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REVIEW

Role of trabectedin in the treatment of soft tissue sarcoma

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Abstract: Interest in marine natural products has allowed the discovery of new drugs and trabectedin (ET-743, Yondelis), derived from the marine tunicate *Ecteinascidia turbinata*, was approved for clinical use in 2007. It binds to the DNA minor groove leading to interferences with the intracellular transcription pathways and DNA-repair proteins. *In vitro* antitumor activity was demonstrated against various cancer cell lines and soft tissue sarcoma cell lines. In phase I studies tumor responses were observed also in osteosarcomas and different soft tissue sarcoma subtypes. The most common toxicities were myelosuppression and transient elevation of liver function tests, which could be reduced by dexamethasone premedication. The efficacy of trabectedin was established in three phase II studies where it was administered at 1.5 mg/m^2 as a 24 h intravenous infusion repeated every three weeks, in previously treated patients. The objective response rate was 3.7%–8.3% and the tumor control rate (which included complete response, partial response and stable disease) was obtained in half of patients for a median overall survival reaching 12 months. In nonpretreated patients the overall response rate was 17%. Twenty-four percent of patients were without progression at six months. The median overall survival was almost 16 months with 72% surviving at one year. Predictive factors of response are being explored to identify patients who are most likely to respond to trabectedin. Combination with other agents are currently studied with promising results. In summary trabectedin is an active new chemotherapeutic agents that has demonstrated its role in the armamentarium of treatments for patients with sarcomas.

Keywords: soft tissue sarcoma, trabectedin, chemotherapy, DNA-minor groove binder

Soft tissue sarcomas

Soft-tissue sarcomas are a highly heterogeneous group of tumors with a low incidence. They account for 1% of all adult and 15% of pediatric malignancies.[1,2] At least 50 different subtypes are distinguished, each with its specific biology and clinical outcome. The majority of these tumors are presumably derived from mesoderm and categorized by the normal tissue they resemble. As they are uncommon, however, they are subsumed under the collective term "soft-tissue sarcoma" and therapeutically approached in similar ways.

Despite adequate local treatment, up to one half of patients develop distant metastases. Most of these patients with advanced (ie, not resectable) or metastatic disease die from the disease after a median survival time from diagnosis ranging between eight to 12 months.[3] The most common approach in all histological subtypes is to use doxorubicin and ifosfamide.[4] Doxorubicin remains the reference in first line therapy with response rate of 20%–30% in large randomized trials.[4–6] As a monotherapy,

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ifosfamide yielded response rate similar to doxorubicin.[5–8] It is widely used as second-line therapy after anthracycline failure with a response rate in the 10%–20% range.[8,9]

Combining these agents might be more effective but has not been shown to improve survival in a significant way compared to their use as single agents.[6] Following suggestions that these agents may have a dose-response relationship,[9–11] various phase-I and -II trials were performed in which they were administered at high doses, yielding response rates of 40%–66%[12–15,16] leading to median overall survival of up to 24 months in some studies. There are also suggestions that applying regimens with high response rates may render an initially inoperable tumor operable, thereby improving the outcome.[17] But in two recent randomized trials,[18–20] neither the response rate nor the overall survival could be improved by the intensification of chemotherapy, even if the progression-free survival was increased in one study.[18]

In recent years, progress has been made in understanding the clinical and biological complexity of sarcomas,[21,22] such that oncologists have increasingly been able to define customized therapies. Paclitaxel has been found to be active mainly in patients with angiosarcomas.[23] Docetaxel combined with gemcitabine has been shown to induce a 53% response rate in leiomyosarcomas of gynecological origin.[24] Topoisomerase inhibitors are active for rhabdomyosarcomas.[25] And the identification of genetic abnormalities with the presence of fusion genes that are strongly associated with specific sarcoma subtypes has contributed towards understanding the biology of these tumors, and has facilitated their diagnosis. It has also opened new avenues for therapies targeted against the transcription pathways induced by these fusion proteins.[26,27]

In the past years, the interest in marine natural products has provided new possibilities of drug discovery.[28] The first clinical agent derived from nucleosides obtained from marine sponge was cytosine arabinoside. Other marine compounds are in development such as aplidine obtained from the Mediterranean tunicate *A. albicans* or bryostatin from the bryozoan *Bugula neritina*. But trabectedin (ET-743, Ecteinascidin-743) is the first marine anticancer drug approved for clinical use in the European Union since 2007.

Trabectedin

Structure, mechanism of action, preclinical antitumor activity and phase I trials

Trabectedin (ET-743, Ecteinascidin, Yondelis) is a DNA-binding agent derived from the marine tunicate *Ecteinascidia turbinata*, now obtained by a semi-synthetic process starting with the antibiotic cynosafracin B.[29,30] The molecule is comprised of three fused tetrahydroisoquinoline rings (Figure 1) of which two are covalently and reversibly bound to the DNA minor groove by a guanine specific alkylation at the N2 position. This induces a DNA bending towards the major groove.[31,32] The third ring, being unbound, could interact with nuclear proteins. The transcriptional activation of inducible genes is inhibited, depending on the presence of the DNA repair systems, particularly the transcription coupled nucleotide excision repair system.[33] The sensitivity to trabectedin is related to the presence of active repair mechanisms.[34] A cell cycle arrest at G2/M and apoptosis is induced through a p53-independent process.[35]

In vitro, trabectedin has a broad spectrum of activity in human primary tumor cells.[36] The drug was very active against several different sarcoma cell lines[37] and the results of activity in human tumor xenografts demonstrated activity in melanoma, non-small cell lung cancer and ovarian cancer.[38,39]

Pharmacokinetics and phase I studies

After infusion, trabectedin is rapidly cleared from plasma, undergoing an extensive hepatic metabolism mainly through the cytochrome P450 3A4.[40] Trabectedin has a high apparent volume of distribution and a half-life of approximately 90 hours.[41] It is mainly excreted in the feces but less than 1% is excreted unchanged in the urine or feces.[42]

In phase I studies (Table 1), different schedules were investigated in patients with solid tumors. This schedules range from an intravenous infusion over 1, 3, 24, or 72 hours every three weeks to a weekly infusion for three out of a four-week cycle.[43–47] The dose-limiting toxicities (DLT) were mainly hematological (neutropenia, thrombocytopenia) and fatigue. Grade 3–4 hepatic toxicities were also DLT in some but not all trials.[45,46] DLT were transient and not cumulative. Nausea and vomiting were managed with 5HT3-antagonists. Fatigue or asthenia grade 3–4 was also frequently described (8% to 50%). Tumors responses were observed in soft tissue sarcoma, osteosarcoma, melanoma, and breast cancer.

Two schedules were recommended for further phase II studies: 1.5 mg/m^2 as a 24-hour continuous infusion given every three weeks and 0.58 mg/m^2 as a three-hour infusion given weekly for three out of four weeks.

Phase II studies of trabectedin in pretreated sarcomas

The efficacy of trabectedin was studied in four phase-II studies in pretreated soft tissue sarcoma from US and

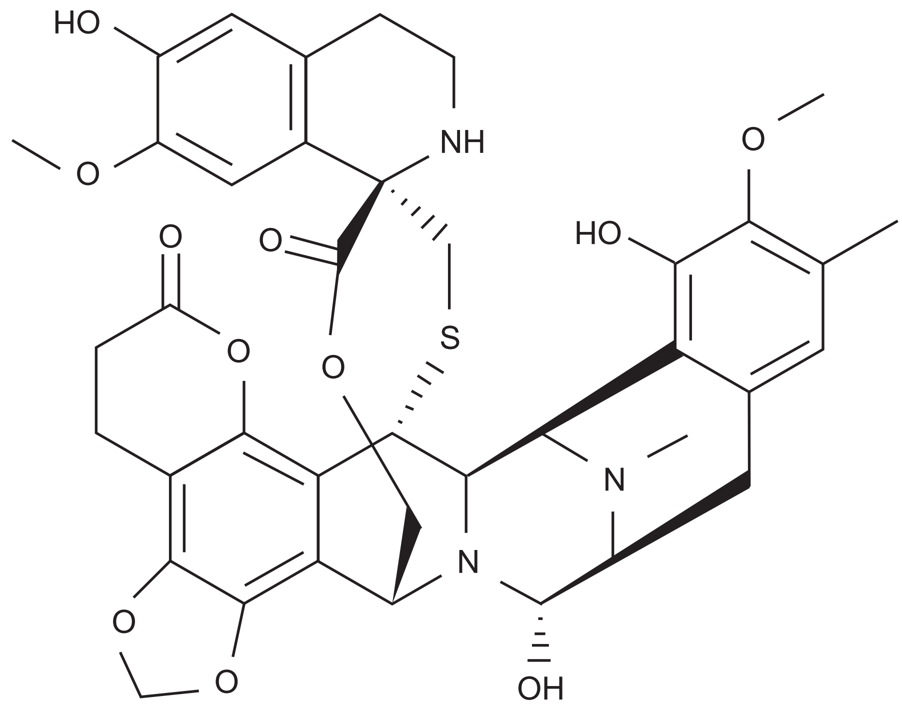

Figure 1 Structure of trabectedin.

Europe[48–50] of which one was a randomized phase II study comparing two different schedules[51] (Table 2).

The first trial, published in 2004 by Yovine and colleagues,[48] investigated trabectedin 1.5 mg/m^2 administered as a 24-hour continuous infusion. Fifty-four patients were enrolled and analyzed in two predefined groups. The first group of 26 patients was pretreated with only one or two single agents or one combination regimen and the second group of 28 patients were heavily pretreated with at least three chemotherapeutic agents or two combinations. The predominant histology was leiomyosarcoma (n = 22, of uterine origin (n = 8)), and liposarcoma (n = 6). Two partial responses (3.7% [95% confidence interval (CI): 0.5%–12.8%]) were reported and both had uterine leiomyosarcoma, four (7.4%) patients had a minor response, and nine (17%) patients had stable disease. Among the six responding patients, five received prior chemotherapy with ifosfamide and doxorubicin. The median progression-free survival was 1.9 months after a median follow-up of 26 months. At three and six months, 38.8% and 24.1% of patients were progression-free, respectively. No difference was noted in progression-free survival between the two groups. But the less pre-treated patients had a longer median overall survival of 13.7 months versus 7.9 months. Overall, the median survival was 12.8 months with 30% of patients alive at two years.

Garcia-Carbonero and colleagues reported the results of the phase II study with trabectedin 1.5 mg/m^2 administered as a 24-hour continuous infusion, conducted in 36 patients pretreated with up to two prior chemotherapy regimens.[50] Some histological subtypes were excluded as gastrointestinal stromal tumors (GIST), mesothelioma, osteosarcoma, carcinosarcoma, Kaposi's sarcoma, or rhabdomyosarcoma. And the main histologies were leiomyosarcomas (n = 13), liposarcomas (n = 10), and synovial sarcomas (n = 6). One complete response (CR) was obtained in a patient with liposarcoma, and two partial responses (PR) in a patient with leiomyosarcoma and in a patient with liposarcoma, for an overall response rate of 8% (95% CI: 2%–23%). Two other patients had a minor response. The median time to progression was 1.7 months and the median overall survival was 12.1 months with an overall survival rate at one year of 53.1%.

The third study published by Le Cesne and colleagues[49] was conducted in eight European centers by the European Organisation for Research and Treatment of Cancer (EORTC) and administered trabectedin at 1.5 mg/m^2 as a 24-hour continuous infusion. One hundred and four patients were accrued with progressive soft tissue sarcomas excluding GIST. Most patients had leiomyosarcomas (n = 43), synovial sarcomas (n = 18), or liposarcomas (n = 10). There were eight partial responses (in leiomyosarcomas [n = 5], synovial sarcoma [n = 1], liposarcoma [n = 1], and malignant fibrous

Table 1 Dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) in Phase I studies

Reference	Schedule	N	MTD	DLT	Recommended dose for phase II
Ryan[44]	72 h infusion every 3 weeks	21	1.2 mg/m^2	Transamanitis, rhabdomyolysis, neutropenia	1.05 mg/m^2
Taamma[45]	24 h infusion every 3 weeks	52	1.8 mg/m^2	Neutropenia, thrombocytopenia	1.5 mg/m^2
Twelves[46]	1 h infusion every 3 weeks	40	1.1 mg/m^2	Fatigue, neutropenia, thrombocytopenia	1 mg/m^2
	3 h infusion every 3 weeks	32	1.8 mg/m^2	Fatigue, hyperbilirubinemia, thrombocytopenia.	1.65 mg/m^2
Villalona-Calero[47]	Daily for five days every 3 weeks	42	0.325 mg/m^2/d	Neutropenia, thrombocytopenia	0.325 mg/m^2/d
Farouzeh[43]	3 h infusion weekly for three weeks of a four week cycle	31	0.65 mg/m^2	Neutropenia and hyperbilirubinemia	0.58 mg/m^2

histiocytoma [n = 1]) and forty-five (40.5%) stable disease. Disease stabilization longer than six months was measured in 26% of patients. Of note the tumor control rate (defined as non progressing patients) reached 56% in leiomyosarcomas, 61% in synovial sarcomas, and 40% in liposarcomas. After a median estimated follow-up of 34 months, the median time to progression was 3.4 months. The progression-free rates at 3, 6, and 12 months were respectively 52%, 29%, and 17%. GIST histology has been evaluated separately. Among 28 patients none responded and the best effect was disease stabilization in nine patients. The median time to progression was less than two months allowing to conclude that trabectedin was ineffective in GIST when given alone with this schedule.[52]

The results of these three phase II[48–50] studies involving 183 patients were pooled and analyzed by Le Cesne.[53] Leiomyosarcoma remained the main histological subtype representing 41% of patients followed by liposarcoma in 14% and synovial sarcoma in 11%. Most patients (95%) were pretreated with anthacyclines or ifosfamide and 113 patients were resistant to anthracyclines, 81 resistant to ifosfamide, and 63 to both agents. The overall response rate was 7.7%. The clinical benefit, defined as the combination of the rate of objective response and of minor response and of stable disease, was 51.5%. The median overall survival was 10.3 months with an overall survival rate at one year of 47.5% and a progression-free survival rate at six months of 19.8%. The clinical benefit was similar in patients with bulky disease, with multiple pretreatments, with short previous progression-free survival or with early resistance to standard chemotherapy, suggesting the lack of cross resistance and a specific mechanism of action.[54]

Table 2 Efficacy and survival data of trabectedin in phase II trials

Reference	Study type	N	Subtype of sarcoma	Overall response		Disease control	Median TTP (months)	Median OS (months)
Yovine[48]	Phase II, second line	28	LMS/Lipo	2	3.7%	24% at six months	1.9	12.8
		22	Other	0				
Garcia-Carbonero[50]	Phase II, second line	23	LMS/Lipo	3	8.3%	ND	1.7	12.1
		13	Other	0				
Le Cesne[49]	Phase II, second line	53	LMS/Lipo	6	8.1%	53.6%	3.5	9.3
		51	Other	2				
Pooled analysis Le Cesne AACR 2003[53]	Phase II, second line	183			7.7%	51.4%	ND	10.3
Morgan ASCO 2007[51]	Phase II, second line	136	3-weekly		5.6	38.9%	3.7	13.8
		134	weekly		1.6	24.3%	2.3	11.8
Garcia-Carbonero[61]	Phase II, first line	24	LMS/Lipo	4	17.1%	20%	1.6	15.8
		12	Other	2				

Abbreviations: ND, no data; LMS, leiomyosarcoma; Lipo, liposarcoma; TTP, time to progression; OS, overall survival.

A phase II randomized trial was conducted in patients with liposarcoma or leiomyosarcoma after failure of anthracyclines and ifosfamide.[51] Two hundred seventy patients received either trabectedin at 0.58 mg/m^2 as a 3 h weekly infusion, three out of four weeks or at 1.5 mg/m^2 as a 24 h continuous infusion every three weeks. A significantly longer time to progression was achieved for the three weekly regimen reaching 3.7 months and 2.3 months for the weekly regimen ($p = 0.0302$, hazard ratio [HR]: 0.734). The median overall survival was 13.8 months for the three weekly and 11.8 for the weekly regimen. The clinical benefit favored the three weekly regimen even if not statistically significant (58% versus 44%). Neutropenia grade 3–4 was described in 47% and 13% on the three weekly and the weekly arm, respectively, but it did not translate to a different rate of febrile neutropenia (0.8%). Similarly grade 3–4 transaminitis (ALAT) was more frequent in the three weekly arm (48% versus 9%), but without clinical consequences.

The results of the compassionate use programs in an unselected population, even though most of them included leiomyosarcomas or liposarcomas, were very similar in term of efficacy and toxicity.[55–59] And in a historical comparison, progression-free survival of trabectedin was better than the other active chemotherapeutic agents.[60]

First line trabectedin in sarcomas

A phase II trial from Garcia-Carbonero and colleagues evaluated 36 patients with advanced or metastatic soft tissue sarcomas.[61] The main histological subtypes were leiomyosarcomas (n = 15) and liposarcomas (n = 9). One complete response and five partial responses were observed for an overall response rate of 17.1% (95% CI: 6.6%–33.6%). Responses occurred in three patients with liposarcoma, and one patient each with leiomyosarcoma, synovial sarcoma, and fibrosarcoma. One patient with a uterine leiomyosarcoma had a minor response. The median progression-free survival was 1.6 months and the progression-free rate at 6 months was 24.4%. Median overall survival was 15.8 months and overall survival at one year was 72%.

Tolerability

The toxicity profile encountered in the phase II trials was similar to the results observed in the phase I studies evaluating the 24-hour continuous infusion of trabectedin at the recommended dose. Myelosuppression and hepatic toxicity were the most frequently observed adverse events (Table 3). Despite neutropenia grade 3–4 in 33%–61% of patients, there was a low incidence of febrile neutropenia (6%–7%), quite different from other agents as doxorubicin (up to 19%)[18,62] or ifosfamide regimens (up to 39%).[5,8,9] Thrombocytopenia and anemia grade 3–4 was seen in 9%–22% of pretreated patients, but not in first line therapy.

Liver toxicity was frequent, but not cumulative and rapidly reversible. Transaminase elevation at more than five times the normal range was described in 20% to 57% of pretreated and 30% of the nonpretreated patients. This toxicity could be improved by dexamethasone premedication. In xenografts models, the hepatotoxicity induced by trabectedin was markedly reduced or avoided after administration of metabolism modulators such as dexamethasone or beta-naphtoflavone 24 h before trabectedin.[63] The authors reported no difference in antitumor efficacy and hypothesized a decreased hepatic exposure to trabectedin, perhaps by regulation of hepatic metabolism.[64] In the clinic dexamethasone premedication was retrospectively analyzed by Grosso and colleagues.[55] Twenty-three patients treated with trabectedin 1.0 to 1.65 mg/m^2 as a three-hour or 24-hour continuous infusion every 21 days did not receive premedication whereas 31 patients received dexamethasone 4 mg per os

Table 3 Incidence of grade 3/4 toxicities in phase II trials

Reference		N	Neutropenia	Febrile neutropenia	Thrombocytopenia	Transaminitis	Nausea	Asthenia/fatigue
Yovine[48]	Phase II, second line	54	33 (61%)	4 (7%)	10 (19%)	26 (48%) ASAT 31 (57 %) ALAT	4 (7%)	8 (15%)
Garcia-Carbonero[50]	Phase II, second line	36	12 (34%)	2 (6%)	6 (17%)	9 (26%) ASAT 7 (20%) ALAT	2 (6%)	0
Le Cesne[49]	Phase II, second line	104	52 (52.5%)	9 (9.1%)	18 (18.2%)	35 (35.3%) ASAT 44 (44.5%) ALAT	7 (7.1%)	0
Garcia-Carbonero[61]	Phase II, first line	36	12 (33%)	0	0	12 (34%) ASAT 13 (36%) ALAT	5 (14%)	4 (11%)

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

bid 24 hours before therapy. The incidence of grade 3–4 transaminitis was reduced from 70% to 3% of patients by the dexamethasone premedication. The incidence of neutropenia and thrombocytopenia was also less important at 10% and 0% instead of 39% and 35%, respectively. The antitumor activity was similar between the two groups and comparable to the ones reported in other phase II trials. The overall response rate was 9% and the median progression-free survival was 2.6 months.

The analysis of four treatment-related deaths in the European phase II study[49] revealed a statistical correlation between these severe toxicities and liver dysfunction. The influence of patient characteristics and pathophysiological variables was analyzed in 69 patients treated in phase II studies.[65] The incidence of severe toxicity was significantly ($p = 0.02$) greater for patients with any baseline liver function test exceeding the upper limit of the normal range. Thus the administration of the full dose of trabectedin at 1.5 mg/m^2 requires normal alkaline phosphatase and transaminase levels, checked before each cycle, and the dose should be reduced to 1.2 mg/m^2 in case of abnormal bilirubin level, and more than 2.5 increase of transaminase or alkalin phosphatase above the normal range.

Other frequent toxicities included nausea, vomiting and fatigue that could be grade 3–4 in up to 9% of patients. Elevation of creatine phosphokinase (CK) has been reported in 26% of patients and a few cases of severe and fatal rhabdomyolysis have been observed in the phase I–II studies.[44,48,66] By an unknown mechanism, trabectedin induced necrosis of skeletal muscle resulting in subsequent release of intracellular contents leading to electrolyte imbalance, renal failure and death. Caution should be taken when used with other medications known to cause muscle injury as statins. CK is the most sensitive indicator of muscle injury and should be $\leq 2.5\times$ upper limit of normal before each treatment and closely monitored during therapy.

Perspective

Predictive factors of response

The mechanism of action of trabectedin is not well understood, but it acts partially through interferences with the transcription pathways.[67–69] The sensitivity of myxoid liposarcoma carrying a specific chromosomal translocation t(12; 16)(q13; p11) or t(12; 22)(q13; q12) that produce a DDIT3-FUS (DDIT3-EWSR1) fusion protein has been clinically recognized.[62] Under treatment, the tissue density changes were consistent with a decrease in cellularity before tumor shrinkage, suggesting that it has modulated adipocyte differentiation. Recent *in vivo* data confirmed that trabectedin was able to induce a cascade of events leading to the activation of adipogenesis differentiation in cell lines presenting similar fusion genes.[70] It opens interesting perspectives for the treatment of other translocation-related sarcomas and for the combination with other differentiating agents.

Trabectedin promotes DNA damage through its binding to N2 guanine in the minor groove causing a structural bending of DNA towards the major groove, and an unusual DNA helix distortion. This lesion is a substrate for the nucleotide excision repair (NER) pathway. In experimental models deficient cells in NER are insensitive to trabectedin.[71] Thus the mRNA levels expression of ERCC1 (NER machinery), XPD (NER machinery), BRCA1 and BRCA2 were analyzed in 92 sarcoma patients by Schoeffski and colleagues.[72] The six-month progression-free survival and median overall survival were improved for patients with high expression levels of ERCC1 (32% versus 15% and 12 months versus seven months, respectively). An increased six-month progression-free rate (33% versus 11%) and a longer median overall survival (15 versus five months) were significantly associated with low expression of BRCA1. No significant effect of XPD and BRCA2 was found on progression-free survival and overall survival. Some patients were highly sensitive to treatment when a high ERCC1 and a low BRCA1 expression were measured. They had a six-month progression-free survival of 50% and a median overall survival of 20.4 months. If the treatment of sarcomas by trabectedin should be based on a specific repair mechanism profile remains to be shown in a prospective study.

Combinations

Combination treatments with doxorubicin,[73] irinotecan,[74] and paclitaxel[75] were evaluated in preclinical studies showing synergistic effects against sarcomas. Trabectedin was combined with doxorubicin,[76,77] pegylated liposomal doxorubicin,[77] paclitaxel,[78] and capecitabine[79] in phase I studies involving patients with solid tumors. The DLT were essentially myelosuppression and hepatotoxicity.

In sarcomas a phase I study combining doxorubicin and trabectedin reported that the maximum tolerated dose was 60 mg/m^2 and 1.1 mg/m^2 respectively. Preliminary pharmacokinetics of both agents were not significantly modified by the combination. Because of severe neutropenia at the first dose level, granulocyte colony-stimulating factor had to be added subsequently. The dose limiting toxicities were neutropenia and thrombopenia. Dose reduction was required in 51% of patients for trabectedin and 27% for doxorubicin. Among the 41 treated patients, five achieved a partial remission and 34 had a disease stabilization that lasted more than six months in 15 patients.[77]

Conclusion

The marine-derived DNA-binding protein trabectedin induces DNA damage and transcriptional interference, which are dependent on the presence of DNA-repair systems. In phase I studies, the optimal dose and schedule of administration of trabectedin was 1.5 mg/m^2 as a 24 h intravenous continuous infusion every three weeks. In pretreated patients, the three phase II studies involving 183 patients showed response rate up to 10% with disease control achieved in approximately 50% and a median overall survival of one year. Significant activity was also found in first line therapy with a response rate of 17% and 72% of patients surviving at one year. The treatment is generally well tolerated at the recommended dose. The most frequent severe toxicities were neutropenia, thrombocytopenia, and transient increase of hepatic tests. These adverse events were not cumulative and were rapidly reversible. Dexamethasone premedication reduced the hepatotoxicity and the myelosuppression. A better understanding of the mechanisms of action of trabectedin and its effect on transcription pathways make it a good candidate for combination with other chemotherapeutic agents or targeted therapy.

Disclosure

The authors report no conflicts of interest in this work.

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