Molecular Therapy
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Commentary

Exploiting divergent mechanisms of trabectedin for bone tumors

Zachary P. Tolstyka[1] and Patrick J. Grohar[1]

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Trabectedin is a natural product chemotherapeutic isolated from *Ecteinascidia turbinata*, a turbinate that lives throughout the southeast coast of North America, the Gulf of Mexico, and the Mediterranean Sea.[1] Extract from this sea squirt was identified as having anti-cancer activity in the 1960s via National Cancer Institute screening. The chemical structure of one of the compounds in the extract, ET-743 (trabectedin), was elucidated in the 1980s, its crystal structure was obtained in 1992, and it was chemically synthesized in 1996.[2] Like many natural products, the drug has a complicated mechanism of action, a broad cytotoxicity profile, and clinical activity in a wide range of tumors from lung carcinoma to sarcoma and is being studied as a combination therapy with a wide array of treatment modalities.[3] Nevertheless, certain tumors, including bone sarcomas, appear to have a heightened sensitivity to the drug. In this commentary, we highlight the three main mechanisms of trabectedin: DNA damage/poisoning of repair, modulation of oncogenic and activated transcription, and immune modulation (Figure 1). We describe how these mechanisms account for the heightened sensitivity of bone tumors to the drug and how complementary approaches can be used to exploit trabectedin's three different mechanisms of action specifically for bone tumors.[1,3,4] In particular, we highlight recent work that exploits the mechanism of immune modulation to sensitize the tumor microenvironment (TME) of bone tumors to oncolytic herpes simplex viroimmunotherapy.[5]

Trabectedin is a minor groove DNA binding agent known to form covalent bonds at the N2 exocyclic amine of guanosine.[6] This covalent bond is somewhat unique in that it is reversible, which allows migration of the adduct to favored triplet double-stranded DNA (dsDNA) sequences (TGG, CGG, AGC, GGC).[1,6] Non-covalent interactions (van der Waals forces and hydrogen bonding) help further stabilize the DNA-drug adduct and interact with the opposite strand to mimic a DNA strand-strand cross-link. Strand separation cannot occur, so replication and transcription stall at the replication fork to generate DNA double-strand breaks.[1] Resolution of this binding and pseudo-cross-link requires the coordinated action of both homologous recombination (HR) and nucleotide excision repair (NER)[1] DNA repair pathways. HR aids in the repair of dsDNA breaks, and cells unable to undergo HR are up to 100 times more sensitive to trabectedin.[7]

In contrast, the NER pathways are integral to the mechanism of the drug and provide an important link to the second major mechanism of trabectedin, suppression of activated transcription. Consistent with this idea, there is as much as a 10-fold reduction in the efficacy of trabectedin in cells deficient in some components of the NER pathway.[7,8] NER is a cellular process that allows for the detection and repair of damaged DNA via global NER (GG-NER) and/or transcription-coupled NER (TC-NER). The trabectedin adduct forms a thermodynamically favored minor distortion of the DNA structure that does not initiate GG-NER, suggesting that GG-NER is not integral to the mechanism of trabectedin. In contrast, TC-NER-deficient cells experience lower DNA damage when treated with trabectedin compared to TC-NER-proficient cells.[4] Typically in TC-NER, damaged DNA is removed and repaired by dual incision reactions on the 5′ side of the DNA-drug adduct by ERCC1/XPF and on the 3′ side by XPG. With trabectedin treatment, the complex generates the expected 5′ single-stranded DNA (ssDNA) breaks; however, the trabectedin-DNA adduct inhibits XPG, so the 3′ single-stranded break (SSB) does not form.[4] This mechanism been exploited to map the impact of trabectedin on transcription genome wide using trabectedin-induced break sequencing (TRABI-seq).[4] Importantly, this work showed that SSBs are more prominent in transcriptionally active regions of chromatin and favor highly expressed genes.[3] Notably, trabectedin does not affect constitutive transcription.

Bone tumors are known to be sensitive to DNA damaging and DNA damage response (DDR)-targeted agents and have an intrinsic dependence on activated transcription. Ewing sarcoma and subsets of osteosarcoma are known to be driven by oncogenic transcription factors. The factors generate high levels of replication and transcription stress, perhaps accounting for the known sensitivity to DNA-damaging agents, which are the mainstay of therapy. Importantly, this same elevated replication stress likely contributes to early seminal observations that bone tumor cells are among the most sensitive cells to the cytotoxic effects of trabectedin.[9] These effects have been further exploited in combination with other targets and agents such as poly (ADP-ribose) polymerase,[10] WRN RecQ like helicase (WRN),[11] insulin like growth factor 1 receptor (IGF1R),[12,13] and others.

The trabectedin-DNA adduct and distortion of DNA structure additionally plays a role in the suppression of specific transcription factors. The adduct and its preferred binding sequences (see above) cause structural changes in DNA that sterically hinder transcription factor binding to consensus sequences with dose-dependent binding inhibition at TBP, E2F, SRF, and CCAAT.[14] This is particularly important for specific

[1]University of Michigan Medical School, Department of Pediatrics, Division of Pediatric Hematology/Oncology, Rogel Cancer Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109, USA

Correspondence: Patrick J. Grohar, University of Michigan Medical School, Department of Pediatrics, Division of Pediatric Hematology/Oncology, Rogel Cancer Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109, USA.
E-mail: grohar@med.umich.edu

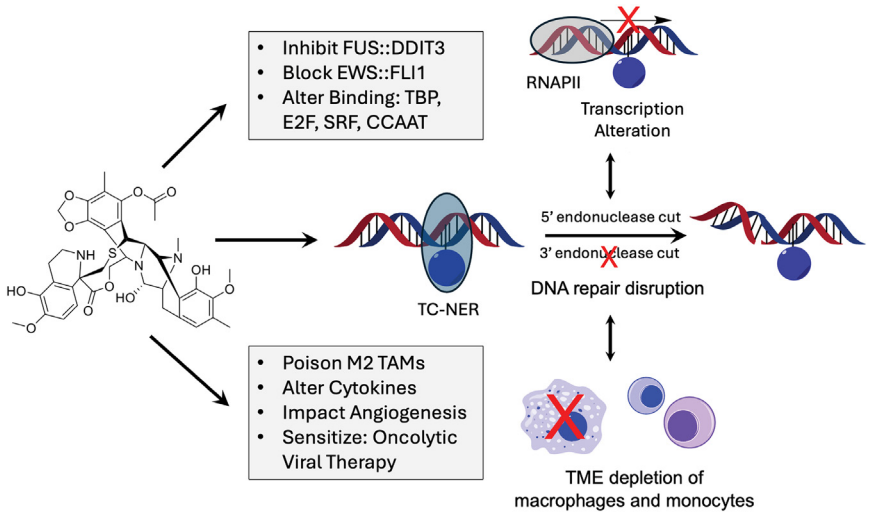

Figure 1. Three major mechanisms of trabectedin
Transcription alteration of specific targets. DNA damage and inhibition of repair. TME modification.

tumors like myxoid liposarcoma, where binding of the driver oncogene of the tumor, the FUS::DDIT3 transcription factor, is disrupted by trabectedin to reverse the oncogenic phenotype.[15] Poisoning of the driver oncogene of Ewing sarcoma, EWS::FLI1, has also been described, but by an alternative mechanism.[16] Activation of the DDR leads to the redistribution of EWS::FLI1 to the nucleolus, an effect that occurs in the setting of UV light DNA damage with wild-type EWSR1.[17,18] This leads to the silencing of EWS::FLI1 enhancers. Because Ewing sarcoma is dependent on EWS::FLI1, this leads to potent suppression of tumor growth, particularly in combination with low-dose irinotecan.[13] This combination is currently under evaluation in the clinic.

Finally, work by a number of groups has established the importance of trabectedin as a modulator of the TME and the immune microenvironment.[3,19] M2 tumor-associated macrophages (TAMs) promote tumor progression and produce immunosuppressive and pro-angiogenic factors. Trabectedin has been found to induce apoptosis in M2 TAMs and other monocytes and macrophages by activating CASP8-dependent apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors. This is evident in patient samples treated with trabectedin where there is a decreased presence of monocytes and macrophages noted by immunohistochemistry staining.[20] This has a number of effects, including altering the production of inflammatory cytokines (CCL2, CCL8, interleukin-6 [IL-6], vascular endothelial growth factor [VEGF], and PTX3) and angiogenic factors such as VEGF and angiopoetin-2 at sub-cytotoxic doses.[19,21] Furthermore, these changes lead to increased $CD8^+$ T lymphocytes/natural killer (NK) cells in tumors and increased expression of interferon-γ, perforin, and granzyme B. Studies show increased effectiveness of combination trabectedin and anti-programmed cell death ligand 1 (PD-L1) in mouse osteosarcoma and ovarian cancer models over anti-PD-L1 alone.[7] Schwarz and coworkers recently showed that depletion of immunosuppressive cells in the TME led to the increased sensitivity of triple-negative breast cancer to IL-12 activated NK cells and enhanced response to anti PD-L1 therapy.[22] As highlighted in the December issue of this journal, in bone tumors, these immunosuppressive effects have been used to improve the efficacy of oncolytic virotherapy. In that report, Ringwalt et al. provided new mechanistic insights into this activity. They demonstrate a 2-fold effect of trabectedin: enhanced viral burden in the cancer cells and disruption of suppressor T cells leading to increased cytotoxic killing and stimulation of granzyme production in T lymphocytes and NK cells.[5] Importantly, the combination of trabectedin and viral therapy caused striking tumor regressions more than either agent alone.

Trabectedin is a chemotherapeutic with activity in a diverse set of malignancies. Bone tumors are highly sensitive to the drug likely due to intrinsic transcriptional and replication stress that sensitizes the cells to both the DNA-damaging and transcription-directed mechanisms of the drug. Furthermore, the drug has been shown to reverse the activity of the oncogenic driver mutation of Ewing sarcoma, the EWS-FLI1 transcription factor. Recent work by Ringwalt et al. exploits the immunomodulatory effects of trabectedin to establish an exciting complementary use of the drug in bone tumors to sensitize these cancers to oncolytic viral therapy.[5] Further understanding and exploitation of the multiple mechanisms of trabectedin could lead to improved outcomes and highly active drug combinations in bone tumors and beyond.

DECLARATION OF INTERESTS

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