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Trabectedin and Campthotecin Synergistically Eliminate Cancer Stem Cells in Cell-of-Origin Sarcoma Models[1]

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Abstract

Trabectedin has been approved for second-line treatment of soft tissue sarcomas. However, its efficacy to target sarcoma initiating cells has not been addressed yet. Here, we used pioneer models of myxoid/round cell liposarcoma (MRCLS) and undifferentiated pleomorphic sarcoma (UPS) developed from transformed human mesenchymal stromal/stem cells (MSCs) to evaluate the effect of trabectedin in the cell type responsible for initiating sarcomagenesis and their derived cancer stem cells (CSC) subpopulations. We found that low nanomolar concentrations of trabectedin efficiently inhibited the growth of sarcoma-initiating cells, induced cell cycle arrest, DNA damage and apoptosis. Interestingly, trabectedin treatment repressed the expression of multiple genes responsible for the development of the CSC phenotype, including pluripotency factors, CSC markers and related signaling pathways. Accordingly, trabectedin induced apoptosis and reduced the survival of CSC-enriched tumorsphere cultures with the same efficiency that inhibits the growth of bulk tumor population. *In vivo*, trabectedin significantly reduced the mitotic index of MRCLS xenografts and inhibited tumor growth at a similar extent to that observed in doxorubicin-treated tumors. Combination of trabectedin with campthotecin (CPT), a chemotherapeutic drug that shows a robust anti-tumor activity when combined with alkylating agents, resulted in a very strong synergistic inhibition of tumor cell growth and highly increased DNA damage and apoptosis induction. Importantly, the enhanced anti-tumor activity of this combination was also observed in CSC subpopulations. These data suggest that trabectedin and CPT combination may constitute a novel strategy to effectively target both the cell-of-origin and CSC subpopulations in sarcoma.

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Abbreviations: MRCLS, myxoid/round cell liposarcoma; UPS, undifferentiated pleomorphic sarcoma; *CSC*, cancer stem cell; hBMSC, human bone marrow-derived mesenchymal stromal/stem cell; CPT, campthotecin; STS, soft tissue sarcomas; NER, nucleotide excision repair; DSB, double strand breaks; SSB, single strand breaks; CI, combination index; TGI, tumor growth inhibition.

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Introduction

Recent advances in sarcoma genomics have contributed to the development of new therapeutic strategies. However, cytotoxic drugs like doxorubicin alone or in combination with ifosfamide, remain as the most utilized agents for first-line treatment of soft tissue sarcomas despite their limited clinical response [1]. A hypothesis to explain the resistance of sarcomas to chemotherapy is the existence of subpopulations of drug-resistant cancer stem cells (CSCs), considered to be responsible for relapses and metastasis. It has been recently established that transformed MSCs and/or their immediate lineage progenitors are the most likely cell-of-origin for sarcomas [2,3]. Accordingly, many efforts have been undertaken to produce models of sarcomas based on MSCs transformed with relevant oncogenic events. This kind of models constitutes unparalleled systems to unravel the mechanisms underlying sarcomagenesis from the cell of origin, to explore the evolution of CSCs subpopulations and to search for CSC-specific therapies. In this regard, we have recently established and characterized pioneer models of undifferentiated pleomorphic sarcoma (UPS) and myxoid/round cell liposarcoma (MRCLS) developed from sequentially mutated human bone marrow-derived MSCs (hBMSCs) [4–6].

Trabectedin (ET-743, Yondelis) is a marine alkaloid originally isolated from the tunicate *ecteinascidia turbinate*. This compound has shown a consistent activity in soft tissue sarcomas (STS) in several clinical trials. Although, it has not demonstrated an objective advantage over first-line standard doxorubicin-based therapy, trabectedin has been approved for the treatment of adult patients with advanced STS, after failure of anthracyclines and ifosfamide, or those who are unsuited to receive these agents [1,7,8]. Trabectedin presents a complex mechanism of action affecting several key processes in both tumor cells and microenvironment [8–10]. Unlike classical alkylating drugs, trabectedin binds covalently to specific triplets of the DNA minor groove leading to a distortion of the double helix structure. These DNA adducts interfere with the binding of several transcription factors to their specific promoter elements. Specifically, trabectedin blocks the transcriptional activity of oncogenic transcription factors such as FUS-CHOP or EWS-FLI-1, which are products of characteristic chromosomal translocations respectively observed in MRCLS and Ewing's sarcoma [9,11–13]. Indeed, trabectedin is particularly efficient in MRCLS patients [14]. In MRCLS pre-clinical models, FUS-CHOP activity inhibition by trabectedin promotes adipogenic differentiation [12,15,16] and inhibits the production of inflammatory mediators by the tumor cells [17]. The structural changes induced by trabectedin cannot be resolved by the nucleotide excision repair (NER) mechanism, which instead are involved in the formation of double strand breaks (DSB) that could be repaired by homologous recombination repair [18–21]. Therefore, trabectedin exhibits reduced efficacy in cells deficient in NER, while it is more potent in homologous recombination repair-deficient cells [22–24]. The formation trabectedin-DNA adducts are also able to trap DNA topoisomerase I, which normally introduce single strand breaks (SSB) to relax DNA supercoiling during replication and transcription. Thus, trabectedin-induced topisomerase I-DNA complexes mediate SSB formation that may evolve to DSB [25].

Several combination strategies have been tested in order to increase the therapeutic index of trabectedin in sarcomas. Many of these strategies are based on the combination with compounds that induce DNA damage while also interfering with DNA damage repair mechanisms, such as doxorubicin, cisplatin, PARP inhibitors or campthotecins [1,8,21,26–28]. Campthotecin (CPT) and its derivatives irinotecan and topotecan are chemotherapeutic drugs with proved activity against several types of sarcoma [29–32]. CPT targets DNA topoisomerase I and inhibits its resealing activity leading to the stabilization of the transiently-generated SSB which can be converted into toxic DSB by collision with replication or transcription complexes [33,34].

In this report, we show that trabectedin inhibits tumor cell growth initiated by cell-of-origin models of MRCLS and UPS, represses the expression of important genes associated to the CSC phenotype and targets CSC subpopulations at rates comparable to that of non-CSC subpopulations. Furthermore, we show that trabectedin efficacy is synergistically increased by combination with CPT.

Materials and Methods

Cell Types and Drugs

Previously developed transformed human BM-MSCs lines (Table S1 and Supplemental information) were cultured as previously described [4–6,35]. Trabectedin and camptothecin were obtained from Pharma-Mar (Madrid, Spain) and Sigma (St Louis, MO), respectively (Supplemental information). All experimental protocols have been performed in accordance with institutional review board guidelines and were approved by the Institutional Ethics Committee of the Hospital Universitario Central de Asturias. All samples from human origin were obtained upon signed informed consent.

Western Blot

Whole cell protein extraction and Western blot analysis were performed as previously described [36]. Antibodies used are described in Supplemental information.

RT-qPCR Assays

The Human Cancer Stem Cells RT2 Profiler PCR Array (PAHS-176-Z; SA Biosciences, Qiagen Iberia, Madrid, Spain) was used to analyze the expression of 84 genes linked to CSCs properties according to the manufacturer instructions and as described before [37,38]. A complete data set including gene information and experimentally obtained C_t values is presented in Table S2.

Cell Viability Assays

The viability of all cell lines in the presence and absence of drugs was determined using the cell proliferation reagent WST-1 (Roche, Mannheim, Germany) as described before [38]. In dose–response experiments cells were treated for 72 hours and all the assayed conditions contain the same concentration of drug solvent (DMSO). The concentration of half-maximal inhibition of viability (IC_{50}) for each treatment was determined by non-linear regression. The existence of synergy in drug combinations was determined by calculating the combination index (CI) according to the Chou and Talalay method using CompuSyn software (ComboSyn) [39]. CI *vs.* the fraction affected (F_a) plots were generated for six combinations of drugs at a fixed ratio according to their IC_{50}s. CI values indicate synergistic (<1), additive (=1) and antagonistic (>1) drug interactions.

Tumorsphere Culture

Tumorsphere formation protocol and the analysis of the effects of drugs on tumorsphere formation ability were previously described [38].

Immunofluorescence Staining

In immunofluorescence staining experiments in adherent cultures (2-D) and tumorspheres (3-D) fixation, staining and mounting of the