2-positive ABC, showing favourable outcome in terms of ORR and prolonged PFS (Araki et al., 2017).

There is only little evidence regarding the role of eribulin in early BC. In the neoadjuvant setting, a phase II trial evaluated for the first time the combination of eribulin with carboplatin in TNBC, reporting an encouraging 43% of pathologic complete response (Kaklamani et al., 2015).

Similarly, in the adjuvant setting, a single pilot experience evaluated the feasibility of the combination of eribulin with capecitabine (days 1–14 of a 21-days cycle) in HR-positive HER2-negative, stage I–II early BC, with preliminary interesting results (Smith et al., 2016).

The most frequent adverse events (AEs) associated with eribulin were neutropenia, fatigue and neuropathy. As reported in the EMBRACE study, neutropenia occurred in 22%–49% of patients and it was easily managed with dose delay, dose reductions or administration of stimulating growth factors. Neuropathy was the most common non-hematologic AEs leading to a limited treatment discontinuation in EMBRACE trial [24 (5%) of 503 patients] (Cortes et al., 2011). Alternative schedules of administration (e.g., biweekly) are being evaluated to derive a better toxicity profile and treatment tolerance.

2.2 Plocabulin

Plocabulin is a novel tubulin-binding agent, isolated for the first time from the Madagascan sponge *Lithoplocamia lithistoides*, currently produced by total synthesis (Pera et al., 2013; Martínez-Díez et al., 2014). Unlike eribulin, plocabulin binds with high affinity to a new site in the β-tubulin plus end, inhibiting the microtubule growing at a very low concentration. The resulting microtubules instability affects the cellular cycle both during interphase and mitosis, leading to alteration in cell shape, trafficking, signalling, transportation, migration and, ultimately, cell apoptosis.

Moreover, the inhibition of microtubule dynamics in endothelial cells leads to alterations in tumour vascular architecture. These antiangiogenic effects, obtained with a lower dose than the cytotoxic one, contribute to enhancing plocabulin's anticancer activity (Galmarini et al., 2018). Worthy of note, plocabulin preserved its effect even in cells expressing the P-gp multidrug efflux pump, typically resistant to vinorelbine and paclitaxel, two well-known and extensively used drugs in BC (Martínez-Díez et al., 2014). Both *in vitro* and *in vivo* studies, Plocabulin exhibits a promising cytotoxic effect on breast tumour cells (Pera et al., 2013).

The first-in-human phase I trial (NCT01299636) of plocabulin in patients with several advanced solid tumour included five patients with ABC. Of them, three achieved stable disease (SD) as the best response, with maximum tumour shrinkage of 28%. The single BC patient who derived the greatest benefit was heavily pretreated (10 prior lines) and reached an interesting PFS of 6 months.

These preliminary signals of anticancer activity came with some toxicities, being the peripheral sensory neuropathy the most common and severe AEs, especially in patients who already had the chemotherapy-induced peripheral neuropathy (CIPN) at baseline (i.e., oxaliplatin). Other common plocabulin-related AEs were mild or moderate, including fatigue, nausea, alopecia, vomiting and abdominal pain. The main severe haematological toxicity was anaemia (23% of grade 3), being neutropenia and thrombocytopenia mild or moderate. Most biochemical abnormalities were grade 1 or 2 and included alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increase and hypoalbuminemia (Elez et al., 2019).

Another prospective phase I trial (NCT02533674) testing plocabulin in combination with gemcitabine in selected advanced solid tumours, including 4 ABC, has been completed, but data on drug efficacy have not yet been reported.

Unfortunately, despite the interesting rationale for the potential role of plocabulin in tumours resistant to other antimicrotubular agents, its activity in advanced BC has not been further investigated in other trials.

3 DNA-targeting agents

3.1 Cytarabine

Cytarabine, also known as cytosine arabinoside (Ara-C), was the very first marine-derived compound approved for its anticancer properties (Figure 3). It had been obtained from a Caibbean sponge, *Cryptotheca crypta*, synthesized for the first time in 1959 and then rendered by *Streptomyces griseus* fermentation. It belongs to the category of drugs known as antimetabolite and exerts its activity interfering with the DNA synthesis. Cytarabine is a pyrimidine analogue and differs from its natural counterpart (cytidine and deoxy cytidine) for the presence of sugar arabinose instead of ribose and deoxyribose. Once inside the cell, cytarabine is rapidly converted into the active triphosphate form, competing with cytidine to incorporate itself into DNA. The modified DNA structure and the inhibition of DNA polymerase caused by cytarabine, prevent DNA replication and repair (Barreca et al., 2020; Wu et al., 2021).

Cytarabine was approved by the FDA in 1969 for the treatment of acute myeloid leukaemia (AML). Later, other haematological malignancies such as lymphoblastic and myeloid leukaemia, both in acute and chronic phase. A liposomal formulation of the drug was developed, that improved the molecular stability and half-life of the drug and also allowed a prolonged exposure to tumour cells in the central nervous system (CNS). For this reason, primary CNS lymphomas are among the many off-label indications for cytarabine, and it has therefore also been tested for the palliative treatment of leptomeningeal carcinomatosis.

A phase III trial (DEPOSEIN) demonstrated that in BC patients with newly diagnosed leptomeningeal metastasis, the addition of intrathecal liposomal cytarabine to systemic treatment versus systemic treatment alone, prolonged disease related PFS. Leptomeningeal metastases PFS was 3.8 months in the combined arm versus 2.2 in the systemic treatment alone arm (HR 0.61, $p = 0.04$) in the intent-to-treat population (Le Rhun et al., 2020).

Despite its demonstrated activity, cytarabine holds severe side effects, with bone marrow suppression and pancytopenia being the most common ones; infection, musculoskeletal and

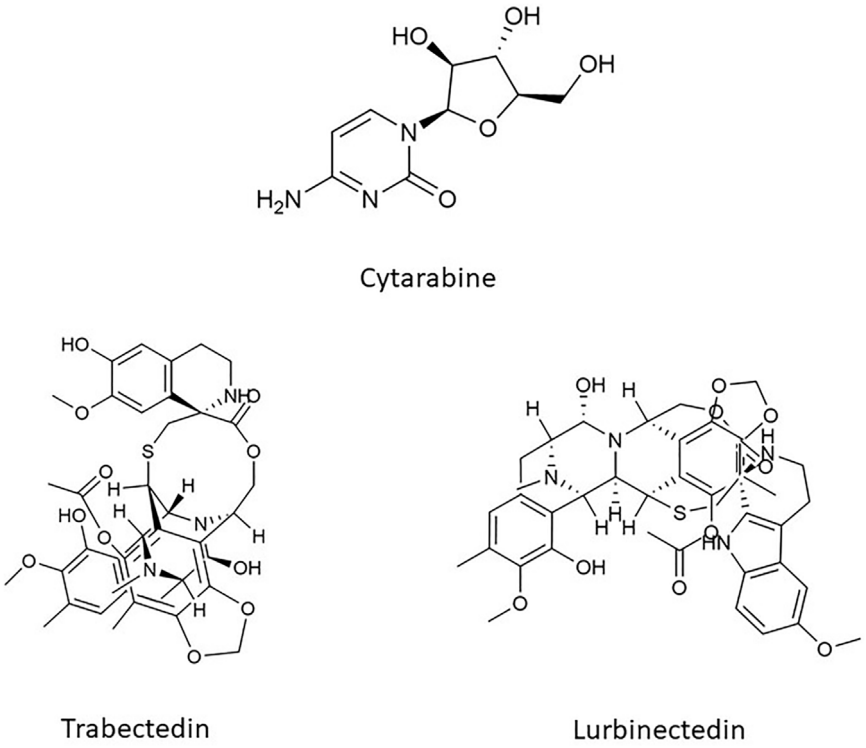

FIGURE 3
Chemical structure of Cytarabine, Trabectedin, and Lurbinectedin.

connective tissue abnormalities arise in a smaller percentage of patients. When administered intrathecally, neurological complications can occur, ranging from a reversible self-limiting cerebellar syndrome, chemical meningitis, myelopathy, up to a more diffuse encephalopathy with seizures (Baker et al., 1991).

3.2 Minor groove binders

About 60 years ago, compounds extracted from the Caribbean Sea squirt *Ecteinascidia turbinata* were found to have a great activity in the inhibition of cell proliferation. Nonetheless, it took about three decades to isolate the bioactive molecule, ecteinascidin 743 (ET-743), that was synthetically produced for the first time only in 1996 (Cuevas and Francesch, 2009).

Trabectedin and its synthetized analogue lurbinectedin are two innovative anticancer alkaloids isolated from extracts of the Caribbean tunicate *Ecteinascidia Turbinate* (Figure 3). The two compounds are structurally and functionally related; they share the same pentacyclic skeleton and differ in the so-called ring C which confers specific pharmacokinetic and pharmacodynamic features (Allavena et al., 2022). For instance, lurbinectedin has a three-fold higher MTD and a four-fold lower volume of distribution than those of trabectedin, thus allowing higher dose-intensities without a meaningful increase in toxicities (Takahashi et al., 2016).

Lurbinectedin and trabectedin belong to the class of "minor groove binders" agents, in light of their cytotoxic effect depending on the interaction with the specific DNA site (Leal et al., 2010). Indeed, the two molecules bind covalently the central guanine of specific nucleotide triplets, mainly located close to promoters of protein-coding genes, inhibiting active transcription by the arrest and degradation of elongating RNA polymerase II (Nuñez et al., 2016). Subsequently, DNA repair systems and especially the transcription-coupled Nucleotide Excision Repair (tc-NER) recognize the lurbinectedin/trabectedin-DNA adduct and induced cell apoptosis, generating double-strand DNA breaks (DSBs). Therefore, the cytotoxic activity of lurbinectedin and trabectedin requires a functional intact NER mechanism while a further enhancement has been described in case of deficient Homologous Recombination Repair (HRR) pathway (Tavecchio et al., 2008). As a consequence, the lack of an efficient DNA repair process in these cells leads to increased unrepaired DSBs induced by the NER-drug complex, eventually resulting in lethal DNA damage and cell death (Soares et al., 2007). Moreover, *in vitro*

and *in vivo* studies have shown that these agents can positively affect the tumour microenvironment by several mechanisms. First, they reduce the viability of tumour-associated macrophages (TAM) and induce their apoptotic cell death (Belgiovine et al., 2017), preventing the production of cytokines involved in cancer growth, downregulation of immune response and resistance to antineoplastic treatments. In addition to mitigation of the TAM-mediated immunosuppression, exposure to lurbinectedin/trabectedin-induced cell death seems to trigger an immune system response by increasing T cell infiltration and activation, questioning a possible synergistic role of this agent with immune checkpoint blockade (Xie et al., 2019).

3.2.1 Trabectedin

Trabectidin an antitumoral drug discovered in 1969 obtained from a Caribbean squirt of the sea: the *Ecteinascidia turbinate*. Trabectedin was approved by FDA for the treatment of advanced soft tissue sarcomas (Demetri et al., 2016) and ovarian cancer (Monk et al., 2010).

Preclinical studies with trabectedin showed potent anticancer activity of the drug against cell lines of solid tumours, including BC, even at very low doses (1–10 ng/ml). However, the small number of BC patients in the 13 different phase I studies testing trabectedin in solid tumours and a very limited number of dedicated experiences challenged the role of trabectedin in ABC (Taamma et al., 2001; Takahashi et al., 2001; Dincalci and Zambelli, 2016). Of note, one of the rare phase I involving ABC patients was reported by Sessa et al. and investigated the safety profile and the anti-tumour activity of the combination of trabectedin and doxorubicin in advanced soft-tissue sarcoma (STS) and ABC, and reported some encouraging data (Sessa et al., 2009).

Trabectedin was also studied in several phase II trials conducted in patients with various forms of solid malignancies, and again the contribution of ABC patients was very limited. Indeed, according to a retrospective review of the role of trabectedin in 35 different phase II clinical trials, only 215/2,298 (9.3%) patients of ABC (Zelek et al., 2006).

A phase II randomized trial evaluating the safety and efficacy profile of trabectedin in ABC patients among different treatment schedules (every 3-weeks versus weekly regimen) showed a greater activity of trabectedin 1.3 mg/m2 administered once every 3 weeks, with a reasonable safety profile. Indeed, no relevant differences were observed as regard the most frequent drug-related AEs (transaminitis, nausea, and asthenia) except for neutropenia (40 vs. 15%); however, the higher ORR (12 vs. 3.7%) and PFS (3.1 vs. 2.0 months) in the 3-weeks arm made this dosing regimen the recommended one in ABC patients (Goldstein et al., 2014).

In terms of activity, trabectedin seems particularly attractive in BC with DNA damage repair defects and especially in BRCA1/2 mutated germline tumours and in the so-called sporadic "BRCAness" BC with specific somatic gene alterations (Peto et al., 1999). At 17%–20% of primary BC are thought to have one of these predictive genomic scars, the impact of the candidate molecular predictors would be substantial in such a prevalent neoplasm (Turner et al., 2004). Based on the previously described MoA, a more pronounced activity of trabectedin in BC harbouring HR deficiency has been postulated (García et al., 2013). Accordingly, Delaloge et al. investigated the efficacy and safety of trabectedin in BRCA1/2 mutant ABC. Of the 35 evaluable BRCA1/2 germline mutation carriers who participated in the trial, PR was documented in six patients (17%) with a median PFS of 3.9 months. Despite the limited sample size, this trial supported trabectedin monotherapy as an active and well-tolerated option in heavily pretreated ABC carrying germline BRCA1/2 mutation (Delaloge et al., 2014).

To further investigate the role of trabectedin in BRCA1 vs. BRCA 2 mutants ABC, a substudy analysis in 39 pretreated ABC suggested that ORR was higher in BRCA2-mutated patients than BRCA1-mutated patients (33.3 vs. 9.1%) with a longer disease stabilisation (25.0 vs. 9.1%) and longer median PFS (4.7 vs. 2.5 months) (Ghouadni et al., 2017).

In addition, a phase II trial explored the role of alternative HR-deficiency genes in the efficacy of trabectedin in ABC. Contrary to expectation, the expression of xeroderma pigmentosum gene (XPG) did not contribute to the prediction of the trabectedin response, raising the question of which genes the main role in the clinical trabectedin susceptibility in the context of HR deficiency (Awada et al., 2013).

3.2.2 Lurbinectedin

Several phase I trials have investigated lurbinectedin activity in advanced solid tumours, including BC, alone or in combination with other drugs. Apart from some quite interesting results observed with the association of lurbinectedin and gemcitabine (one PR and five SD among six evaluable patients with ABC) (Paz-Ares et al., 2017), no particularly noteworthy clinical effects were found with other companion drugs, such as paclitaxel (Drilon et al., 2016) and capecitabine (NCT02210364).

As for trabectedin, the role of HR deficiency in strengthening lurbinectedin efficacy suggested a possible strong activity in BRCAness tumours. Accordingly, Cruz et al. (2018) performed a phase II trial investigating the activity of lurbinectedin in pre-treated germline BCRA1/2 mutant ABC. Patients were divided into two groups based on BRCA1/2 status: 54 patients with BRCA1/2 mutation vs. 34 with wild-type (WT) or unknown status. Lurbinectedin was administered at a flat dose of 7 mg (then modified to a dose of 3.5 mg/sqm) every 3 weeks. Among the BRCA mutated cohort, the primary endpoint of ORR was 41 vs. 9% in the WT cohort (crossing the futility border). As regards the safety profile, the most common toxicities were haematological (neutropenia,

lymphopenia, and anaemia) and biochemical (AST, ALT and creatinine increased) abnormalities; most frequent non-laboratory AEs included fatigue and nausea, without differences between the two cohort of patients. The BSA-dose adjustment meaningfully reduced the overall incidence of grade 3 or 4 AEs. Translational analysis showed that resistance to lurbinectedin relied widely on alterations in NER-related genes. As previously reported for trabectedin (Ghouadni et al., 2017), an interesting higher benefit of lurbinectedin was found in BRCA2 vs. BRCA1 mutant ABC patients (ORR 61 vs. 26%, respectively), possibly due to the specific role of the BRCA2 protein in preventing the formation of RNA-DNA hybrids (R-loops) during the transcriptional process (Bhatia et al., 2014) with a more pronounced genomic instability under the pressure of the minor-groove binding drugs.

Apart from a completed but not yet published phase II trial (NCT02454972) regarding 21 germline BRCA1/2 mutant ABC, currently there are no ongoing trials of lurbinectedin in BC. Noteworthy, on June 2020, lurbinectedin received its first approval by the FDA for second-line treatment of patients with metastatic small-cell lung cancer (SCLC) progressing after platinum-containing chemotherapy, based on positive results of a single-arm, phase II basket trial (NCT02454972) (Trigo et al., 2020). However, a subsequent phase III trial (NCT02566993) comparing lurbinectedin plus doxorubicin with the common second-line treatments (topotecan or cyclophosphamide, doxorubicin plus vincristine regimen) eventually failed to show a significant OS benefit (Paz-Ares et al., 2021).

4 Antibody-drug conjugates

ADC are considered the latest achievement in the landscape of tailored cancer treatment. The mechanism of action of these drugs is to deliver a cytotoxic payload attached, *via* a cleavable linker, to an antibody that targets a specific surface antigen expressed by the cancer and its niche (Boni et al., 2020). This smart way of delivering chemotherapy is currently used in HER2-positive BC with the advent of trastuzumab-emtansine (T-DM1) (von Minckwitz et al., 2019), and trastuzumab deruxtecan (Cortés et al., 2022; Modi et al., 2022) and in TNBC with Sacituzumab govitecan (Bardia et al., 2021), having different linkers and payloads. Among these payloads, a marine compound used in several ADCs is the monomethyl auristatin E (MMAE), a potent cytotoxic agent derived from the *dolastatins*, pseudopeptides extracted from shell-less marine mollusc *Dolabela Auricularia* (Dosio et al., 2011) (Figure 4). Isolation dates back to 1987 when auristatins, synthetic analogues of the natural antimitotic agent *dolastatin 10,* were extracted from *Dolabella auricularia* by Pettit and colleagues (Bai et al., 1990). Its ability to inhibit microtubule polymerisation and tubulin-dependent GTP hydrolysis leads to cell death. Aside with its potency, significant toxicities have been observed at doses insufficient to achieve clinical efficacy (Kumar et al., 2017). The release of MMAE molecules in circulation leads to cell apoptosis, inhibition of cell growth and angiogenesis. The main AEs associated with MMAE payload are myelotoxicity (anaemia and neutropenia) and peripheral neuropathy. In addition to these AEs, antibody-dependent side effects must also be considered.

The linker that binds MMAE to the antibody is stable in the extracellular fluid, but is cleaved by cathepsin once ADC has bound to and entered the antigen of the target cancer cell, whereupon ADC releases the toxic MMAE and activates the potent antimitotic mechanism (Caculitan et al., 2017).

Most ADCs have a particular property called the "bystander effect." After ADC binding, the cytotoxic molecules are released not only to cells expressing the target but also to adjacent or nearby cells. At the same time, this particular mechanism also damages stromal tumour cells and vascularisation, thus increasing the killing effect of cancer cells. Another important feature is the Drug-to-Antibody Ratio (DAR), defined as the number of payload molecules linked to each antibody, which is fundamental for determining the toxicity and the activity of the drug.

4.1 Enfortumab vedotin

Enfortumab vedotin (EV) targets cells expressing nectin-4. Nectin-4 is a member of the nectin family of immunoglobulin-like adhesion molecules mediating Ca^{2+}-independent cell–cell adhesion processes (Rikitake et al., 2012). The immunohistochemistry analyses demonstrated a moderate to strong expression (H-score > 100) of nectin-4 in about 50% of BC specimens. AGS-22M6, a fully human antibody targeting nectin-4, was studied *in vitro* and *in vivo* for affinity, cross-reactivity and ability to induce cell apoptosis. AGS-22M6 conjugated to MMAE showed a dose-dependent activity *in vivo*, inhibiting cancer cell growth at low doses and inducing tumour regression at higher doses (Challita-Eid et al., 2016).

The EV-202 trial is an open-label phase II study (NCT04225117) evaluating ORR in previously treated locally advanced/metastatic malignant solid tumours, including HR-positive and HER2-negative BC and TNBC. All patients receive EV 1.25 mg/kg IV on Days 1,8, and 15 of each 28-days cycle until progression or unacceptable toxicity (Bruce et al., 2020). The study is currently ongoing and is expected to be completed in April 2024.

Noteworthy, in December 2019 EV obtained the FDA approval for locally advanced or metastatic urothelial cancer in previously pre-treated patients, based on the significant longer OS results observed with EV compared to standard chemotherapy (Powles et al., 2021).

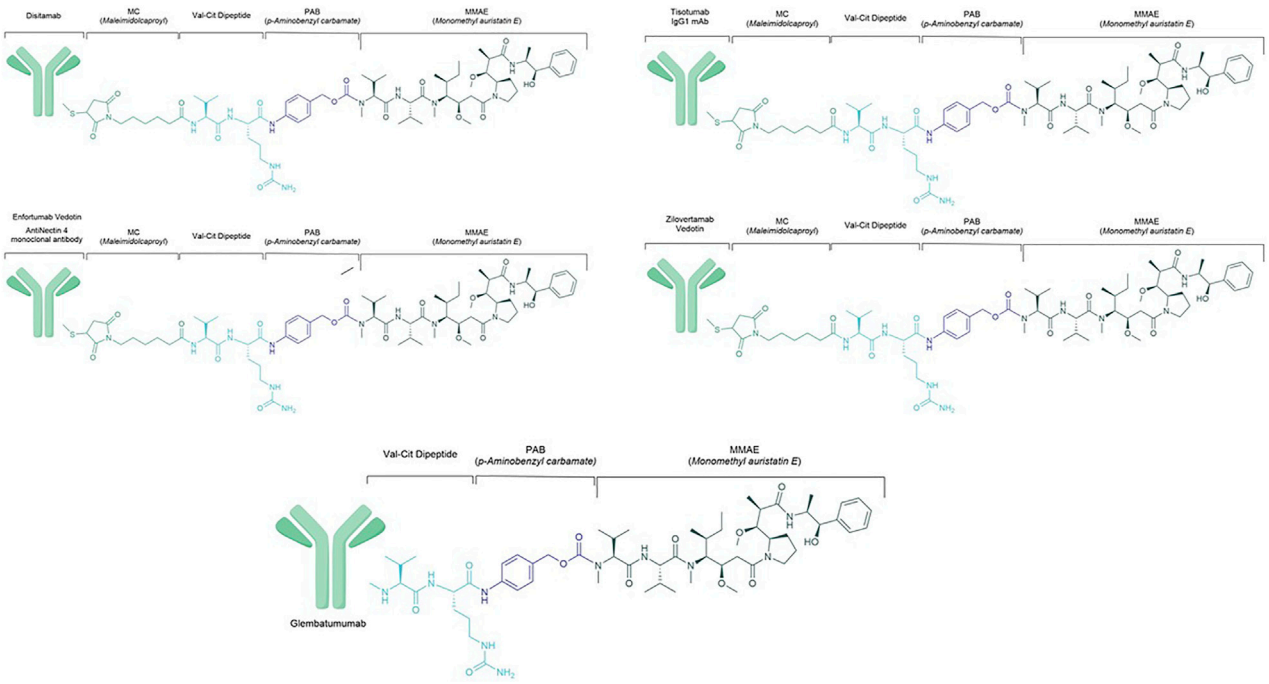

FIGURE 4
Chemical structure of main monomethyl auristatin E ADCs.

4.2 Ladiratuzumab vedotin

Ladiratuzumab vedotin (LV) is an ADC whose IgG1 antibody targets the zinc transporter LIV-1, expressed in ER + BC cells, with a MMAE payload. A discrete tolerability profile and activity have been proven in pre-treated advanced TNBC patients at the recommended dose of 2.5 mg/kg every 21 days, with a disease control rate (DCR) up to 59%, mostly with SD (Modi et al., 2018). Recently presented data showed an ORR around 28% (95% CI: 13, 47) with a weekly schedule at 1.25 mg/kg (Tsai et al., 2021). Ongoing studies on safety and tolerability profile in metastatic BC are exploring LV alone or in combination with trastuzumab (NCT01969643). Other combinations regard immunotherapy with PDL1 inhibitors. An open-label phase Ib/II trial (SGNLVA-002/KEYNOTE 721) is currently investigating the activity of LV combined with pembrolizumab (200 mg every 3-weeks) in treatment-naïve (locally) advanced TNBC. The rationale lies behind the concept that LV might produce an advantageous microenvironment for the engagement of the immune-response enhanced by the anti-PD-1 drug. This combination showed encouraging data in 26 TNBC patients, with an ORR of 54% (95% CI: 33.4, 73.4) and a manageable toxicity profile comprising fatigue, alopecia, gastrointestinal symptoms, and peripheral neuropathy, mostly low grade. Further immunotherapy-based regimens are being investigated in an umbrella randomised trial which includes LV alone or in combination with atezolizumab (anti-PD-L1) in advanced TNBC (NCT03424005). The use of LV was also investigated in the neoadjuvant setting in the I-SPY2 trial (NCT01042379), resulting similar in pathological complete response (pCR) and AEs to paclitaxel, despite less incidence of CIPN(Beckwith et al., 2021).

4.3 Tisotumab vedotin

Tisotumab vedotin is an ADC directed toward tissue factor (TF) linked with MMAE. Monoclonal antibodies or pathway inhibitors directed to TF have been demonstrated to inhibit cancer cell growth, metastases spreading and angiogenesis. In preclinical studies, high levels of TF are expressed in invasive tumours, particularly in TNBC (Zhang et al., 2017). No other studies are currently recruiting patients with BC. However, based on this evidence, there is room for exploration of this drug, particularly in TNBC.

On 20 September 2021, tisotumab vedotin was approved by the FDA for the treatment of previously treated metastatic cervical cancer based on an ORR of 24% (95% CI: 16, 33) in the NCT03438396 phase II trial (Coleman et al., 2021).

TABLE 1 Studies on marine-derived compounds in breast cancer. Abbreviations: TPC, treatment physician's choice; BC, Breast Cancer; ABC, advanced breast cancer; LA, locally advanced; HR, Hormone Receptor; TNBC, triple-negative breast cancer; LM, leptomeningeal metastasis; OS, overall survival; ORR, objective response rate; PFS, progression free survival; DCR, disease control rate; pCR, pathological complete response; PR, partial response; SD, stable disease; DTL, Dose Limiting Toxicity; AE, Adverse Events; MTD, maximal tolerance dose; RP2D, recommended phase II dose; NA, not available.

Drug	Phase	Sample size (BC)	Main results/Primary endpoint	Authors/NCT number
Marine-drugs with activity in BC treatment - APPROVED				
Eribulin Mesylate	III	762	mOS = 13.1 vs 10.6 months (HR 0.81, p = 0.0041) mPFS = 3.7 vs 2.2 months (HR 0.87, p = 0.137) ORR = 12% vs 5% (p = 0.002)	Cortes et al. (2011)
	III	1102	mOS = 15.9 vs 14.5 months (HR 0.88, p = 0.056) mPFS = 4.1 vs 4.2 months (HR 1.08, p = 0.30) ORR = 11 vs 11.5% (p = 0.85)	Kaufman et al. (2015)
Intrathecal Liposomal cytarabine	III	74	mLM-PFS = 3.8 vs 2.2 months (HR 0.61, p = 0.04)	Le Rhun et al. (2020)
Marine-drugs with activity in BC treatment - NOT APPROVED				
Ladiratuzumab vedotin	I	44	ORR=32%	Modi et al. (2018)
	I	29	ORR = 28% (95% CI: 13, 47)	Tsai et al. (2021)
	I	26	ORR = 54% (95% CI: 33.4, 73.4)	Han et al. 2020
Disatamab vedotinI	I	70	Dose of 1.5 mg/kg: ORR = 22.2% (95% CI: 6.4, 47.6); mPFS = 4.0 months (95% CI: 2.6, 7.6) Dose of 2.0 mg/kg: ORR = 42.9% (95% CI: 21.8, 66.0); mPFS = 5.7 months (95% CI: 5.3, 8.4) Dose of 2.5 mg/kg: ORR = 40.0% (95% CI: 21.1, 61.3); mPFS = 6.3 months (95% CI: 4.3, 8.8)	Wang et al. (2018)
		48	ORR = 39.6% (95% CI: 25.8, 54.7)mPFS = 5.7 months (95% CI: 4.1, 8.3)	
ALT-P7	I	22	DCR at 6 weeks = 77.3%(17/22) PR = 13.3% (2/15)	Park et al. (2020)
Glembatumumab vedotin	II	83	ORR = 6% (5/83) for GV vs 7% (3/41) for ChemotherapyORR = 30% (7/23) vs 9% (1/11) for gpNMB overexpression (≥ 25% of tumor cells)	Yardley et al. (2015)
	IIB	213	mPFS = 2.9 months (95% CI: 2.8, 3.5) for the GV arm vs 2.8 months (95% CI: 1.6, 3.2) months for the capecitabine arm (HR = 0.95; 95% CI: 0.71, 1.29; p = 0.7607)	Vahdat et al. (2021)
Trabectedin	II	27	ORR = 14% (95% CI: 3.5–32%) mOS = 10 months (95% CI: 4.88–15.18 months)	Zelek et al. (2006)
	I	9	PR 55.5% (5/9), SD 33.3 (3/9)	Sessa et al (2009)
	II	44	mPFS = 1.9 months (95% CI: 1.8-3.5)PR 15.9% (7/44)	Awada et al, (2013)
	II	40	ORR = 17% (95% CI: 7,34)	Delaloge et al. (2014)
Lurbinectedin	II	54	ORR = 41% (95% CI: 28% to 55%)	Cruz et al. (2018)
		34	ORR = 9% (95% CI: 2% to 24%)	
	I	11	ORR = 17% (1/6); SD 67% (4/6)	Paz-Ares et al. (2017)
Plocabulin	I	5	SD 60% (3/5)	Elez et al. (2019)
	Marine-drugs with activity in BC treatment - ONGOING CLINICAL TRIALS			
RC48-ADC	II	20	pCR	NCT05134519
	III	366	PFS	NCT04400695
	Ib	112	RP2D	NCT03052634
	II/III	301	PFS	NCT03500380
CAB-ROR2-ADC (BA3021)	I/II	420	Safety Profile; AEs (Phase I)ORR (Phase II)E	NCT03504488
Enfortumab Vedotin	II	280	ORR	NCT04225117
Ladiratuzumab Vedotin		4000	pCR	NCT01042379
	b/II	211	ORR; AEs; Incidence of laboratory abnormalities; DLT	NCT03310957
	Ib/II	280	ORR; AEs; Incidence of laboratory abnormalities; DLT	NCT01969643
W0101	I/II	316	AEs	NCT03316638
Zilovertamab vedotin	II	210	ORR	NCT04504916

4.4 Disitamab vedotin

Another effective ADC is disitamab vedotin (RC48-ADC), which consists of a HER2 monoclonal antibody bound to the MMAE payload with a DAR of 4 by a cleavable protease linker. Upon binding, the MMAE is released into lysosomes and produces a variety of compounds that are conjugated or non-conjugated to trastuzumab in varying proportions, enhancing the cytotoxic activity of both drugs with high affinity and specificity (Yao et al., 2015; Abdollahpour-Alitappeh et al., 2019). *In vitro* studies confirmed that conjugated trastuzumab is more effective than unconjugated trastuzumab in inhibiting colony formation in HER2-positive cells, making the RC48-ADC a potential therapeutic option in HER2-positive BC (Yaghoubi et al., 2021).

A pooled analysis of two phase I studies on RC48-ADC (NCT02881138 and NCT03052634) have shown increasing response in terms of tumour shrinkage and PFS at higher doses, achieving an ORR of 40.0% (95% CI: 21.1, 61.3) and PFS of 6.3 months (95% CI: 4.3, 8.8) with a dose of 2.5 mg/kg in pre-treated HER2-postive BC (Wang et al., 2018). Similar results were observed in the HER2-low subgroup of BC. Hepatic function alteration and neuropathy were reported in 3/4 of cases, and neutropenia in nearly half. Most treatment-related adverse events (TRAEs) were mild to moderate in severity. The most favourable profile in terms of benefit-risk ratio occurred with a fortnightly administration at a dose of 2.0 mg/kg (Wang et al., 2021). Ongoing studies in previously treated HER2-positive BC are currently recruiting patients to test the drug efficacy in phase II and III clinical trials (NCT03500380; NCT04400695) and in neoadjuvant settings (NCT05134519). Noteworthy, to date, disitamab vedotin has received its first approval in China for the treatment of HER2-positive advanced gastric cancer (Deeks, 2021).

4.5 ALT-P7

A phase I trial on ALT-P7, a trastuzumab conjugated with two MMAE molecules, enrolled patients with advanced HER2-positive BC previously treated with at least two anti-HER2 therapies. ALT-P7 was well tolerated up to a dose of 4.2 mg/kg with DLT observed at 4.8 mg/kg. In pilot experience, twenty-two patients had an assessment at 6 weeks with a disease control rate of 77.3% (17/22), and a partial response in 2/15 cases with measurable disease (Park et al., 2020).

4.6 Zilovertamab vedotin

The expression of receptor tyrosine kinase-like orphan receptors (ROR) activated by noncanonical Wnt signalling pathway could represent a potential target for ADC therapy in BC (Zhang et al., 2012). ROR1 is targeted by an ADC called zilovertamab vedotin (ZV) which showed a fast internalisation and effective MMAE release. Preliminary evidence of strong anticancer activity in terms of ORRs have been documented for lymphoma; studies in TNBC are still ongoing (NCT04504916).

4.7 Glembatumumab vedotin

Glembatumumab vedotin (GV) consists of an antibody directed against NMB glycoprotein (gpNMB), a negative prognostic marker overexpressed in cancer cells, conjugated to MMAE (Maric et al., 2013). The randomised phase II trial EMERGE demonstrated no significant differences in ORR as compare to standard chemotherapy (6 vs. 7%), but the activity of GV appeared to be increased in TNBC and especially in case of gpNMB-overexpression (≥25% epithelial cancer cells staining positive by IHC) (Yardley et al., 2015). In the METRIC trial, patients with pretreated advanced TNBC overexpressing gpNMB were selected to receive GV or capecitabine at randomisation. Interestingly, the GV showed greater activity in tumour shrinkage compared with capecitabine, but with a shorter and transient duration of response (Vahdat et al., 2021).

4.8 Lonigutamab Ugodotin

Lonigutamab Ugodotin (W0101) is an ADC which targets the Insulin-like Growth Factor 1 receptor (IGF-1R) and delivers MMEA as a payload. A phase I trial has shown that it is able to induce tumour regression in BC models with IGF-1R overexpression without affecting normal cells (Akla et al., 2020). A first-ever pilot clinical trial (NCT03316638) is currently evaluating the safety profile of the drug in advanced or metastatic tumours, including BC.

5 Conclusion

To date, the only marine-derived drug approved for BC treatment is eribulin. As discussed, several other agents have been (or are still being) evaluated in clinical trials for the treatment of BC. Even though these agents have not yet entered the phase III phase, we believe that interesting progress is being made in studying these drugs in BC as well (Table 1).

Pharmacologic agents from natural products have always been used in the treatment of human diseases. The success rate for natural products to be developed into drugs is higher than in case of synthetic compounds (0.3 vs. 0.001%) (Atanasov et al., 2021). Among these, marine natural compounds show higher incidence of significant bioactivity which is associated with their rare and unique chemical structure. Indeed, they typically present both a direct and an indirect action on tumour cells and tumour microenvironment contrary to

classical chemotherapy agents with a specific cytotoxic activity (i.e., alkylating agents, antimetabolites, topoisomerase II inhibitors). This is particularly evident for eribulin, plocabulin, trabectedin and lurbinectedin. Eribulin causes tumor cells apoptosis by microtubule-targeting mechanisms but it also acts on tumour microenvironment, angiogenesis and epithelial-mesenchymal transition. Plocabulin has both a peculiar microtubule dynamics inhibition and a powerful vascular-disrupting activity. The mechanism of action of trabectedin and lurbinectedin also involves a direct cytotoxic mechanism on cancer cells and a modulation of transcription regulation of cancer and normal cells (i.e., macrophages) thus leading to microenvironment changes. As a result of this mechanism of action, as known from the experience of trabectedin in soft tissue sarcomas, it seems likely that trabectedin antitumor activity is more frequently associated with a disease stabilization, even prolonged, but not necessarily with an objective response rate according to RECIST criteria (De Sanctis et al., 2016). Furthermore, ADCs act not only on cells expressing the target antigen but also on off-target cells and tumor microenvironment, throughout the bystander effect. Some pharmacological characteristics, such as the hydrophobicity of the marine-derived payload auristatin, seem to play a major role in this effect.

Therefore, the antitumor activity of these compounds seems to arise from a combination of more than one mechanism and this may explain their predominant use as single agents as opposed to classical chemotherapeutic agents (especially in the adjuvant and neoadjuvant settings, where cytotoxic agents with different mechanisms of action are used in order to inhibit the emergence of broad spectrum drug resistance).

Notably, there must be thousands or even millions of, as yet, undiscovered marine organisms that may provide interesting new anticancer agents in the future. However, developing a new drug from a natural product is challenging and requires an interdisciplinary approach. Indeed, this long-term process typically takes 20–30 years and includes basic research, preclinical and clinical trials, but we believe that it is worth to be funded. Furthermore, a better knowledge of the factors involved in the sensitivity of individual tumour (and tumour subtype) might lead to a more rational and effective use of newly discovered marine-derived drugs.

Data availability statement

No new data were created or analysed in this study. Data sharing is not applicable to this article.

Author contributions

RD: methodology, investigation, writing—original draft, writing—review, and editing, and visualization; FJ: methodology, investigation, writing—original draft, writing—review, and editing; CB: methodology, investigation, writing—original draft, writing—review, and editing. MG: methodology, investigation, writing—original draft, writing—review, and editing; RF: drawing of chemical structures, writing—original draft and review; RT: methodology, investigation, writing—original draft, writing—review, and editing; PP: methodology, investigation, writing—original draft, writing—review, and editing; AS: methodology, investigation, writing—original draft, writing—review and editing; AZ: conceptualization, methodology, investigation, writing—original draft, writing—review, and editing, supervision.

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Figure 1 was created using Figure created in the Mind the Graph platform (https://mindthegraph.com/). Chemical structures Figures 2–4 were drawn using ChemDraw (https://perkinelmerinformatics.com/products/research/chemdraw).

Conflict of interest

AS: Advisory Board: Bristol-Myers-Squibb (BMS), Servier, Gilead, Pfizer, Eisai, Bayer, Merck Sharp & Dohme (MSD). Consultancy: Arqule, Sanofi, Incyte. Speaker's Bureau: Takeda, BMS, Roche, Abb-Vie, Amgen, Celgene, Servier, Gilead, Astrazeneca, Pfizer, Arqule, Lilly, Sandoz, Eisai, Novartis, Bayer, MSD (all outside the submitted work). AZ: personal fees and non-financial support from Novartis, AstraZeneca, Lilly, Pfizer, Daiichi Sankyo, MDS (Merck Sharp & Dome), Roche, Seagen, Exact Sciences, Gilaed, Istituto Gentili (all disclosures are outside the submitted work). RD: honoraria for advisory board consultancy from Novartis, Istituto Clinico Gentili, Amgen, and EISAI (all outside the present work).

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

Publisher's note

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Trabectedin and lurbinectedin: Mechanisms of action, clinical impact, and future perspectives in uterine and soft tissue sarcoma, ovarian carcinoma, and endometrial carcinoma

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The ecteinascidins trabectedin and lurbinectedin are very interesting antineoplastic agents, with a favorable toxicity profile and peculiar mechanisms of action. These drugs form adducts in the minor groove of DNA, which produce single-strand breaks (SSBs) and double-strand breaks (DSBs) and trigger a series of events resulting in cell cycle arrest and apoptosis. Moreover, the ecteinascidins interact with the tumor microenvironment, reduce the number of tumor-associated macrophages, and inhibit the secretion of cytokines and chemokines. Trabectedin has been approved by the Federal Drug Administration (FDA) for patients with unresectable or metastatic liposarcoma or leiomyosarcoma who received a prior anthracycline-based regimen. Moreover, trabectedin in combination with pegylated liposomal doxorubicin (PLD) has been approved in the European Union for the treatment of platinum-sensitive recurrent ovarian cancer. Lurbinectedin has been approved by the FDA for patients with metastatic small cell lung cancer with disease progression on or after platinum-based chemotherapy. The review assesses *in vitro* and *in vivo* experimental studies on the antineoplastic effects of both ecteinascidins as well as the clinical trials on the activity of trabectedin in uterine sarcoma and ovarian carcinoma and of lurbinectedin in ovarian carcinoma and endometrial carcinoma.

KEYWORDS

trabectedin, lurbinectedin, tumor microenvironment, uterine sarcoma, ovarian cancer, endometrial cancer

Introduction

Trabectedin is the lead compound of ecteinascidins originally isolated from the extracts of the tunicate *E. turbinate* (1, 2), with antitumoral activity in patients with sarcoma and especially in those with liposarcoma or leiomyosarcoma after prior anthracyclines (3, 4). The toxicity profile of the drug is favorable, especially with corticosteroid premedication, with the most adverse events (AEs) being grade 1–2, reversible and non-cumulative liver and hematological toxicity (5, 6). In a phase Italian 2 study that administered trabectedin 1.3–1.5 mg/m^2 to elderly patients with advanced sarcoma, the trabectedin plasma clearance and distribution volume were 39.98 L/h/m^2 and 1460 L/m^2, respectively (7). In October 2015, trabectedin has been approved by the Federal Drug Administration

(FDA) for patients with unresectable or metastatic liposarcoma or leiomyosarcoma who received a prior anthracycline-based regimen (8). Trabectedin is also active in relapsed ovarian cancer (9–12). In a randomized phase study, trabectedin + pegylated liposomal doxorubicin (PLD) was associated with a significantly longer progression-free survival (PFS) compared with single-agent PLD in patients with platinum-sensitive recurrent ovarian cancer, with the greatest benefit observed in patients with a platinum-free interval (PFI) of 6–12 months (13, 14). Since 2009, trabectedin in combination with PLD has been approved in the European Union and in other countries for platinum-sensitive recurrent ovarian cancer (15).

Lurbinectedin is a new synthetic alkaloid structurally related to ecteinascidins, with different pharmacokinetic and pharmacodynamic properties compared with trabectedin (16, 17). The analysis of data from several phase II trials with lurbinectedin found that the plasma clearance and apparent volume at the steady state of this drug were 11.2 L/h and 438 L, respectively (18). This first-in-human study identified a 7.0 mg flat dose (1-h infusion) every 3 weeks (q3wk) as the phase II recommended dose for lurbinectedin (17). The primary toxicity was myelosuppression, with neutropenia nadir occurring during and without treatment delays in most cases. Other common AEs were mild/moderate fatigue, nausea, and vomiting. A subsequent phase I study supported the administration of lurbinectedin 5 mg 1-h infusion on days 1 and 8 3qwk and suggested to test this novel schedule in future phase II studies (19). Some phase I and II studies on lurbinectedin combined with gemcitabine (GEM) or doxorubicin (DOX) have confirmed good clinical tolerability (20–22). A phase I trial of lurbinectedin + GEM found that the recommended dose was lurbinectedin 3.0 mg flat dose + GEM 800 mg/m^2 on days 1 and 8 q3wk (20). This regimen had manageable toxicity, mainly consisting of grade 3–4, not cumulative myelotoxicity. DOX 50 mg/m^2 + lurbinectedin 4.0 mg flat dose q3wk was the recommended dose in a phase I trial including patients with recurrent small cell lung cancer (SCLC) (21).

Lurbinectedin has significant antitumor efficacy with tolerable AEs in patients with platinum-sensitive and platinum-resistant SCLCs and in those with recurrent SCLCs after second-line treatment, and this agent has been approved by the FDA for patients with metastatic SCLCs with disease progression on or after platinum-based chemotherapy (23, 24). Lurbinectedin has also shown activity against malignant pleural mesothelioma (25, 26); sarcoma, especially leiomyosarcoma, myxoid liposarcoma, and dedifferentiated liposarcoma (22); and ovarian (27–29) and endometrial carcinoma (29–31).

This narrative review of the literature performed through PubMed assesses the *in vitro* and *in vivo* experimental studies as well as the clinical trials on trabectedin and lurbinectedin in gynecological cancers.

Mechanisms of actions of trabectedin and lurbinectedin

The tumor microenvironment (TME), especially tumor-associated macrophages (TAMs), can release growth factors, cytokines, and chemokines that promote inflammation and neoangiogenesis (32, 33). Therefore, agents targeting TAMs and the other components of TME, such as trabectedin and lurbinectedin, can offer interesting perspectives of biological and clinical research in cancer treatment.

Trabectedin forms adducts in the minor groove of DNA that produce single-strand breaks (SSBs) and double-strand breaks (DSBs) and trigger a series of events resulting in cell cycle arrest and apoptosis. Moreover, trabectedin reduces the number of TAMs and myeloid-derived suppressor cells (MDSCs) and inhibit the secretion of inflammatory cytokines and chemokines (34, 35). Trabectedin selectively induces apoptosis in monocytes/macrophages *via* the activation of caspase-8 but not in other leukocyte subsets, probably because of a differential expression of the functional tumor necrosis factor (TNF)–related apoptosis-inducing ligand receptors (TRAIL-Rs). In blood leukocytes, functional TRAIL-Rs (TRAIL-R1 and TRAIL-R2) are exclusively detected in monocytes, while neutrophils and T cells express only the decoy non-signaling TRAIL-R3 and are spared by trabectedin. As shown in *in vitro* and *in vivo* studies on lipomixoid sarcoma, trabectedin inhibits the transcription of CCL2, CXCL8, interleukin (IL)-6, and the vascular endothelial growth factor (VEGF) (36). These anti-inflammatory effects have also been demonstrated in tumor xenografts and in human soft tissue sarcoma samples from patients treated with trabectedin (35). These mechanisms of action have been confirmed by the persistent *in vivo* antitumor activity of trabectedin in mice injected with tumor cells resistant to trabectedin *in vitro*. Therefore, the effects of the drug on the TME and TAMs play a major role in its antitumor and antimetastatic activity (34).

Lurbinectedin is a next-generation DNA minor groove binder that exerts potent antitumor activity in a low nanomolar range (16, 17). In several human cancer cell lines, lurbinectedin blocks the transcription process through binding to CG-rich sequences near the promoters of protein-coding genes (37). Moreover, this drug

triggers both the degradation of phosphorylated RNA polymerase II (Pol II) on the DNA template and the generation of SSBs and DSBs that drive tumor cells to apoptosis. The ovarian cells resistant (IGROV-ET) to ecteinascidin-743 ovarian cancer cells, which overexpress P-glycoprotein and are resistant to DOX, etoposide, and trabectedin, are less sensitive to lurbinectedin. Therefore, lurbinectedin must accumulate in the cell to exert its antiproliferative effect. In murine models subcutaneously xenografted with A549 lung adenocarcinoma cells, the tumor growth inhibition following lurbinectedin treatment correlates with both Pol II degradation and DNA damage induction. *In vitro* studies, a short exposure to 5 nM lurbinectedin significantly reduced the production of CCL2, CXCL8, and VEGF by lipopolysaccharide-stimulated monocytes and decreased the migration of monocytes (38). A gene profiling analysis of the monocytes after exposure to lurbinectedin, trabectedin, and DOX showed that the transcriptomes modulated by lurbinectedin and trabectedin were similar each other and quite different from those modulated by DOX (38). Several genes of the RhoGTPase family, involved in different cell functions such as actin cytoskeleton organization and cell motility (39), were sharply downregulated by both ecteinascidins (38). *In vitro* and *in vivo* experimental studies have shown that lurbinectedin exerts the same selective effects of trabectedin on the TME (38). Lurbinectedin elicits the caspase-8-dependent apoptosis in monocytes/macrophages that express functional TRAIL-R1 and TRAIL-R2, but not in neutrophils and T cells that express the decoy TRAIL-R3 (35, 40). Moreover, lurbinectedin reduces the secretion of CCL2, CXCL8, and the VEGF (38). It has been hypothesized that lurbinectedin at high doses promotes the apoptosis of monocytes and TAMs, whereas the drug at low concentrations impairs monocyte migration and adhesion through the inhibition of genes involved in the regulation of the actin cytoskeleton and suppresses the secretion of inflammatory cytokines and the VEGF in the TME.

Both trabectedin and lurbinectedin activate the ataxia-telangiectasia mutated (ATM)/checkpoint kinase (Chk)2 and ATM and RAD3-related (ATR)/Chk1 pathways in HeLa cells (41). The simultaneous inhibition of both ATM and ATR enhances the activity of ecteinascidins by suppressing the generation of γ-H2AX, BRCA1, and Rad51 foci after exposure to these agents. Moreover, this double inhibition significantly improves the cytotoxicity of both ectainescidins against cisplatin (CDDP)-sensitive and CDDP-resistant ovarian cancer cells. Therefore, ATR and ATM seem to be the major regulators of the DNA damage response to ecteinascidins.

Ecteinascidins generate DSBs that are processed through homologous recombination (HR), thus rendering HR-deficient cells very sensitive to these agents (42, 43). *In vitro* studies on different mammalian isogenic cell lines showed that the sensitivity to trabectedin and lurbinectedin was 2–4-fold greater in Nucleotide excision repair (NER)-proficient cells and 150–200-fold greater in HR- deficient cells (43).

The cytotoxicity of ecteinascidins against human ovarian cancer cells was reduced by the addition of ascitic fluid from either nude mice or ovarian cancer patients (44). The cytotoxicity of lurbinectedin was completely abolished, whereas that of trabectedin was sharply decreased. The same effects were observed when a culture medium was added with α1-acid glycoprotein, usually present at relatively high concentrations in ascites, which appeared to suggest that this protein was involved in cytotoxicity inhibition.

Antineoplastic activity of trabectedin: *In vitro* and *in vivo* experimental studies and clinical studies in sarcoma and ovarian cancer

Trabectedin shows significant antitumor activity in ovarian clear cell carcinoma cells ***in vitro*** and in mice inoculated with ovarian clear cell carcinoma cell lines ***in vivo*** (45). Trabectedin induces mammalian target of rapamycin (mTOR) activation in an V-akt murine thymoma viral oncogene homolog (AKT)-dependent manner, and mTOR inhibition by everolimus prevents ovarian clear cell carcinoma cells from acquiring resistance to trabectedin. Therefore, the combination of trabectedin and everolimus deserves further investigation for the treatment of this histological type.

The combined administration of trabectedin and the anti-PD1 antibody suppressed the peritoneal tumor formation in mice transplanted intraperitoneally 10 days previously with murine ID8 ovarian cancer cells. Long-term surviving mice were resistant to the rechallenge by the subcutaneous injection of ID8 ovarian cancer cells but not the subcutaneous injection of unrelated TC1 lung cancer cells, which suggested the development of a tumor-specific memory immune response. The analysis of peritoneal washing of mice 7 days after treatment start revealed a significant increase of the effector $CD4^+FoxP3^-$ T cells and $CD8^+$ T cells and a significant decrease of the immunosuppressive T-reg cells and MDSCs.

Poly(ADP-ribose) (PAR) polymerase (PARP) inhibitors (PARP-is) have been assessed and evaluated in patients with BRCA-mutated ovarian, breast, prostate, and pancreatic cancers (46). Through the suppression of base excision repair (BER), PARP-is promote synthetic lethality in HR-deficient cells (47). Moreover, PARP-is exert many several pharmacological effects other than synthetic lethality and they can also be active in patients with wild-type BRCA and HR-proficient tumors (48–55). The combination of PARP-i and DNA-damaging agents could be very interesting, but its feasibility is usually limited by myelosuppression (56–58). However, trabectedin could be an ideal agent to combine with PARP-i (34). In preclinical models,

trabectedin activates PARP1 and the combined use of trabectedin and olaparib produces a greater antineoplastic antitumor activity than each single drug (59). An open-label multicenter, phase 1b study on patients with recurrent bone and soft-tissue sarcoma showed that trabectedin + olaparib had a favorable toxicity profile and that trabectedin 1.1 mg/m^2 (24-h infusion) q3wk + olaparib 150 mg twice daily (BID) were the recommended doses for a two-phase study (60).

Trabectedin is active is second or further line of therapy in patients with heavily pretreated uterine leiomyosarcoma (61–64), and a significant proportion of these patients obtain a long-term clinical benefit (Table 1). It is noteworthy that in the Trabectedin Activity in Uterine Leiomyosarcoma (TAUL) study, including pretreated patients with metastatic or locally relapsed uterine leiomyosarcoma, the activity of trabectedin (1.3 mg/m^2 24-h infusion q3wk) was independent of the number of prior chemotherapy lines (64). Trabectedin has also shown promising activity in undifferentiated uterine sarcoma (65).

DOX 60 mg/m^2 followed by trabectedin 1.1 mg/m^2 (3-h infusion) q3wk with granulocyte-colony stimulating factor support was administered to 108 patients with advanced or metastatic uterine or soft tissue leiomyosarcoma in a multicenter phase II trial (66). Median PFS and median OS were 10.1 and 34.4 months in the whole series, 8.3 and 27.5 months in patients with uterine leiomyosarcoma, and 12.9 and 38.7 months in patients with soft tissue leiomyosarcoma, respectively. Toxicities were predominantly hematological and hepatic. The NCT02997358 randomized phase III trial is currently comparing DOX + trabectedin followed by trabectedin *versus* single-agent DOX as first-line therapy in patients with metastatic or unresectable uterine or soft tissue leiomyosarcoma.

As for ovarian cancer, docetaxel 60 mg/m^2 followed by trabectedin 1.1 g/m^2 (3-h infusion) q3wk with G-CFS support was given to 71 patients with recurrent disease after up to three prior regimens (67). The response rate, median PFS, and median OS were 30%, 4.5 months, and 16.9 months, respectively. Grade 3–4 leukopenia, neutropenia, thrombocytopenia, and metabolic AEs occurred in 29.6%, 29.6%, 9.9%, and 14.1% of the patients, respectively.

In the OVA-301 trial, trabectedin 1.1 mg/m^2 (3-h infusion) + PLD 30 mg/m^2 q3wk was associated with significantly better PFS and OS compared with single-agent PLD 50 mg/m^2 q4wk in recurrent ovarian cancer patients with a PFI of 6–12 months (14). The patients of the trabectedin + PLD arm experienced a significantly longer interval time from randomization to subsequent platinum as well as significantly longer survival from the start of platinum rechallenge. A subset analysis of this trial appeared to evidence the superiority of the combination in terms of PFS and OS in patients with mutated BRCA but not in those with wild-type BRCA (68). A phase 3 randomized trial, aimed to assess trabectedin + PLD as a third-line chemotherapy in patients with platinum-sensitive recurrent ovarian cancer who had received two prior platinum-based regimens, detected that patients with both a mutated BRCA and a PFI of 6–12 months had 62.6% reduction in the risk of death with this combination compared with single-agent PLD (69). On the other hand, a prospective European phase IV trial of trabectedin + PLD found no differences in response rates and PFS according to the BRCA status in patients with platinum-sensitive recurrent ovarian cancer (15). Real-word evidence has confirmed that trabectedin + PLD is an effective non-platinum combination in this clinical setting (70).

In vitro and *in vivo* studies on trabectedin-resistant ovarian cancer and myxoid liposarcoma cell lines have revealed that tumor cells that are persistent after trabectedin are NER deficient and sensitive to platinum compounds (71). Casado et al. (72) retrospectively assessed patients with recurrent ovarian cancer who received trabectedin at initial doses ranging between 1.1 and 1.5 mg/m^2 (3-h infusion) q3wk. The agent achieved an objective response and a disease control in 18.2% and 59.1% of the 22 evaluable patients. Afterward, 17 patients underwent a platinum rechallenge, with an objective response rate and a disease control rate of 41.2% and 47.0%, respectively. Therefore, trabectedin could sensitize neoplastic cells to platinum retreatment, through both interaction with NER components in tumor cells and the inhibition of inflammatory mediators in the TME (73).

Antineoplastic activity of lurbinectedin: *In vitro* and *in vivo* experimental studies and clinical studies in sarcoma, ovarian cancer, and endometrial cancer

A phase II study on heavily pretreated metastatic and/or unresectable sarcomas reported a 24-week disease control in 8

TABLE 1 Trabectedin in patients with recurrent uterine leiomyosarcoma.

Authors	pts	No. of priorchemotherapy lines	ORR (%)	SDR (%)	Clinical outcome
Judson (61)	62	0–6	17.7	32.3	6-month PFS = 30.7%
Sanfilippo (62)	66	1–5	16.7	34.8	6-month PFS = 33%
Hensley (63)	134	1–4 or more	11.2	19.4	Median PFS = 4 months (range: 2.43–4.60)
Gadducci (64)	108	1–3	23.5	7.4	6-month PFS = 35.2%

pts, patients; ORR, objective response rate; SDR, stable disease rate; PFS, progression- free survival

(40%) of 20 anthracycline-naïve patients treated with DOX 50 mg/m^2 + lurbinectedin 2 mg/m^2 on day1 q3wk, in 2 (20%) of the 10 patients with prior anthracyclines who received GEM 800 mg/m^2 + lurbinectedin 1.6 mg/m^2 on days 1 and 8 q3wk, and in none of the 12 patients with prior anthracyclines and GEM treated with single-agent lurbinectedin 3.2 mg/m^2 q3wk (22). Leiomyosarcoma, myxoid liposarcoma, and dedifferentiated liposarcoma were the subtypes with greater clinical benefit with DOX + lurbinectedin.

Similarly to trabectedin, lurbinectedin exerts antitumor activity against human ovarian clear cell carcinoma cells *in vitro* as well as against mouse ovarian clear cell carcinoma cell xenografts *in vivo* (74). Lurbinectedin shows a significantly greater cytotoxicity on human ovarian clear cell carcinoma cells compared with PTX, DOX, SN-38 (which is an active metabolite of irinotecan), and CDDP. The combination of lurbinectedin and SN-38 has a stronger synergistic effect. The lurbinectedin-resistant subline RMG1-LR derived from the human ovarian clear cell carcinoma cell line RMG1 has an increased P-glycoprotein expression compared with the parental cell line. SN-38 is able to reduce the expression of this protein involved in lurbinectedin resistance in a dose-dependent manner. In nude mice injected with RMG1 cells, the administration of lurbinectedin and irinotecan decreased tumor burden by 85.1% compared with phosphate-buffered saline treatment, and this growth-inhibitory activity was significantly stronger than that obtained with each single agent. Irinotecan has been employed in *in vivo* studies on xenograft models because the use of SN-38 was limited by its poor aqueous solubility (75).

mTORC1 is often activated in the clear cell carcinoma of the ovary (76). The mTORC1 inhibitor everolimus significantly increases the antitumor effects of both lurbinectedin alone and lurbinectedin + SN-38 in clear cell carcinoma cell lines (74). The phase II trial NCT01196429 was planned to assess the combination of temsirolimus with carboplatin (CBDCA) + PTX followed by temsirolimus maintenance as a first-line therapy in patients with stage III–IV ovarian clear cell carcinoma. This treatment was well tolerated but failed to improve 12 month-PFS when compared to historical controls (77).

Orthotopic tumor graft models, which retain the characteristics of the original primary tumor, are useful tools for identifying novel therapeutic targets and for testing new drugs (78, 79). The tumor tissue named OVA1X, collected from a patient who had not received CDDP-based chemotherapy, and the CDDP-resistant tumor named OVA1XR, developed through repeated *in vivo* exposures to the CDDP of OVA1X, were transplanted into nude mice (79). When the tumors reached a homogeneous palpable size, the animals were randomly assigned to receive placebo, lurbinectedin, CDDP, and a combination of the two drugs. Compared with placebo, CDDP, lurbinectedin, and lurbinectedin + CDDP obtained tumor weight reductions of 95.3%, 88.3%, and 87.2%, respectively, in CDDP-sensitive tumor grafts and of 48.2%, 93.6% and 96.7%, respectively, in CDDP-resistant tumor grafts. Lurbinectedin-induced tumor responses were mediated by both anti-proliferative and pro-apoptotic effects.

Poveda et al. (27) planned a two-stage, phase II trial including heavily pretreated patients with platinum-resistant/refractory ovarian cancer. The first stage assessed the activity of lurbinectedin 7.0 mg flat dose (1-h infusion) q3wk in 22 women, whereas the second stage randomized 59 patients to receive either lurbinectedin with the same dose and schedule or topotecan (either 0.75–1.5 mg/m^2 on days 1–5 q3wk or 2.4–4 mg/m^2 on days 1, 8, and 15 q4wk). An objective response was detected in 23.1% of the 52 patients treated with lurbinectedin, with a median duration of response of 4.6 months (Table 2). In the second randomized stage of the study, an objective response was noted in 17% of 30 patients treated with lurbinectedin *versus* 0% of the 29 treated with topotecan. The corresponding median PFS was 3.9 months *versus* 2.0 months (p= 0.0067), and the corresponding median OS was 9.7 months *versus* 8.5 months (p= 0.2871). Severe neutropenia, febrile neutropenia, and severe 3–4 thrombocytopenia occurred in 85%, 21%, and 33% of the patients treated with lurbinectedin.

The CORAIL phase III trial randomized 442 heavily pretreated patients with platinum-resistant ovarian cancer to receive either

TABLE 2 Lurbinectedin-based chemotherapy in recurrent ovarian and endometrial cancer.

Authors	CT	pts	PFImonths	ORR(%)	Median PFSmonths	Median OSmonths
Poveda(27)	Lurbenectedin	52^	<6	23.1	4.0	10.6
Gaillard (28)	Lurbinectedin	221^	<6	14.5	3.5	11.4
Poveda (30)	Lurbinectedin + olaparib	46^	NA§	6.6	4.5	-
Poveda (30)	Lurbinectedin + olaparib	26*	NA§	15.4	4.8	–
Kristeleit (31)	Lurbinectedin + DOX	19*	NA§§	42.1	7.7	14.2

CT, chemotherapy, pts, patients; PFI, platinum-free interval; ORR, objective response rate, PFS, progression-free survival, OS, overall survival; NA, not available.
^pts with ovarian cancer, *pts with endometrial cancer.
§1–4 or more prior chemotherapy lines.
§§1–2 prior chemotherapy lines (not including anthracycline).

lurbinectedin 3.2 mg/m^2 (1-h infusion) 3qwk or investigator choice's therapy (consisting of either PLD 50 mg/m^2 q4wk or topotecan 1.5 mg/m^2 on days 1–5 q3wk) (28). Median PFS was 3.5 months in the lurbinectedin arm *versus* 3.6 months in the control arm (HR = 1.057, 95%CI = 0.854–1.309), respectively; the corresponding median OS was 11.4 months *versus* 10.9 months (HR = 0.956, 95%CI = 0.772–1.183), and the corresponding objective response rates were 14.5% *versus* 12.7% (p = 0.6772) (Table 2). The analysis of the BRCA status in tumor tissues from the patients of lurbinectedin arm showed better median OS for patients with mutant BRCA than for those with wild-type BRCA (16.9 months *versus* 10.8 months *p*= 0.0495). Severe AEs, mainly hematological, were more common in the control arm. The elevated incidence of bone marrow toxicity in the control arm was probably due to the administered doses of topotecan, which were higher than those currently used in the clinical practice.

The phase I PM01183 in Combination With Olaparib in Advanced Solid Tumors (POLA) study tested the combination of lurbinectedin on day 1 + olaparib BID on days 1–5 3qwk in 20 patients with ovarian and endometrial cancer previously treated with systemic chemotherapy (29). Lurbinectedin 1.5 mg/m^2 + olaparib 250 mg BID was found to be the recommended phase II dose. None of the patients achieved an objective response, but 60% of these obtained disease stabilization. In the subsequent phase II POLA trial, the combination of lurbinectedin 1.5/m^2 on day 1 + olaparib 250 mg BID on days 1–5 3qwk was administered to heavily pretreated patients with high-grade ovarian cancer, endometrial cancer, and triple-negative breast cancer (30). There was a trend to a better overall response rate in the patients with endometrial cancer than in those with ovarian cancer (p = 0.057) (Table 2). No correlation was found between response to treatment and the HR status. The most common severe AEs were hematological, predominantly neutropenia reported in 38.3% of the patients. This combination deserves further investigation in patients with recurrent ovarian and endometrial cancer.

A two-stage, phase I study assessed 34 anthracycline-naïve patients with an advanced endometrial cancer of any histological type who had been treated with one or two prior chemotherapy lines and who received a combination of DOX + lurbinectedin q3wk (31). In the escalation phase, DOX 50 mg/m^2 + lurbinectedin 3.0–5.0 mg (1-h infusion) achieved an objective response in 26.7% of 15 patients, with a median PFS of 7.3 months. In the expansion cohort, this combination at the recommendation dose of DOX 40 mg/m^2 + lurbinectedin 2.0 mg obtained an objective response in 42.1% of 19 patients (Table 2). Transient severe anemia, neutropenia, and thrombocytopenia occurred in 31.6%, 78.9%, and 15.8%, of the patients, respectively. These results compared favorably with those previously observed with several drugs tested in the second-line setting and were similar to those reported with the combination of lenvatinib + pembrolizumab (80, 81). In fact, DOX 40 mg/m^2 + lurbinectedin 2.0 mg and lenvatinib 20 mg daily + pembrolizumab 200 mg q3wk achieved the objective response rates of 42.1% and 38.3%, respectively (31, 82)

Conclusions

Trabectedin and lurbinectedin, which affect both tumor cells and the TME, are also very interesting antineoplastic agents in gynecological cancers with a peculiar mechanism of action and an acceptable toxicity profile. Trabectedin is commonly used in the second and further line therapy of patients with recurrent uterine leiomyosarcoma, with a significant proportion of patients still in treatment after several months. This reflects both the paucity of drug-related AEs and the prolonged tumor control. The anti-inflammatory and immunomodulatory properties of the drug could play a major role in long-term responders. Trabectedin + PLD is an effective combination for the treatment of patients with platinum-sensitive recurrent ovarian cancer and especially in those with a PFI of 6–12 months. In a phase I study on heavily pretreated patients with advanced endometrial cancer, the combination of lurbinectedin + DOX obtained the same results as the combination of lenvatinib plus pembrolizumab in a similar clinical setting.

As far as future perspectives are concerned, since *in vitro* and *in vivo* experimental studies suggest that both trabectedin and lurbinectedin are active against ovarian clear cell carcinoma, these ecteinascidins should be tested in clinical trials including patients with this histological type that is poorly sensitive to platinum-based chemotherapy. A phase III clinical trial on heavily pretreated patients with platinum-resistant ovarian cancer showed that lurbinectedin had similar antitumor activity and a favorable safety profile compared to the control arm consisting of PLD or topotecan. Additional biological and clinical research is warranted to detect biomarkers predictive of response to lurbinectedin and to assess the combination of lurbinectedin with other agents.

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

Conceptualization: AG. Data curation: AG. Methodology: AG. Project administration: AG and SC. Writing—original draft: AG. Revision and editing: AG and SC. 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.

Publisher's note

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