A Review of Trabectedin (ET-743): A Unique Mechanism of Action

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Abstract

Trabectedin (ET-743) is a marine alkaloid isolated from the Caribbean tunicate *Ecteinascidia turbinata*, with a chemical structure characterized by three fused tetrahydroisoquinoline rings. Two of these rings (subunits A and B) provide the framework for covalent interaction with the minor groove of the DNA double helix, whereas the third ring (subunit C) protrudes from the DNA duplex, apparently allowing interactions with adjacent nuclear proteins. The compound's chemical interactions trigger a cascade of events that interfere with several transcription factors, DNA binding proteins, and DNA repair pathways, likely to be different from other DNA-interacting agents. Trabectedin also causes modulation of the production of cytokines and chemokines by tumor and normal cells, suggesting that the antitumor activity could also be ascribed to changes in the tumor microenvironment. The promising data on the combination of trabectedin with other anticancer agents, observed in preclinical systems, have prompted several clinical studies that are currently ongoing. One of these combinations (trabectedin-pegylated liposomal doxorubicin) was recently authorized by the European Commission for the treatment of patients with relapsed platinum-sensitive ovarian cancer.

Introduction

Trabectedin (Yondelis; ET-743) is a tetrahydroisoquinoline alkaloid that was initially isolated from the marine ascidian *Ecteinascidia turbinata* and is currently prepared synthetically (1). The compound was selected for clinical development on the basis of its novel chemical structure and its striking activity against tumor cell lines of different origins in *in vitro* and *in vivo* models. In 2007, trabectedin obtained marketing authorization from the European Commission for the treatment of patients with advanced soft tissue sarcoma after failure of anthracyclines and ifosfamide, or who are unsuited to receive these agents. In 2009, it received marketing authorization from the European Commission in combination with pegylated liposomal doxorubicin for the treatment of patients with relapsed platinum-sensitive ovarian cancer. Its clinical activity is currently being evaluated in other neoplasms, including prostate and breast cancer. Trabectedin's mechanism of action seems to be different from that of the available DNA-damaging agents used in cancer chemotherapy to date. Understanding the molecular and cellular mechanisms responsible for its antitumor activity is potentially useful in the design of new therapeutic strategies alone, or in combination with other drugs. Furthermore, the unique features of this compound make it a useful tool for elucidating complex mechanisms related to gene transcription regulation and DNA repair. Here, the most relevant observations related to trabectedin's mechanism of action are summarized, in addition to identifying the questions requiring further preclinical and clinical investigations.

DNA Binding

Trabectedin (Fig. 1) is formed by a monobridged pentacyclic skeleton composed of two fused tetrahydroisoquinoline rings (A and B), linked to a 10-member lactone bridge through a benzylic sulfide linkage, and attached through a spiro ring to an additional ring system made up of a tetrahydroisoquinoline (subunit C). In contrast to traditional alkylating agents that bind guanine at the N7 or O6 position in the DNA major groove, trabectedin binds to the exocyclic N2 amino group of guanines in the DNA minor groove through an iminium intermediate generated *in situ* by dehydration of the carbinaloamine moiety present in the ring A. The carbinaloamine moiety is imperative for the pharmacological activity of trabectedin, as related compounds without this reactive group (e.g., ET-745) were 100 times less active than trabectedin. The resulting adduct is additionally stabilized through van der Waals interactions and one or more hydrogen bonds between rings A and B, with neighboring nucleotides in the same or opposite strand of the DNA double helix, thus creating the equivalent to a functional...
interstrand crosslink (Fig. 2). In fact, hydrogen-bonding rules seem to determine the DNA-binding sequence specificity of trabectedin, with a guanine located in the central position of triplets 5′-purine-GC and 5′-pyrimidine-GG. Therefore, favored triplets are TGG, CGG, AGC, and GGC sequences, whereas CGA is completely refractory (2). The binding of the drug in the minor groove induces the formation of DNA adducts and bends DNA toward the major groove in a fashion that seems unique for this compound (3). The ring C apparently does not participate in DNA binding, and it was proposed to protrude out of the DNA duplex, apparently allowing an interaction with adjacent macromolecules.
DNA Repair

Several studies have been conducted on cell lines with well-defined defects of DNA repair mechanisms. These studies suggested a role for transcription-coupled nucleotide excision repair (TC-NER) and homologous recombination in the cytotoxic activity of trabectedin, whereas mismatch repair deficiency status does not affect it (Fig. 3).

Transcription-Coupled Nucleotide Excision Repair

The observation that TC-NER–deficient cells are 2 to 10 times less sensitive to trabectedin is very peculiar for this compound, and contrasts with what was reported to other DNA-damaging agents (e.g., cisplatin) used in cancer treatment (4–7). TC-NER involves recognition of DNA damage and the recruitment of various factors to the damaged site in order to repair lesions that distort the DNA backbone. Molecular modeling studies based on results obtained in Rad-13 (orthologue to human XPG) mutants of S. pombe (8), suggested that the ring C would form a hydrogen bond with an arginine residue (Arg961), located in a 46-amino acid region of Rad13, which is homologous to a DNA-binding region of human nuclease FEN-1. The formation of this Rad13-DNA–trabectedin ternary complex would then induce DNA strand breaks, which should result in cell death. Although, when comparing the antiproliferative activities of trabectedin side by side with an analog without the ring C (ET-673), no major differences were found (9).

DNA Double Strand Repair

Homologous (HR) and nonhomologous end joining (NHEJ) are involved in DNA double strand break (DSB) repair. It was found that HR is particularly important for trabectedin as deficient cells were approximately 100 times more sensitive to the drug. In contrast, these differences were not observed in NHEJ-deficient cells. The lack of HR was associated with the persistence of unrepaired DSBs during the S phase of the cell cycle and apoptosis (10, 11). The particular sensitivity of cells deficient in HR seems to be confirmed in the clinic, as the tumor expression of BRCA1 or BRCA2 seems inversely related to the response in a series of patients with sarcomas treated with trabectedin (12). Although the majority of the DSBs are replication dependent and involve the phosphorylation of H2AX by ATM, recently it has been proposed that some of them are also replication independent, transcription coupled, and dependent on Mre11-Rad50-Nbs1 (13). As mentioned above, the DNA monoadducts are stabilized by hydrogen bonds between trabectedin and nucleotides located in the same or in the opposite strand, resulting in a DNA lesion similar to interstrand crosslinks. This characteristic explains why human cells with mutations in Fanconi anemia genes such as FANCA, FANCC, FANCE, FANCG, or FANCD1 are highly sensitive to trabectedin (14).

Transcription Regulation

Structural changes to DNA caused by trabectedin might hinder the recognition of transcription factors to specific GC-enriched DNA consensus sequences (15). Although the binding properties of some transcription factors are unaffected (e.g., MAF, MYB, and MYC), a concentration-dependent inhibition was reported for TBP, E2F, and SRF (10-30 μmol/L), and for the CCAAT box factor NF-Y (50-300 μmol/L; ref. 15). These data were in keeping with previously obtained data for other DNA minor groove binders, such as distamycin or CC-1065 derivatives, which have been reported to inhibit some transcription factors in a sequence-specific manner.
(AT enriched), different from trabectedin’s preferential sequences (16). At the cell level, trabectedin does not seem to affect constitutive transcription, but only the transcription of activated genes (17). At nanomolar concentrations, trabectedin effectively blocked the activated transcription of genes such as HSP-70 (18) or MDR1 (19). Further studies indicated that, although trabectedin is a DNA minor groove binder, it also has effects on promoters regulated by transcription factors that bind to the major groove (e.g., Sp1; ref. 20), and studies also indicated that the drug is not active on all activated genes (e.g., methallothionein, CYP3A4; ref. 21). In addition, trabectedin can inhibit (e.g., DHFR, TK, or cyclin B2) or stimulate (e.g., cyclin E) the activated transcription process. Of interest, trabectedin treatment induces the rapid degradation of transcribing RNA polymerase II (PolII), only in cells with normal TC-NER function, but not in XPD-, XPA-, XPG-, and XPF-deficient cells (22).

The observation that trabectedin can induce either up-regulation or downregulation of the same genes in different cell lines suggests that cell-specific cofactors play a role in the ability of the drug to modulate transcription regulation. This finding certainly requires further research.

**Effects on Tumor Microenvironment**

At lower concentrations causing modest cytotoxicity, trabectedin inhibits the in vitro production of the proinflammatory mediators CCL2 and interleukin (IL)-6 by monocytes, macrophages, and tumor-associated macrophages isolated from ovarian cancer biopsies (23). The modulation of cytokines and chemokines occurs at the transcriptional level, thus indicating that the mechanism of trabectedin on transcription regulation can be effective both in cancer cells and in some normal cells, which produce factors that are relevant for the tumor growth and progression. Note that CCL2 plays a major role in monocyte recruitment at tumor sites, whereas IL-6 is a growth factor for several tumors. Similar results were recently seen in a tumor particularly sensitive to trabectedin: myxoid liposarcoma (24). The production of CCL2, CXCL8, IL-6, vascular endothelial growth factor (VEGF), and the matrix binder protein pentraxin 3 (PTX3), by primary cultures or cell lines of myxoid liposarcomas, was selectively inhibited by nontoxic concentrations of trabectedin. These findings were further confirmed in a patient-derived myxoid liposarcoma xenograft mouse model in which trabectedin treatment caused a marked reduction in the tumor expression of CCL2/CXCL8/CD68+ infiltrating macrophages and CD31 tumor vessels (24).

**Mechanism of Action in Specific Subtypes of Sarcomas**

Soft tissue sarcomas are a very heterogeneous group of more than 50 mesenchymal tumors, with different molecular pathogenesis, histopathology, clinical features, and differential sensitivity to drugs. The overall response rate to trabectedin observed in several phase II studies in patients who have failed one or two lines of chemotherapy is around 10%, with disease stabilization exceeding 50%. The more relevant antitumor efficacy is observed in patients with advanced myxoid liposarcoma, in which trabectedin induces objective responses in approximately 50% of cases and progression-free survival exceeding 2 years in 80% of patients (25, 26). Myxoid liposarcoma pathogenesis is related to characteristic chromosomal translocations such as t(12;16)(q13;p11) or less frequently t(12;22)(q13;q12), resulting in the expression of FUS-CHOP and EWS-CHOP fusion genes, respectively (27). In some patients, trabectedin sensitivity seems to be associated with radiological changes in tissue density, similar to those previously reported for GIST after administration of molecular targeted therapies. Moreover, trabectedin treatment was associated with significant histopathologic changes, characterized by regression of the capillary network and a presence of mature lipoblasts. A possible explanation for these changes could be that, by blocking the trans-activating ability of FUS-CHOP chimeric protein, trabectedin might modulate the transcription of genes that are crucial for adipocytic differentiation (28). The findings observed in myxoid liposarcoma could also be extrapolated to other sarcomas in which the translocation results in an altered regulation of the expression of transcription factors. For example, some responses observed in patients with Ewing’s sarcomas could be explained by this mechanism. This disease is characterized by a translocation, most frequently resulting in the creation of an aberrant transcription factor (EWS-Fli1 fusion protein), known to regulate more than 70 genes required for the survival of Ewing’s cells. It was recently shown that trabectedin has a direct inhibitory effect on EWS-Fli1 transcriptional activity (29).

**Resistance to Trabectedin**

Only a few cell lines have been described that show specific resistance to trabectedin. The resistance to trabectedin of the colorectal carcinoma ER5 cell line (derived from the HCT116 cells) is associated with a loss of heterozygosity at 13q33, where the gene encoding for XPG is located. Sequential analysis of the XPG gene showed an insertion of adenine at codon 240, which resulted in a stop codon at position 243 (5, 30). Another mechanism associated with trabectedin’s resistance has been described in human surgically resected chondrosarcoma cells (CS-1; ref. 31). Alteration in the cytoskeleton architecture, mainly related to modified types I and IV collagen expression, has been shown in this cell line model (32). In a different study, continuous exposure for 10 months to increasing doses of trabectedin (up to 800 nmol/L) generated highly resistant, overexpressing P-gp ovarian cancer cells (Igrov1/25ET; ref. 33).
Combinations

*In vitro* and *in vivo* experiments have shown additive or synergistic activity in combination with doxorubicin (DOX). *In vitro*, concomitant exposure of human sarcoma cells (TE671, HT-1080, HS-18) to trabectedin and DOX produces additive or synergistic effects that allowed concentration reductions of 1.5 fold for trabectedin and 6.7 fold for DOX at the 90% kill level (34, 35). In U-2OS and Saos-2 sarcomas, the trabectedin and DOX combination provided additional evidence that a significant inhibition of cell growth can be achieved (36). Similarly, additive effects have been reported in MCF7 and MX-1 breast cancer cell lines (34). *In vivo*, synergistic interaction with DOX has also been described in murine fibrosarcoma UV2237 and human rhabdomyosarcoma TE671 xenografts (35). The combination was effective regardless of the schedule. In a different experiment involving TE671 sarcoma xenografts, the combination was again more effective than each drug alone. In patients, the combination trabectedin-Doxyl was recently authorized by the European Commission for the treatment of patients with relapsed platinum-sensitive ovarian cancer.

In platinum compounds, *in vitro* synergism was observed in human ovarian (Igrov-1 and A2780), colon (HCT116), breast (MCF7), and sarcoma (TE671, U-2OS, and Saos-2) cancer cell lines (36–39). The synergism of the combination was independent of the schedule, even though the most pronounced effects occurred when cisplatin was administered before trabectedin. *In vivo*, the antitumor activity of trabectedin-cisplatin combinations was also synergistic in ovarian and sarcoma xenografts.

![Schematic of the unique and complex mode of action of trabectedin.](image_url)

**Figure 4.** Schematic of the unique and complex mode of action of trabectedin. The antitumor effects of trabectedin are due to multiple mechanisms involving DNA binding in the minor groove, interactions with DNA repair mechanisms, modulation of transcription regulation, and induction of microenvironment changes.
models (40). Finally, a number of investigations have studied trabectedin combined with other anticancer drugs. In vitro combinations of trabectedin and camptothecin showed slight synergistic effects in MC7F and MX-1 cell lines (39). Similar results were observed when combined with paclitaxel, in sarcoma (HT-1080) and breast (MC7F and MX-1) cancer cell lines (34, 39). In vivo, the combination of trabectedin and oxaliplatin or paclitaxel presented greater antitumor effect in different xenograft models (38, 39).

Concluding Remarks

Figure 4 provides a schematic of trabectedin's mechanism of action, which is unique compared with that of other anticancer agents identified to date.

The available data indicate that trabectedin is a minor groove binder, able to induce DNA damage that will finally alter the normal function of DNA repair and transcription processes, resulting in arrest of proliferation, differentiation, and cell death. However, three principal mechanisms are still not fully understood: the precise molecular links between DNA repair, cell response, and the effects on transcription regulation; the role of tumor-stroma interactions in trabectedin's antitumor activity; and the relevance of each mechanism in different tumor types. For example, the high activity of trabectedin against myxoid liposarcomas seems to be related to its ability to counteract the biological activity of the FUS-CHOP fusion gene, the most frequent hallmark of this disease. However, other tumor types that do not express this fusion gene are also highly trabectedin sensitive. In other tumor types, such as ovarian or breast cancers, the tumor's sensitivity to trabectedin might be related to altered expression of DNA repair genes (e.g., BRCA1 or BRCA2). These tumors are very sensitive to trabectedin. In other cases, the drug's ability to modulate the tumor microenvironment might be mainly responsible for the antitumor effects. In this sense, trabectedin is effective in modulating gene transcription of tumor-associated macrophages, with a decrease of factors potentially relevant for tumor growth, progression, and metastasization. The promising preclinical results obtained combining trabectedin with other cytotoxic agents, such as cisplatin, anthracyclines, or taxanes, is under clinical evaluation. Nonetheless, and in spite of these uncertainties, trabectedin, either as a single agent or in combination, has been shown to be a clinically valid option for the treatment of advanced soft tissue sarcomas or relapsed platinum-sensitive ovarian cancer.

Disclosure of Potential Conflicts of Interest

CM. Galmarini: employee and shareholder of Pharma Mar. No other potential conflicts of interest were disclosed.

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