Role of trabectedin in *BRCA*-mutated patients

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Abstract
Trabectedin, approved for the treatment of adult patients with advanced soft tissue sarcoma (STS), after failure of anthracyclines and ifosfamide, or who are unsuited to receive these agents, and in combination with pegylated liposomal doxorubicin (PLD) for the treatment of patients with relapsed platinum-sensitive ovarian cancer (ROC), has a complex multi-targeted mechanism of action. Not only does trabectedin interact with DNA binding proteins and modulate transcription leading to cell-cycle arrest, growth inhibition and cancer cell death, it also has activity in the tumour microenvironment and may inhibit epithelial-mesenchymal transition, thus preventing epithelial cancer cells becoming more aggressive and thus reducing their metastatic potential and preventing development of resistance to chemotherapy. Differences in the cancer cell DNA repair mechanism appear to affect sensitivity to trabectedin, with those deficient in homologous recombination (HR) being highly sensitive to trabectedin and nucleotide excision repair-deficient cells being less sensitive to trabectedin. As trabectedin-induced damage requires repair of DNA-double-stranded breaks, this pathway appears to be pivotal for drug-induced cytotoxicity and response to trabectedin. It was hypothesized that differential expression of factors involved in DNA repair, such as the protein nibrin, may affect clinical outcomes of patients with ROC. Indeed low nibrin expression seems to be associated with significantly better clinical outcome in patients with platinum-sensitive disease treated with the combination of trabectedin plus PLD, but not in with PLD alone. The accumulating evidence of the existence of a sub-set of tumours with defects in the HR-DNA repair pathway, – the BRCAness phenotype – suggests that these patients should benefit more from trabectedin-based therapy. Early data show that this may be true for patients with STS and metastatic breast cancer (MBC) suggesting a potential role for trabectedin monotherapy in the management of these conditions. This may also be the case in high-grade serous ovarian carcinomas for which improved outcomes have been seen in patients with germline *BRCA* mutations. The BRCAness phenotype is expected to identify tumour cells likely to be more vulnerable to trabectedin on the basis of its mechanism of action and we await further data from ongoing evaluations of response to trabectedin according to *BRCA* mutation status and DNA polymorphism of other genes involved in DNA repair with interest. Expression levels of DNA repair genes could be used as molecular biomarkers to select patients who will obtain greater benefit from trabectedin.

Key words: *BRCA*-mutation, BRCAness, DNA repair, nucleotide excision repair, relapsed ovarian cancer, soft tissue sarcoma, trabectedin

Introduction

Trabectedin, a tetrahydroisoquinoline alkaloid, is an anti-tumour compound registered in Europe and in several other countries, for the treatment of adult patients with advanced soft tissue sarcoma (STS), after failure of anthracyclines and ifosfamide, or who are unsuited to receive these agents, and in combination with pegylated liposomal doxorubicin (PLD) for the treatment of patients with relapsed platinum-sensitive ovarian cancer (ROC). This compound was originally extracted from a marine organism, the tunicate *Ecteinascidia turbinata*, and is now synthetically produced.

Trabectedin binds to the minor groove of the DNA, in a different way from conventional alkylating agents, forming DNA adducts on the N2 position of guanine and bending the DNA towards the major groove. A part of the molecule protrudes out of the DNA and presumably plays an important role by interacting with DNA binding proteins (e.g., DNA repair proteins and transcription factors).

Trabectedin causes double-strand breaks (DSBs) and modulates transcription regulation in a promoter-and gene-dependent fashion. The consequences of this include cell-cycle arrest, growth inhibition, differentiation

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and cancer cell death [1]. Recently, it was discovered that trabectedin also has activity in the tumour microenvironment: trabectedin has been shown to selectively deplete blood monocytes and tumour-associated macrophages in tumour-bearing mice, as well as in STS and ovarian cancer patients, suggesting that part of the anti-tumour activity of the drug could be ascribed to its ability to act as a tumour microenvironment modifier [2].

Recent research also suggests that trabectedin may have important inhibitory activity on the epithelial-mesenchymal transition, a process whereby cancer cells of epithelial origin become more aggressive by developing a mesenchymal phenotype. The epithelial-mesenchymal transition is connected with metastatic potential and the development of resistance to chemotherapy [1]. Indeed, trabectedin has a multi-target mechanism of action, with a selective cytotoxic effect on tumour stromal macrophages, a profound anti-angiogenic activity, and selective inhibition of inflammatory mediators (Figure 1) [3]. These effects not only restrict tumour growth and angiogenesis, but also limit tumour aggressiveness (i.e., invasiveness and metastatic potential).

The DNA repair mechanism is particularly important to trabectedin activity. That is, cancer cells deficient in homologous recombination (HR) are highly sensitive to trabectedin. This is of major clinical significance, since many solid tumours have DNA HR deficiency (e.g., mutations or decreased expression of *BRCA1/2* proteins involved in HR), which seems to be a common feature of high-grade serous ovarian cancer [1].

It has been reported that nucleotide excision repair (NER)-deficient cells are significantly less sensitive to trabectedin than NER-proficient cells; the formation of lethal DNA breaks was reported to be related to a functional transcription-coupled NER pathway (TC-NER) [4]. Findings from a study in *Schizosaccharomyces pombe* have suggested that the action of trabectedin in eukaryotic cells might be the result of NER inactivation through the formation of an inactive Rad13/DNA/trabectedin ternary complex, thus conveying the idea that NER components may represent the primary targets of the drug [4]. In the same study, it was shown that trabectedin-induced damage requires DNA-DSB repair, suggesting a pivotal role of this pathway in the drug-induced cytotoxicity. Very recent data also suggest that in mammalian cells the HR repair of DSB is important in modulating the cellular response to trabectedin [5]. Mammalian HR-deficient cells displayed hypersensitivity to the drug and, in addition, data produced in the fission yeast *S. pombe* suggested a crosstalk between the NER and HR pathways to deal with trabectedin-induced lesions [4]. In the proposed model, the trabectedin adducts in the minor groove are recognized by the NER system, in particular the XPG protein. The catalytic endonuclease activity of the Rad13 protein was found to be dispensable, while its C terminal region was essential for the formation of 'cytotoxic complexes' that during the S phase give rise to lesions, probably DSBs, that need to be repaired by HR.

Tavecchio et al. [6] studied the involvement of the DNA-DSB repair pathways (HR and non-HR) both in budding yeasts and in mammalian cells, and the possible cross-talk between NER and these repair pathways. Budding yeasts and mammalian cells deficient in the non-homologous end-joining pathway were moderately sensitive to trabectedin, while systems deficient in the HR pathway were extremely sensitive to the drug, with a 100-fold decrease in the IC50, suggesting that trabectedin-induced lesions are repaired by this pathway. The induction of Rad51 foci and the appearance of c-H2AX were chosen as putative markers for DNA-DSBs and were studied at different time points after trabectedin treatment in NER-proficient and

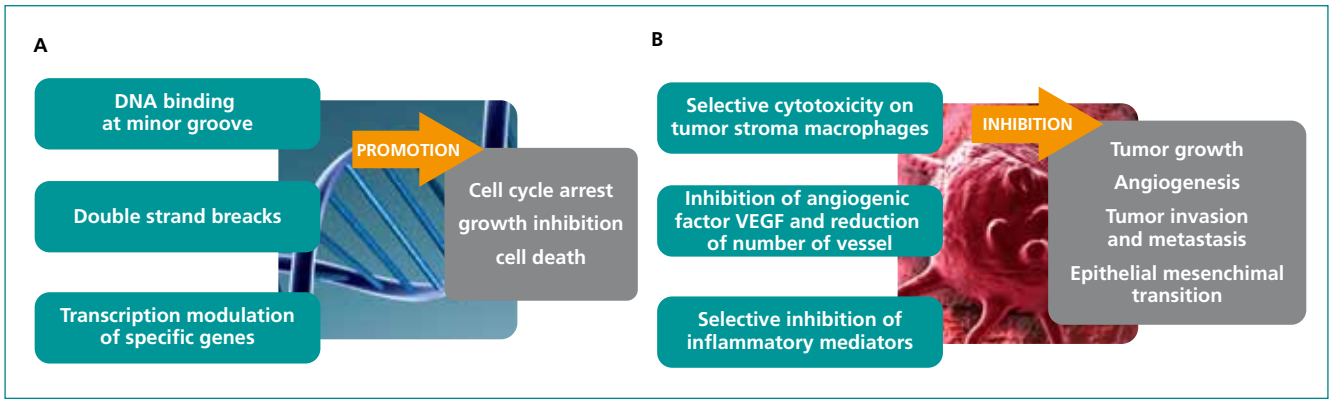

Fig. 1. Mechanism of action of trabectedin. (A) Trabectedin interacting at DNA level and (B) Trabectedin effects on tumour microenvironment (modified from [3]).

Table 1. High levels of DNA repair-related protein nibrin predict worse trabectedin response in ovarian cancer (modified from [7])

Predicative factors	Progression-free survival			Overall survival		
	HR	95% CI	p-value	HR	95% CI	p-value
Treatment arm	0.59	0.35–1.00	0.05*	–	–	–
Age	–	–	–	1.04	1.01–1.07	0.004*
PFI	0.93	0.90–0.97	< 0.001*	0.95	0.92–0.98	0.001*
BD	2.59	1.46–4.57	0.001*	1.67	1.03–2.70	0.04*
Ascites	–	–	–	1.92	1.20–3.06	0.007*
High nibrin	1.02	1.01–1.03	0.001*	1.01	1.00–1.02	0.006*
High BRCA2	0.98	0.97–1.00	0.03*	–	–	–

BD, presence of bulky disease; CI, confidence interval; HR, hazard ratio; PFI, platinum-free interval.
*Statistically significant ($p<0.05$) based on multivariate stepwise Cox regression analysis

-deficient systems. Both were clearly detected only in the presence of active NER, suggesting that the DSBs are not directly caused by the drug, but are formed during the processing/repair of the drug-induced lesions. In conclusion, trabectedin shows decreased activity (from 2- to 8-fold) in NER-deficient cell lines, while cells deficient in HR repair are approximately 100 times more sensitive to the drug, indicating that trabectedin causes DNA-DSBs.

In addition, Monk et al. [7] showed that differential expression of factors, involved in DNA repair, may affect the clinical outcomes of patients with ROC. In particular, low expression of nibrin seems to be associated with a statistically significant better clinical outcome (higher response rate and longer progression-free survival [PFS] and overall survival [OS]) in patients with platinum-sensitive disease treated with the combination of trabectedin plus pegylated liposomal doxorubicin (PLD) [Table 1], but not in those patients treated with PLD alone [7]. These results support the hypothesis that HR can impair DNA repair caused by DNA-damaging agents and result in higher survival rates due to an improved response in HR-deficient patients.

The unravelling of the molecular mechanism of the DNA repair pathways and the cumulating evidence of the existence of a sub-set of tumours with specific defect in the HR DNA repair pathway, the so called *BRCA*ness phenotype [8-10] clearly suggest that patients whose tumours harbour those specific defects should benefit more from a trabectedin-based therapy. This concept will have to be tested in the clinic.

Trabectedin and sarcomas

In agreement with preclinical observations, a retrospective clinical trial of trabectedin in STS showed longer survival times in 245 patients who had active TC-NER machinery and low expression levels of the recombination proteins *BRCA1* and *BRCA2*. In marked contrast, high expression of *BRCA1*/*BRCA2* was associated with lack of clinical response to trabectedin [11].

In tumour samples from 113 trabectedin-treated patients with advanced STS and different *BRCA1* expression, patients with at least one allele of the most common AAAG haplotype had significantly greater PFS (median: 5.6 *vs* 2.5 months; p=0.03) and OS (median: 14.1 *vs* 5.4 months; p=0.0095), compared with patients without an AAAG allele [12]. This suggests that trabectedin induces direct DNA damage more readily in tumour cells with defects in the HR system than those without defects.

Trabectedin and breast cancer

Trabectedin has shown activity against locally advanced or metastatic breast cancer (MBC) [13], including complete (CR) or partial responses (PR) in patients with tumours that overexpressed HER2 or were basal-like. Since 20% of basal-like tumours have been shown to bear a germline and/or a somatic *BRCA1* or *BRCA2* mutation, this subgroup of patients could particularly benefit from trabectedin treatment [14].

In vitro studies have shown that breast cancer cell lines with a defective *BRCA1* gene are more sensitive to DNA-damaging chemotherapy agents, including trabectedin, than those with normal *BRCA* function [15]. Recently a phase II clinical trial was therefore carried out to evaluate the efficacy and safety of trabectedin 1.3 mg/m^2 given as a 3-hour intravenous infusion every 3 weeks (q3w) in patients with MBC [16]. This trial included three parallel cohorts of breast cancer patients (triple-negative, HER2-overexpressed and *BRCA1/2* mutants). In the cohort of patients with germline *BRCA1/2* mutation, a total of 40 patients were included, 26 patients had *BRCA1* mutation, 13 had *BRCA2* mutations and one had both *BRCA1* and *BRCA2* gene mutations. All patients had received previous chemotherapy, with a median of four lines (range: 1–10 lines) per patient. Six assessable patients had confirmed

PR as per Response Evaluation Criteria In Solid Tumours [RECIST] (objective response rate [ORR]=17%; 95% CI=7–34%), in addition, two patients had unconfirmed PR according to RECIST, and six had prolonged (>4 months) disease stabilization. Median PFS was 3.9 months (95% CI=1.6–5.5 months); CA 15.3 levels decreased in 18 of 29 assessed patients (62%), including four responders; 14 patients (40%; 95% CI=24–58%) had clinical benefit (PR or stabilization of the disease ≥4 months) [15]. Altogether, these results suggest a potential role for trabectedin monotherapy in the management of *BRCA*-mutation-associated MBC, which could be enhanced in combination with other anticancer drugs.

Trabectedin and ovarian cancer

Ovarian cancers are heterogeneous at the cellular and molecular levels, and have multiple genetic and epigenetic abnormalities. Some pathophysiologic understanding is evident, for example, in the case of high-grade serous ovarian carcinoma, which is associated with *TP53* mutation and loss of function in 60–80% of familial and sporadic cases.

Important information about *BRCA* alterations is provided by the Cancer Genome Atlas project, which obtained DNA sequence data from 489 tumour samples from patients with high-grade serous ovarian carcinoma [17]; 22% of the samples showed evidence of alterations in the *BRCA* system. These alterations comprised germline mutations, somatic mutations and epigenetic silencing via hypermethylation. Previous reports have documented that up to 10–15% of ovarian cancers are linked with germline mutations of *BRCA1* or *BRCA2* [18].

The Cancer Genome Atlas project demonstrated, in analysis of the entire homologous repair (HR) pathway, that 51% of patients with ovarian cancer had genetic alterations in the HR system. It is known that DNA repair defects lead to multifocal aggressive disease with faster progression, and that lack of functional *BRCA* genes leads to increased genomic instability and disease progression [18].

Regarding the association of *BRCA1/2* mutation with survival and sensitivity to platinum-based chemotherapy, this and other genomic analyses in ovarian cancer (OC) have confirmed improved OS and ORR in patients with germline *BRCA* mutations as compared with non-carriers [19]. Indeed, deficiencies of the HR pathway in DNA repair can impair DNA cross-links repair introduced by platinum-based chemotherapy and result in higher survival rates [20]. Typically patients with repeated platinum sensitivity are considered to have an impaired HR DNA repair system and the clinical selection of BRCAness phenotype is made according to the number of previous responses to platinum [8-10].

In a randomized phase II study, del Campo and co-workers reported that trabectedin had promising activity in 107 patients with platinum-sensitive ROC; the ORR was 35.8–38.9% [21]. Also, trabectedin monotherapy was evaluated in pooled data from three phase II trials, with a total of 295 patients with ROC; 189 (64%) of them had platinum-sensitive disease and (n=106; 36%) had refractory/resistant disease. Response rate was also evaluated according to the prior platinum containing lines received [22]. The ORR to trabectedin was markedly greater in patients with platinum-sensitive *versus* platinum-resistant disease (33–46% *vs* 5–9%), irrespective of whether trabectedin was administered as second- or third-line therapy (Table 2).

In a retrospective, multicentre study [23] aimed at investigating the efficacy and toxicity of trabectedin as a single agent in a large series of 88 very heavily treated ROC patients, we showed that trabectedin provided an ORR of 27.5%. These results seem quite relevant, especially considering that 94% of patients had already received ≥2 previous treatments before trabectedin administration (median number of prior regimens = 4). Moreover, the rate of ORR of 34.3% in the subset of patients with platinum-sensitive disease is of particular interest and compares well with what has been previously reported [21]. It has been suggested that serous, grade 3 ovarian carcinoma could exhibit the BRCAness phenotype, which is

Table 2. Efficacy of trabectedin treatment is maintained across multiple previous platinum-based chemotherapy lines (modified from [22])

Tumour response	Line of trabectedin therapy			
	Second line (n=199)		Third-line or greater (n=95)	
	Platinum-resistant disease (n=67)	Platinum-sensitive disease (n=132)	Platinum-resistant disease (n=40)	Platinum-sensitive disease (n=55)
ORR, %; (CR+PR; 95% CI)	9 (3.4–18.5)	33 (24.7–41.3)	5 (0.6–16.9)	46 (32.0–59.4)
SD (%)	40	39	48	40

CR, complete response; ORR, objective response rate; PR, partial response; SD, stable disease

expected to identify tumour cells likely to be more vulnerable to trabectedin on the basis of its mechanism of action [6]. The need to investigate in more depth the role of *BRCA1/2* mutation, and BRCAness phenotype in conditioning tumour responsiveness to trabectedin has to be acknowledged. Recently Monk et al. [24] reported increased OS (27.3 *vs* 18.7 months) and PFS (13.4 *vs* 5.5 months) in *BRCA1*-mutated ovarian cancer patients treated with trabectedin-PLD combination *versus* PLD monotherapy, suggesting that *BRCA1* may predict improved outcome among trabectedin-PLD treated subjects.

In the same context, the recently published results of the phase II MITO 15 study (NCT01772979, http://www.clinicaltrials.gov) aimed at determining the efficacy of single-agent trabectedin in ROC patients with *BRCA* mutation or *BRCA*ness phenotype, are of great interest [25]. The trial reported an outstanding 50% response rate in a population of 100 ROC patients who had responded to at least two previous platinum-based chemotherapy lines (clinical definition of *BRCA*ness). The evaluation of response to trabectedin according to *BRCA* mutational status and DNA polymorphism of genes involved in DNA repair is ongoing [25].

Conclusion

Although we are currently experiencing a shift toward molecular-targeted anticancer treatments, chemotherapies either alone or in combination with other diverse biological treatment will continue to be a mainstay of treatment for patients with ROC. Treatment of patients with ROC will possibly benefit most from the careful alignment of new cytotoxic chemotherapies and regiments, new trial designs and the addition of therapies targeting critical pathways responsible for tumour progression. All preclinical and clinical studies suggested that maximal trabectedin cytotoxicity requires a synergistic action of both repair systems, and that the expression levels of DNA repair genes (e.g. *BRCA1* and *BRCA2*) could be used as molecular biomarkers to select patients who will obtain greater benefit from trabectedin.

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