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Trabectedin, a drug acting on both cancer cells and the tumour microenvironment

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Trabectedin is the first marine-derived anti-neoplastic drug approved for the treatment of advanced soft tissue sarcoma and, in combination with pegylated liposomal doxorubicin, for the treatment of patients with relapsed platinum-sensitive ovarian cancer. From the beginning of its development, trabectedin showed some peculiar properties that clearly distinguished it from other anti-cancer drugs. In this mini-review, we will outline the current state of knowledge regarding the mode of action of trabectedin, which appears to represent a new class of anti-neoplastic drugs acting both on cancer cells and on the tumour microenvironment.

Trabectedin is a tetrahydroisoquinoline alkaloid that was initially isolated from the Caribbean tunicate *Ecteinascida turbinata* and currently prepared by chemical synthesis (Figure 1A) (Cuevas and Francesch, 2009). It was previously described that trabectedin binds to specific selected triplets of the DNA minor groove, whereas a part of it protrudes out of the DNA and probably interacts with proteins at the site of adduct such as XPG or RNA polymerase II (Pol II) (Figure 1B) (Hurley and Zewail-Foote, 2001; Gago and Hurley, 2002; Herrero *et al*, 2006; D'Incalci and Galmarini, 2010; Feuerhahn *et al*, 2011). Besides these molecular mechanisms, trabectedin also induces unexpected biological effects; for example, it is active at low nM concentrations whereas DNA-interacting agents (e.g., temozolomide or platinum compounds) act in the high μM range. It is also worthwhile noting that trabectedin affects slow growing cells and cells in the G1 phase of the cell cycle; furthermore, the pattern of sensitivity observed in cells deficient in DNA repair mechanisms is different. For example, cells deficient in nucleotide excision repair (NER) are generally more sensitive to cisplatin while they are partially resistant to trabectedin (Damia *et al*, 2001; Erba *et al*, 2001; Takebayashi *et al*, 2001; Damia and D'Incalci, 2007). On the other hand, cells deficient in homologous recombination (HR) (e.g., with mutations of BRCA1 or BRCA2 genes) are sensitive to trabectedin as well as to platinum compounds (Damia and D'Incalci, 2007; Soares *et al*, 2007; Tavecchio *et al*, 2008).

gene-dependent manner. This finding, initially demonstrated for the promoters of heat-shock proteins (HSPs) and MDR1 genes, was subsequently extended to several other promoters and appears to be cell dependent (Jin *et al*, 2000; Minuzzo *et al*, 2000; Feuerhahn *et al*, 2011). Moreover, it was reported that trabectedin affects trans-activated but not basal transcription using different mechanisms (Friedman *et al*, 2002). First, trabectedin inhibits transcription by preventing the binding of transcription factors to DNA (see below) (Forni *et al*, 2009; Di Giandomenico *et al*, 2013). Second, the adducts formed by trabectedin functionally mimic an inter-strand cross-linking lesion, despite the fact that the drug binds covalently to only one DNA strand (its interaction with the opposite strand being only through van der Waals forces and hydrogen bonds). This type of lesion is highly effective in blocking transcription through stabilisation of the ds-DNA (Bueren-Calabuig *et al*, 2011; Feuerhahn *et al*, 2011). Finally, trabectedin interacts directly with the elongating Pol II regardless of the strand they are located on (Feuerhahn *et al*, 2011). This eventually arrests Pol II during transcription elongation and induces its rapid and massive degradation via the proteasome pathway in a transcription coupled NER-dependent manner (Aune *et al*, 2008). Therefore, it seems that the activity of trabectedin is mainly related to direct effects on transcription regulation.

INHIBITION OF TRANS-ACTIVATED TRANSCRIPTION

What appears unique for trabectedin is that, at pharmacological concentrations, it modulates gene expression in a promoter- and

SPECIFIC MECHANISMS AGAINST SOME TRANSLOCATION-RELATED SARCOMAS

Myxoid liposarcomas are mainly associated with the presence of t(12;16) or t(12;22) translocations that fuses the gene CHOP

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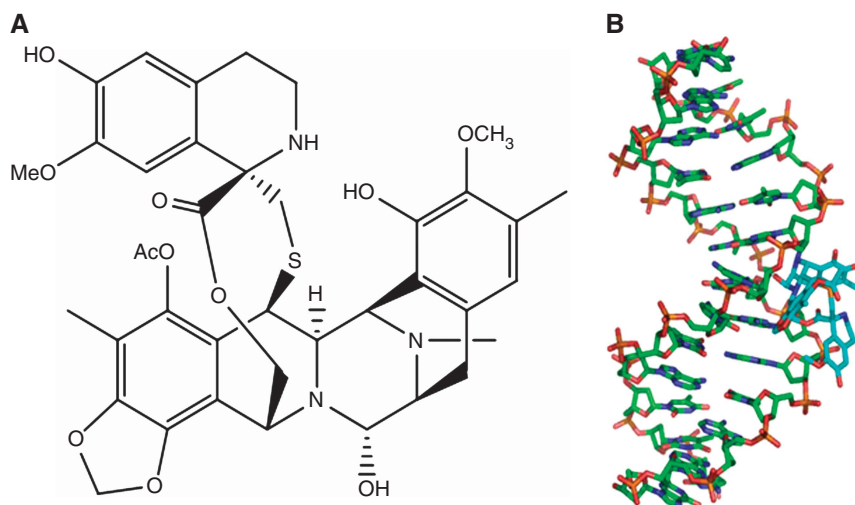


Figure 1. (A) Chemical structure of trabectedin; (B) trabectedin binding to the DNA minor groove. The figure illustrates that a part of the molecule binds to DNA whereas another part protrudes out of DNA.

(at 12q13.1–q13.2) with FUS (at 16p11.2) or EWS (at 22q12.2), respectively. The fusion proteins FUS-CHOP or EWS-CHOP act as abnormal transcription factors and are involved in the pathogenesis of the disease. Recent experiments have demonstrated that trabectedin blocks the trans-activating ability of these chimaeras by displacing the oncogenic fusion protein FUS-CHOP from its target promoters (Forni *et al*, 2009; Di Giandomenico *et al*, 2013). This eventually induces adipogenic differentiation of myxoid liposarcoma cells. This effect has been observed in FUS-CHOP expressing experimental models (tumour cells, myxoid liposarcoma xenografts and transgenic models) and in tumour biopsies taken before and after trabectedin therapy in patients with myxoid liposarcoma (Charytonowicz *et al*, 2012; Di Giandomenico *et al*, 2013). It was also found that, while the displacement of FUS-CHOP chimaera variants (e.g., subtypes I, II and III) from target promoters is similar, the kinetic of re-attachment of the fusion protein subtypes appears to differ. In fact, re-attachment is faster for type III than for types I and II. This is possibly due to the larger size of type III chimaera that makes the complex of transcription factor cofactors and DNA more stable. Of note, type III myxoid liposarcoma experimental models appear to be less sensitive to the differentiation and anti-angiogenic effects of trabectedin, suggesting that the inhibition time of the chimaeric protein is apparently important for its pharmacological effects. It is also interesting to note that FUS-CHOP expression influences several genes involved in tumour angiogenesis and cancer inflammation (Germano *et al*, 2010). Therefore, the peculiar activity of trabectedin in myxoid liposarcomas (Grosso *et al*, 2007, 2009) could be related to an effect not only on genes involved in adipocytic differentiation, but also on genes that code for factors which affect the tumour microenvironment (TME).

Modulation of transcription by trabectedin has also been shown in Ewing's sarcoma, a neoplasm driven by the oncogenic fusion gene EWS-FLI1 (Grohar *et al*, 2011; Grohar and Helman, 2013). Recently, it has been shown that this modulation can be exploited to select a very effective combination treatment of trabectedin followed by irinotecan (Grohar *et al*, 2013). The basis of this synergism is that, by blocking the trans-activating ability of EWS-FLI1 protein, trabectedin induces a downregulation of Werner Syndrome protein (WRN) that increases susceptibility to topoisomerase I poisons (Grohar *et al*, 2011; Grohar and Helman, 2013). The sequential use of trabectedin and irinotecan has been found to be very effective not only *in vitro* but also in tumour

xenografts derived from Ewing's sarcoma patients (Grohar *et al*, 2011; Grohar and Helman, 2013). These results may provide the rationale for clinical studies on the sequential combination of trabectedin and irinotecan in Ewing's sarcoma, a tumour for which there is an urgent and unmet need for more effective therapies.

EFFECTS OF TRABECTEDIN ON THE TME

Since the last decade, tumours are considered as complex tissues with a dynamic and reactive TME. This TME is populated by different non-neoplastic cells (e.g., inflammatory leukocytes, activated fibroblasts and endothelial cells) that actively communicate with cancer cells via chemokines or cytokines. It is now well established that the persistence of inflammatory pathways in the TME is linked with tumour promotion (Bissell and Hines, 2011). Among stromal cells, tumour-associated macrophages (TAMs), derived from blood circulating monocytes and functionally 'educated' by tumour cells, display a number of pro-tumoral functions, such as production of growth factors, switch-on of neo-angiogenesis, enhanced protease activity and support in tumour cell dissemination (Mantovani *et al*, 2002; Pollard, 2004; Qian and Pollard, 2010; Allavena and Mantovani, 2012). It was therefore of interest to find that, in a tumour, trabectedin was affecting not only the neoplastic compartment but also monocytes and macrophages, which are quiescent cells and therefore considered as poorly sensitive to classical DNA-damaging agents (Galmarini *et al*, 2014). These findings opened an important area of research for the implications of macrophage pro-tumoral functions and disease progression.

The exquisite selectivity of trabectedin against mononuclear phagocytes (monocytes and macrophages) was recently related to the rapid activation of caspase 8 by membrane signalling TRAIL receptors; these receptors are expressed more in monocytes/macrophages than in neutrophils and lymphocytes (Germano *et al*, 2013). It was also shown that short exposure to trabectedin caused a strong decrease in the production of several cytokines and chemokines secreted by monocytes/macrophages and tumour cells. For example, trabectedin treatment downregulates the expression of IL-6, CCL2, CXCL8, Angiopoietin 2 or VEGF, but not of other biological mediators such as TNF α (Allavena *et al*, 2005; Germano *et al*, 2010). The anti-inflammatory effects of trabectedin were

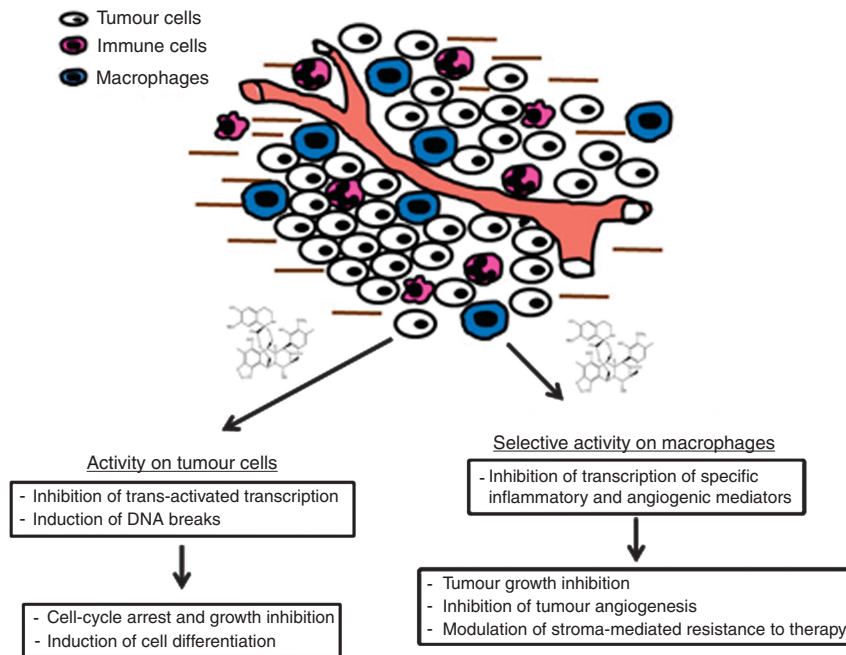


Figure 2. Schematic summary of the different mechanisms of action and biological effects on cancer cells and tumour microenvironment.

confirmed in different tumour xenografts and in human soft tissue sarcoma samples from patients receiving trabectedin as neoadjuvant therapy (Germano *et al*, 2013). The importance of this mechanism of anti-cancer activity was definitely proven in experiments where tumour cells resistant to trabectedin *in vitro* were injected into mice. Interestingly, in the *in vivo* setting, trabectedin still showed anti-tumour activity (Germano *et al*, 2013). The results clearly indicated that the macrophage-targeted effect was sufficient to significantly reduce neoplastic growth, under conditions where tumour cells were unresponsive to the drug. Thus, trabectedin effects on the TME and on macrophages are—at least in part—important contributors of its anti-tumour and anti-metastatic activity. A summary of these effects are depicted in Figure 2.

CLINICAL IMPLICATIONS AND OPEN QUESTIONS

According to the above findings, trabectedin not only inhibits the growth of cancer cells but also affects the TME by limiting the numbers of macrophages and by inhibiting the production of macrophage products that eventually promote tumour growth. In reference to its activity on neoplastic cells, the ability of trabectedin to modulate oncogenic transcription factors is certainly very appealing for several tumours whose pathogenesis is related to translocations involving transcription factor activity. This is the case for some soft tissue sarcoma, but the concept could be extended to other solid tumours and haematological malignancies. To our knowledge, trabectedin is the first compound able to displace an oncogenic transcription factor from its target promoters with high specificity. Unpublished data from our laboratories, however, suggest that in some cases (e.g., in Ewing's sarcoma) the inhibition of transcription factor binding to target sequences does not last very long, and transcription modulation occurs for a short time, possibly not sufficient to exert a significant anti-tumour effect. Nevertheless, a transient change in the transcription of some genes can be exploited to identify appropriate combinations, as recently highlighted in the case of Ewing's sarcomas by the work of Grohar *et al* whose approach

deserves further investigation (Grohar and Helman, 2013). On the other hand, the specific pattern of sensitivity of tumour cells deficient in DNA repair mechanisms opens the possibility to new therapeutic strategies. For example, based on the NER profile, it is possible to envisage the sequential administration of trabectedin followed by drugs such as cisplatin; clinical results in ovarian cancer appear to support this strategy (Callata *et al*, 2013). Also, the high sensitivity of cells that are deficient in HR repair mechanisms suggests that the drug can be particularly effective against breast or ovarian tumours with BRCA1 or BRCA2 mutations (Schoffski *et al*, 2011; Romano *et al*, 2013).

Certainly, the ability of trabectedin to decrease TAM and to modulate the production of cytokines and angiogenic factors is also of relevance. Several clinical and preclinical observations advocate a significant effect of trabectedin on the TME (Galmarini *et al*, 2014). It has also been observed that the response to trabectedin often takes long time, becoming evident only after several courses of therapy, a finding that is difficult to explain whether the drug acts only on cancer cells (Grosso *et al*, 2007; Sanfilippo *et al*, 2011; Sanfilippo and Casali, 2013). Changes in tumour density before size reduction have been documented (Hollebecque *et al*, 2010; Sanfilippo *et al*, 2011). For instance, in liposarcomas, a decrease in tumour density may occur without tumour shrinkage, eventually resulting in size decrease only after several courses (Grosso *et al*, 2007; Sanfilippo and Casali, 2013). Furthermore, the drug often induces a very prolonged disease stabilisation indicating that trabectedin appears to keep tumours under control, suggestive of an oncostatic effect (Del Campo *et al*, 2013; Ferrandina *et al*, 2013; Le Cesne, 2013). This was documented in a series of studies carried out by the Gynecologic Oncology Group (GOG) group with uterine leiomyosarcoma patients treated with different drugs. Trabectedin showed an increased PFS over any other treatment despite a lower rate of tumour reduction in the same time of study (Monk *et al*, 2012). This remarkable observation demonstrates a distinct pattern of response for trabectedin when compared with classical agents like ifosfamide, doxorubicin and gemcitabine.

Furthermore, D'Incalci *et al* (2012) reported significant correlations between patient weight gain and improved survival. Positive correlations occurred during the first cycles of treatment and included small weight differences, suggesting that weight gain

is a visible effect of other underlying changes induced by the treatment. The possibility that responders to trabectedin experience changes in inflammatory cytokines such as IL-6, known to be downmodulated by the drug (Allavena *et al*, 2005), coupled to the fact that this favours weight gain, is worthy of further testing in treated patients.

More studies are needed to elucidate the overall effects of trabectedin on different immunological mechanisms, one example being to assess the relationship between the decrease in the number of immune-suppressive TAM and corresponding effects on adaptive immune response mechanisms. Trabectedin could represent a paradigm to be combined with other therapies directed to elicit anti-tumour cytotoxic lymphocytes. It would be also important to understand whether the different mechanisms of action of trabectedin could be dose and/or treatment schedule dependent. It might be hypothesised that, to obtain a more significant and prolonged anti-inflammatory and anti-angiogenic effect, it would be necessary to use a metronomic administration approach. Work is in progress in our laboratories to evaluate whether this is the case at least at the preclinical level.

CONCLUSIONS

As reviewed here, trabectedin not only has direct effects against cancer cells but also has host-modulating properties that appear to be of great importance for its therapeutic effect. Strong preclinical and clinical evidence reveals the ability of this drug to decrease the number of TAMs and to modify the TME and angiogenesis at therapeutically relevant doses. Therefore, it seems plausible to hypothesise that the multiple mechanisms of action may have different roles in different tumours, and thus the determinants of the drug action can be dissimilar in the diverse contexts. It is reasonable to believe that there is a relationship between the effects on cancer cells and the effects on the TME, resulting in a therapeutic synergism. Subsequent studies should address how to exploit the unique mechanistic features of trabectedin to combine it either with immunological or microenvironmental modulators or with cytotoxic agents in a rational manner.

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