

Over 225 000 new cases of ovarian cancer are diagnosed each year. Symptoms are often vague, so most cases are detected when the disease is at an advanced stage. There is a need to find new drugs which will be able to treat ovarian cancer effectively. One of the most promising antineoplastic agents is trabectedin (Yondelis), derived from the marine tunicate *Ecteinascidia turbinata*, approved by the European Union in July 2007 for the treatment of soft-tissue sarcomas. This drug shows a mechanism of action based on the inhibition of the nucleotide excision repair system. Trabectedin shows anti-tumour activity *in vitro* and *in vivo* in ovarian, breast, prostate, renal, melanoma and non-small cell lung cancer cell lines. Trabectedin in combination with pegylated liposomal doxorubicin demonstrates synergistic antineoplastic activity.

Key words: trabectedin, chemotherapy, pegylated liposomal doxorubicin, ovarian cancer.

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Trabectedin as a single agent and in combination with pegylated liposomal doxorubicin – activity against ovarian cancer cells

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Introduction

Ovarian cancer is called “the silent killer” because of its not-so-obvious symptoms such as fatigue, weight change, abdominal distention and pain. The lack of efficient and early detection is the reason for the high mortality rate. This neoplasm is responsible for 125 000 deaths annually [1–3]. The “gold standard” of ovarian cancer treatment consists of surgery followed by carboplatin alone, or a combination of chemotherapy with carboplatin and paclitaxel (PTX). Currently, first line therapy in early disease (stages I–IIa) is only surgery. In 25% of patients, this protocol does not cure, and the disease will recur. In advanced stages (IIa–IV), the current standard of care is cytoreduction followed by platinum-based chemotherapy. Unfortunately, this treatment does not work for most women. Because of frequent relapses, most patients die [4]. Furthermore, side effects are considered a very important problem connected with chemotherapy. The most common adverse effects include haematological disorders, nausea, vomiting, disruption of the bone cycle and pain. According to most patients, alopecia is one of the most distressing side effects. Chemotherapeutic agents often cause the loss of hair. Drugs commonly used in ovarian cancer treatment (paclitaxel, docetaxel) cause severe alopecia which markedly lowers the quality of patients' lives [5, 6].

There is a need to investigate and find new drugs which will selectively and effectively treat ovarian cancer, and also be able to overcome multidrug resistance (MDR) – the factor responsible for many cases of recurrent epithelial ovarian cancer (EOC).

Trabectedin – structure and mechanism of action

Trabectedin, also known as Yondelis or ET-743, is an anti-tumour drug, originally derived from the marine tunicate *Ecteinascidia turbinata*. Now it is obtained from the antibiotic cynosafracin B [7]. It is composed of three fused tetrahydroisoquinoline rings (Fig. 1). Two of the rings covalently bind the N₂ amino group in the guanine residue in the DNA minor groove, in contrast to traditional alkylating drugs that bind guanine at the N7 or O6 position in the DNA major groove. Favoured triplets are TGG, CGG, AGC, and GGC. The CGA triplet is refractory. The adducts are stabilized by van der Waals interactions and hydrogen bonds between rings A and B and DNA [7, 8]. The third unbound C ring affects critical nuclear proteins, mainly transcription factors. Trabectedin induces DNA alkylation and DNA-protein crosslinks which cause formation of DNA strand breaks [7, 9, 10]. The mechanism of action is based on inhibition of DNA transcription. Trabectedin causes disruption of transcription by inhibition of the transcription-dependent nucleotide excision repair system (NER), followed by G2/M arrest and activation of the extrinsic and intrinsic apoptotic pathways, occurring through p53-independent pro-

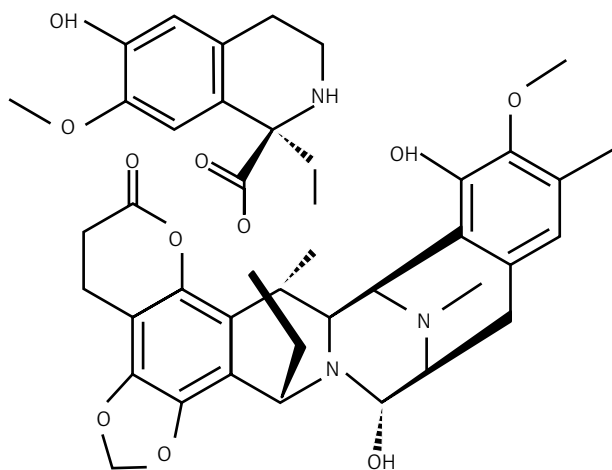


Fig. 1. Structure of trabectedin

Table 1. Natural derivatives of trabectedin isolated from *Ecteinascidia turbinata* (based on [20])

Analogues	R ¹	R ²	R ³	R ⁴
ET-743	CH ₃	OH	–	–
ET-729	H	OH	–	–
ET-745	CH ₃	H	–	–
ET-759B	CH ₃	OH, S-oxide	–	–
ET-759A	CH ₃	=O (lactam)	–	–
ET-759C	CH ₃	OH, N ¹² -oxide	–	–
ET-770	CH ₃	CN	–	–
N ¹² -formyl ET-729	CHO	OH	–	–
ET-815	CH ₃	CH(CHO) ₂	–	–
ET-731	H	H	–	–
ET-745B	H	OH, S-oxide	–	–
ET-736	CH ₃	OH	–	–
ET-722	H	OH	–	–
ET-838	CH ₃	H	–	–
ET-808	CH ₃	CH(CHO) ₂	–	–
ET-752	CH ₃	OH, S-oxide	–	–
ET-637	Ac	OH	H	NHAc
ET-594	Ac	OH	–	=O
ET-552	H	OH	–	=O
ET-652	Ac	OH	H	NHCOCH ₂ NH ₂
ET-583	H	OH	H	NH ₂
ET-597	CH ₃	OH	H	NH ₂
ET-596	CH ₃	OH	–	=O
ET-641	CH ₃	H	H	NHAc

cess [7, 11, 12]. Data on HeLa, Chinese hamster and human leukaemia cells obtained by Soares *et al.* [13] indicated that DNA synthesis and cellular viability were reduced by 50% after 1 h exposure to, respectively, 30 nM and 20 nM trabectedin. It was associated with formation of difficult-to-repair drug-DNA adducts, converted later into DNA double-strand breaks (DSBs). The phosphorylated histone H2AX (γ -H2AX) was considered as a marker. Furthermore, trabectedin toxicity was 8-fold higher toward BRCA2 deficient cells compared with the parental cell lines. Unrepaired DSBs in BRCA2-deficient cells led to chromosomal aberrations [13]. Due to the promising outcomes of trabectedin, many of its natural derivatives were isolated from *Ecteinascidia turbinata*. Some of these analogues are presented in Table 1.

Trabectedin as a single agent

Trabectedin was approved in the European Union in July 2007 for the treatment of soft-tissue sarcomas (STS) in adults after failure of conventional therapy including anthracyclines and ifosfamide [7, 9, 11]. In a preclinical study, it demonstrated antineoplastic activity *in vitro* and *in vivo* in ovarian, breast, prostate, renal, melanoma and non-small cell lung cancer [11]. It is effective in ovarian carcinoma xenografts and ovarian cancer explants and can be combined with cisplatin [10]. Unfortunately, *in vitro* studies with mammalian cells transfected with MDR1 genes and an *in vivo* study in mice with P-gp overexpression showed that trabectedin is a substrate of P-glycoprotein (P-gp), the molecule responsible for multidrug resistance in human cancer cells [14].

Anti-tumour activity of trabectedin in ovarian cancer has been studied in phase I and phase II clinical trials [11]. The phase I trial study included women with platinum-sensitive and platinum-resistant ovarian cancer after therapy based on platinum and taxanes. Trabectedin caused 43% response rates in patients with platinum-sensitive ovarian cancer, with a time to progression of 7 to 9 months. There were also observed two partial responses among women with platinum-resistant ovarian cancer. The time to progression was 5 months. The most common adverse effects were nausea and vomiting (78%), neutropenia (41%), asthenia (78%) and thrombocytopenia (7.5%) [15]. Phase II clinical trials included women with recurrent ovarian cancer after one or two platinum-based chemotherapy regimens. Trabectedin showed 29% and 6.3% response rates in women with platinum-sensitive and platinum-resistant ovarian cancer respectively, with a median progression time of between 5.1 and 2 months. The side effects were neutropenia (8%), nausea, vomiting, and fatigue (5%) [16].

Anti-tumour effectiveness of trabectedin in monotherapy was evaluated by Mabuchi *et al.* [11] *in vitro* using 3 lines of human ovarian clear cell carcinoma (CCC) and human ovarian serous adenocarcinomas (SAC) – 3 lines. It inhibited growth and proliferation of CCC and SAC. CCC showed higher sensitivity to trabectedin than SAC cell lines. The effect was maintained in cisplatin- and paclitaxel-resistant CCC cells. Then, the anti-tumour effect of trabectedin was evaluated *in vivo* using a xenograft model with mice inoculated with CCC cells. Trabectedin was generally well tolerated. After 4 weeks of treatment the tumour mass was reduced by greater than 70%, compared with PBS-treated mice [11].

The drug is generally well tolerated. It is metabolized in the liver with a half-life of approximately 90 hours. Cytochrome P 450 CYP3A4 isoenzyme is involved in this process. The major tissue toxicities are connected with the bile duct (elevated plasma bilirubin, bile acids and aspartate transaminase (AST), alanine transaminase (ALT), γ -glutamyl transferase (GGT) and alkaline phosphatase activities). The most common side effects are fatigue, nausea, anorexia, vomiting, constipation, atelectasis, dyspnoea, neutropenia, haemorrhoids, and intestinal obstruction. There were no indications of renal toxicity in rats (or mice and dogs) [7, 14, 17, 18]. There is also no cardiac toxicity and no ECG changes (considered as prolongation of QT/QTc interval) after treatment with trabectedin [17].

Trabectedin in combination with pegylated doxorubicin

Very interesting data were obtained for the combination of trabectedin with pegylated liposomal doxorubicin (PLD, Doxil). It is a unique form of anthracycline antibiotic – doxorubicin, packed in a liposome coated with polyethylene glycol. This modification prevents plasma protein adsorption to the liposome surface and showed that in contrast to non-pegylated liposomes, PLDs are able to remain in the circulation much longer. Due to the enhanced permeability and retention (EPR) effect, the liposomes delivered drugs more specifically to the cancer tissues and limited exposure of normal cells to the drugs. The use of PLD for ovarian cancer treatment gave promising results [19, 20].

Based on the results of a randomized phase III trial (ET-743-OVA-301) (comparing PLD alone with a combination of PLD and trabectedin), on 672 patients diagnosed with a recurrent ovarian cancer from 21 countries in 2009, the European Commission approved the combination with PLD: 30 mg/m² (as a 3 h infusion), every 3 weeks, for the treatment of patients with a recurrent platinum-sensitive ovarian cancer, for whom a first line platinum-based chemotherapy had failed [14, 21, 22]. The study demonstrated that trabectedin in combination with PLD improves progression-free survival (PFS) and overall response rate (ORR) in comparison to Doxil alone as a second-line treatment of recurrent ovarian cancer. Among the 672 patients, 522 (77.7%) deaths were observed (including 258 in the trabectedin and Doxil arm and 264 in the Doxil arm). The median overall survival (OS) for trabectedin plus Doxil and Doxil alone was 22.2 and 18.9 months respectively. This effect was observed in all age groups except > 65. Surprisingly, the progression-free interval (PFI) favouring the PLD arm (PLD – PFI) was 13.3 months, whereas trabectedin + PLD – PFI was 10.6 months. Additionally, this combination was well tolerated, with manageable toxicity. Furthermore, a decrease of PLD-associated toxicity was observed, which supports the thesis that trabectedin in combination with PLD is a good solution for patients with recurrent ovarian cancers [21–23].

Future combined therapies with trabectedin

Angiogenesis – the creation of new blood and lymphatic vessels – is a crucial process for tumour development. Most types of tumours respond to a hypoxic environment by secreting a key pro-angiogenic growth factor called vascular endothelial growth factor (VEGF). These particles bind to its receptors (VEGFR-1, VEGFR-2, VEGFR-3) on the surface of cancer cells, leading to metastasis. Studies by Kłasa-Mazurkiewicz *et al.* [24] showed that the overexpression of VEGFR-2 occurred among patients with early ovarian cancer stages (FIGO – I, IIa), which may indicate the important role of apoptosis during this phase, whereas an increased level of VEGFR-3 was detected in advanced stages of cancer, and correlated with a positive response to chemotherapy. A high level of VEGFR-3 is also connected with aggressiveness of ovarian cancer and indicates poor prognosis.

Angiogenesis is not the only mechanism of a tumour vasculature. Vasculogenic mimicry is a phenomenon con-

nected with the formation of fluid-conducting channels, not lined with endothelium. This process occurs during tumour development and concerns undifferentiated cancers especially in the advanced stage of the disease. The presence of vasculogenic mimicry may indicate a poor prognosis. Additionally, some researchers claim that vasculogenic mimicry may protect the neoplasm against anticancer agents (as tumours demonstrating this phenomenon are often drug-resistant) [25].

Currently the most studied agent among anti-angiogenic drugs is bevacizumab – a humanized IgG1 monoclonal antibody which selectively inhibits VEGF activity. Two phase III clinical trials, GOG-218 and ICON7, showed a significant benefit in PFS when the standard (carboplatin-paclitaxel) chemotherapy was combined with bevacizumab. Based on these results, the European Medicines Agency (EMA) approved this combination of drugs for the front line treatment of advanced epithelial ovarian cancer [26]. Interestingly, studies by Takano *et al.* [27] indicated that bevacizumab in combination with trabectedin and oxaliplatin causes complete remission of recurrent ovarian clear cell carcinoma, with manageable general toxicity. It is thought that bevacizumab blocks vascular repair and survival, which enhances the activity of trabectedin and oxaliplatin.

The latest studies indicate that angiogenesis and vasculogenic mimicry are important in tumour development; therefore deeper understanding of the individual angiogenic patient's phenotype may be helpful in designing an appropriate and effective anticancer therapy.

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