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Predictive impact of DNA repair functionality on clinical outcome of advanced sarcoma patients treated with trabectedin: A retrospective multicentric study

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ARTICLE INFO

Article history:
Received 2 December 2010
Received in revised form 6 January 2011
Accepted 20 January 2011
Available online 4 March 2011

Keywords:
Sarcoma
DNA repair
Trabectedin
Yondelis
ET-743

ABSTRACT

Aim: Trabectedin sensitivity is increased in cells with functional nucleotide excision DNA repair, whereas efficient homologous recombination repair leads to resistance. On this basis, a retrospective study of mRNA expression of *BRCA1* (breast cancer susceptibility 1 gene), *XPG* (*Xeroderma pigmentosum* group G gene) and *ERCC1* (excision-repair cross complementing group 1 gene) in tumour samples from sarcoma patients treated with trabectedin was conducted, to correlate DNA repair profiles with patient outcome.

Materials and methods: Quantification of expression in paraffin embedded tumour samples from 245 patients with advanced sarcomas was performed by qRT-PCR (quantitative real-time polymerase chain reaction). Median values were used as cut-off to define low/high mRNA expression.

Results: Low *BRCA1* mRNA expression in tumour samples correlated with statistically significant better response to trabectedin. In contrast to other DNA interacting agents, high expression of *XPG* was significantly correlated with increased response to the drug and high *ERCC1* or *XPD* (*Xeroderma pigmentosum* group D gene) expression did not have a detrimental impact. A composite signature including low *BRCA1* and high *ERCC1* and/or *XPG*

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.01.016

identifies a highly sensitive population of sarcomas with significantly improved treatment outcome.

Discussion: This retrospective study indicates that the DNA repair profile predicts improved outcomes in advanced sarcoma patients when treated with trabectedin. This clinical utility of this signature should be evaluated in prospective enriching studies in sarcoma and other malignancies for patients sensitive to trabectedin.

1. Introduction

Trabectedin (Yondelis®, ET-743) is a marine-derived isoquinoline discovered in the tunicate *Ecteinascidia turbinata*[1] and currently produced using a semi-synthetic technique. The drug is approved in Europe and in other countries for use in patients with advanced previously treated sarcomas[2] and in ovarian cancer.

The clinical data generated for trabectedin in sarcoma patients have consistently reported long lasting remissions and tumour control in a clinically relevant proportion of patients.[3,4] Moreover, the full set of phase II and comparative studies with trabectedin in advanced pre-treated sarcomas[5] have reported 6-month progression free survival (PFS6) rates of 20-80% and median survival of 10 -14 months, with 30-40% of the patients alive at 24 months, which compares well with other active drugs used in this setting.

Experimental data has provided evidence of a unique mechanism of action of trabectedin as compared to conventional agents active in sarcomas.[6] Beyond the action of trabectedin as a minor groove DNA binder,[7] a set of elegant studies have reported diverse interactions at the transcriptional level.[8,9]

Trabectedin binds to G residues in DNA through the minor groove,[7] inducing DNA damage that is recognised by the nucleotide excision repair (NER) pathway, resulting in stalled DNA-protein repair complexes and cell death.[10,11] Therefore, in contrast to what would be expected from a DNA damaging agent, sensitivity to trabectedin is correlated to functional NER *in vitro*.[12] In addition, trabectedin induces DNA double strand breaks, chiefly during early S phase, and this damage is repaired by the homologous recombination repair (HRR) pathway.[13]

Taking into account both clinical and molecular rationales, we investigated DNA repair-based signatures linked to sensitivity/resistance to trabectedin in sarcoma patients. We report the final results from a large retrospective clinical-pharmacogenomic study with trabectedin in patients with previously treated sarcomas. The data gathered in this study suggest the possibility of enriching patient populations for future clinical trials with trabectedin based on this signature.

2. Materials and methods

2.1. Patients

A total of 245 tumour samples were retrospectively collected from sarcoma patients treated with single agent trabectedin in the context of a compassionate use programme, to determine *BRCA1* (breast cancer susceptibility 1 gene), *ERCC1* (excision-repair cross complementing group 1 gene), *XPD* (*Xeroderma pigmentosum* group D gene) and *XPG* (*Xeroderma pigmentosum* group G gene) mRNA expression levels. Paraffin-embedded tissue samples obtained at diagnosis were collected between May 1999 and February 2007. The baseline clinical information and patient outcome data were retrieved from each participating institution by filling out a customised case report form. This study was carried out following approval by the institutional review board at each participating institution.

The characteristics of this unselected sarcoma population are summarised in Supplementary data, Table S1. The following clinical parameters were used for correlative analyses: Objective response was assessed according to RECIST 1.0. The disease control rate was the percentage of patients achieving RECIST complete or partial responses or stable disease. Progression free survival (PFS) and overall survival (OS) were assessed according to clinical research standards. The PFS6 was considered as the time related parameter indicative of drug-induced disease control.[14] Both response and PFS/OS data were used according to investigators' assessment; central review was not conducted.

2.2. Laboratory methods

Tumour samples were evaluated by a pathologist. Those samples having less than 80% of tumour cells were laser-captured microdissected. The remaining samples were analysed without microdissection.

We examined the mRNA gene expression levels of *BRCA1*, *ERCC1*, *XPG* and *XPD* in formalin-fixed, paraffin-embedded specimens as previously described.[15] Methods for RNA expression quantitation and statistical analysis are briefly described as Supplementary data.

3. Results

3.1. Treatment outcome in the overall population

Patients were exposed to a median of 3 cycles of trabectedin (range 1–43). In line with previous phase II data[5] one third of this population received 6 or more cycles of therapy. Information on objective response was retrieved in 177 cases; data on PFS and survival were obtained in 181 patients. Patient characteristics are detailed in Supplementary data, Table S1.

The objective response rate by RECIST in this patient cohort was 14% (95% CI 9–20%); 25 patients achieved a partial response (PR). In addition, 11 cases (6%) had minor tumour

shrinkage (less than 30%); 57 patients (32%) had stable disease (SD), 35 of them (61%) with PFS longer than 6 months. A total of 84 patients (48%) had progressive disease as the best response to trabectedin. In 23 (12%) patients, the response to trabectedin was unknown.

The rate of trabectedin-induced tumour control (PR + MR + SD $\geqslant$ 6 months) was 40% (95%CI 29–43%). Seventy-six patients (37%) were progression-free at 6 months (PFS6). The median PFS was 3.1 months (95% CI 2.5–4.1 mo); the Kaplan–Meier PFS6 was 36% (95% CI 29–43%), and the median survival was 11.9 months (Kaplan–Meier estimates, 95% CI 9–17.9 mo) with an estimated 2-year survival rate of 31% (95% CI 23–39%) (Table 1). Patients who survived beyond 2 years included a high proportion of initially symptomatic patients (60%) and 28% of them had bulky disease at entry.

3.2. *RNA expression analyses*

Tissue samples were available from 245 individual patients. Due to the limited amount of tumour material available, not all samples were successfully analysed for the expression of all marker genes. Two hundred patient samples provided expression data for at least one of the markers studied. Data were obtained from 182 out of 200 samples for *ERCC1* (91%), and 165/200 samples for *BRCA1* (83%), 130/162 samples for *XPG* (80%) and 128/162 (79%) samples for *XPD* mRNA expression levels.

The median expression was used to establish the cut-off value to distinguish between high and low expression levels. The median values and ranges of expression determined were 4.99 (0.78–29.71) for *ERCC1*, 2.36 (0.17–31.75) for *BRCA1*, 1.55 (0.26–8.68) for *XPG* and 2.22 (0.16–13.68) for *XPD*.

3.3. *Impact of HRR functionality in treatment outcome*

BRCA1 mRNA expression levels are a solid indicator of HRR functionality. The median *BRCA1* mRNA expression value of 2.36 determined in 165 patients analysed was considered the cut-off for the purpose of the analysis. There were no significant differences in baseline clinical parameters between patients bearing low and high *BRCA1* mRNA expression levels (data not shown).

The objective response rate (CR + PR) in the low and high mRNA expression groups was 8% and 16% ($p = 0.16$). However, low levels of *BRCA1* expression correlated with a statistically significant better tumour control rate, 48% versus 26% ($p < 0.01$), and with a higher number of PFS6 patients; 42% versus 21% ($p < 0.01$). The median PFS were 4.7 months and 2.0 months in the low and high *BRCA1* expression cohorts, respectively (Kaplan–Meier estimates, $p = 0.002$). The PFS6 were 43% and 23% in the low and high groups (Kaplan–Meier estimates, $p = 0.012$). The estimated median survival in low *BRCA1* patients was 15.4 months and 7.1 months in patients bearing high *BRCA1* expression levels ($p < 0.002$) (Table 1). The Kaplan–Meier plots on PFS and OS according to the *BRCA1* mRNA expression levels in tumour samples, median (2.36) as cut-off, are depicted in Figure 1.

The impact of *BRCA1* mRNA expression levels gains weight when the treatment outcomes are clustered by the 3 different terciles. In fact, PFS6 rates in patients carrying the 0–33% versus 66–100% *BRCA1* mRNA expression levels were 33% and 19% ($p = 0.03$): The median survival in the low versus high terciles was 11.7 versus 6.0 months ($p < 0.001$, data not shown).

These results indicate that sensitivity to trabectedin is associated with a low expression of *BRCA1*, a key gene in the HRR machinery.

3.4. *Impact of NER functionality in treatment outcomes*

3.4.1. ERCC1 and XPD expression levels and sensitivity to trabectedin

The impact of *ERCC1* mRNA expression on treatment outcomes indicates a superior although not statistically significant objective response rate (17% versus 11%, $p = 0.27$), tumour control rate (47% versus 33%, $p = 0.08$), PFS6 (43% versus 29%, $p = 0.05$), median PFS (3.4 versus 2.6, $p = 0.28$) and median survival (17 versus 10 months, $p = 0.67$) in patients having high *ERCC1* mRNA expression levels. These data suggest that, in contrast to the data applicable to other DNA damaging agents, *ERCC1* functionality does not have a detrimental effect on the

Table 1 – Impact of RNA biomarkers expression in the outcome of sarcoma patients treated with trabectedin.

Expression	Subgroup Total STS	CR + PR 25 (14.1%)	Tumour control 71 (40.1%)	Median PFS (months) 3.1	PFS 6 36.0%	Median OS (months) 11.9
BRCA1	Low	13/82 (15.8%)	37/77 (48.1%)	4.7	43%	15.4
	High	7/83 (8.4%)	18/70 (25.7%)	2.0	23%	7.1
	p-Value	0.1601	0.0064	0.002	0.0119	0.0018
ERCC1	Low	9/82 (10.9%)	27/82 (32.9%)	2.6	29%	10.2
	High	14/81 (17.3%)	38/81 (46.9%)	3.4	43%	16.9
	p-Value	0.2691	0.0795	0.2754	0.0516	0.6682
XPG	Low	6/59 (10.2%)	21/59 (35.6%)	2.5	30%	9.3
	High	11/57 (19.3%)	32/57 (56.1%)	7.1	52%	19.1
	p-Value	0.1957	0.0399	0.0021	0.0107	0.2367
Profile	Low *BRCA1* + High (*ERCC1* or *XPG*)	6 (20.7%)	20 (69%)	7.1	60.2%	25.7
	Other	7 (11.5%)	18 (29.5%)	2.4	25.6%	9.3
	p-Value	0.3361	0.0006	0.0015	0.001	0.0107

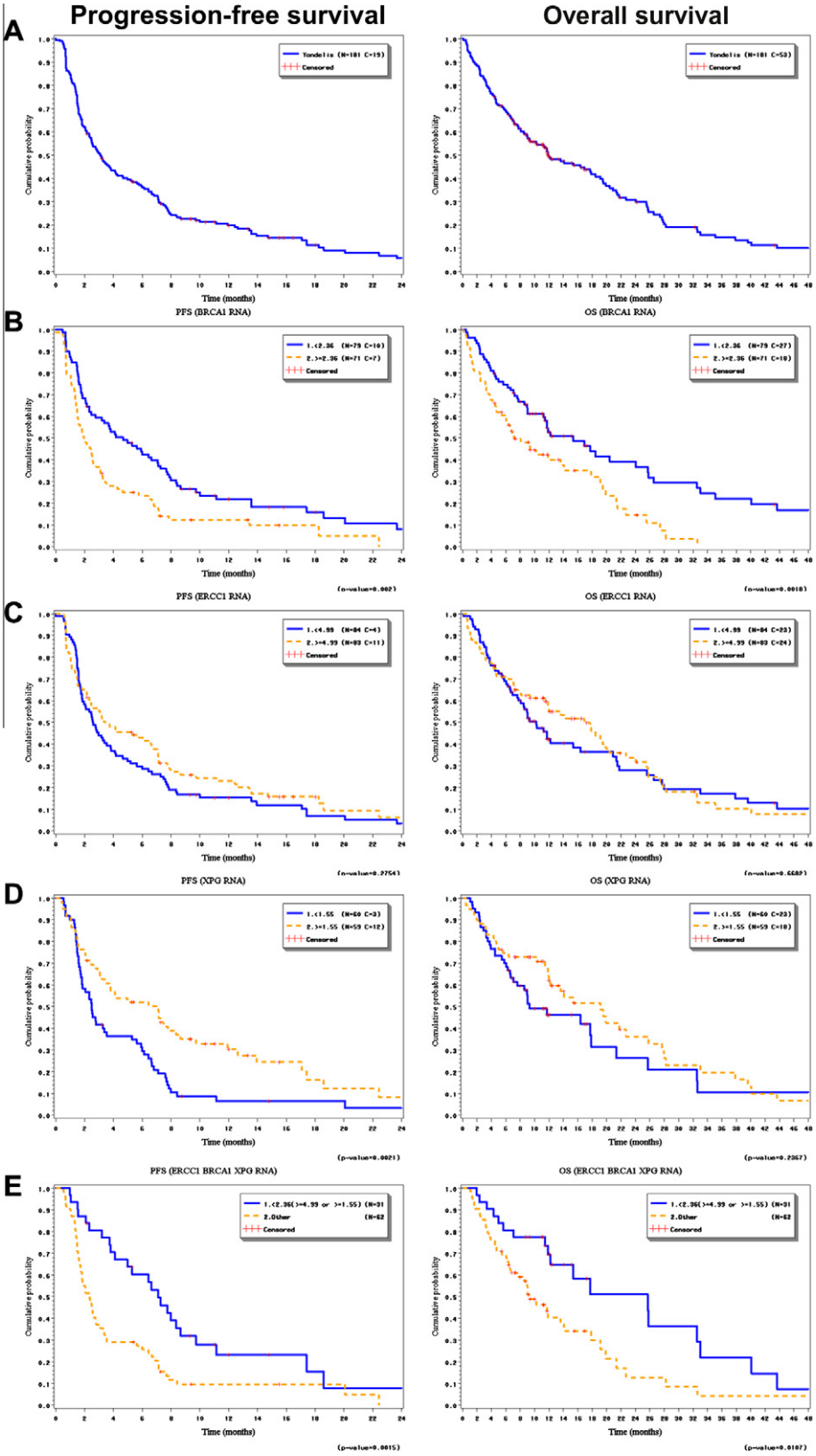

Fig. 1 – Kaplan–Meier plots of progression-free survival and overall survival of patient population (A), according to the mRNA expression of *BRCA1* (B), *ERCC1*(C), *XPG*(D) or the profile (low *BRCA1* + High *ERCC1* or *XPG*) (E) in patients' tumour samples.

outcome of treatment of sarcoma patients treated with trabectedin. Similar results (data not shown) are applicable to the correlation between *XPD* expression levels, and sensitivity and outcome to trabectedin therapy.

3.4.2. *XPG* expression levels and sensitivity to trabectedin

Although gender distribution was well balanced in the full cohort (48% males), a significantly higher proportion of males (61%) were observed in the high *XPG* mRNA expression group ($p = 0.01$).

The analysis of treatment outcome correlated with *XPG* mRNA expression levels (Table 1) demonstrates higher, but not statistically significant, objective response rates in patients bearing high *XPG* mRNA expression levels. CR + PR was attained in 19% versus 10%, ($p = 0.2$) of cases with high versus low levels of expression, respectively. Moreover, patients with high levels of *XPG* expression had a statistically better rate of tumour control (56% versus 36%, $p = 0.04$) and a clear trend to a better PFS6 rate (48% versus 30%, $p = 0.06$). The projected Kaplan–Meier PFS6, median PFS and median survival estimates are 52% versus 30% ($p = 0.01$), 7.1 versus 2.5 months ($p = 0.002$) and of 19.1 versus 9.3 months ($p = 0.24$), respectively, in patients with high versus low levels of expression (Fig. 1). The expression data gathered indicate a direct correlation between NER functionality and sensitivity to trabectedin.

3.5. *Multivariate analysis*

The results of the multivariate analysis of independent markers of clinical benefit are shown in Supplementary data, Table 2. The variables studied were: age, sex, performance status, myxoid liposarcoma, time from diagnosis > 24 mo, sample precedence, *BRCA1*, *ERCC1*, *XPD* and *XPG* expression, both as a binary variable (above/below median) or as a continuous variable. Multivariate analyses for binary categorical variables were done by stepwise logistic regressions, and by stepwise Cox regressions in case of 'time to event' variables. When the mRNA expression data are analysed as a categorical variable (high or low expression), the myxoid liposarcoma subtype was the independent variable with the strongest association to the outcome of trabectedin treatment. Myxoid liposarcoma becomes a variable of statistical significance when correlated with objective response, tumour control and PFS. The mRNA expression of DNA repair genes, such as *BRCA1*, *ERCC1* and *XPD*, are also significantly associated with tumour control and survival. In this multivariate model, performance status (PS), known to be associated with survival, was also found to be an independent variable. *XPG* expression emerges as an independent variable only when the expression data were analysed as a continuous variable (data not shown).

3.5.1. Two- and three-gene composite signatures based on HRR deficiency and NER functionality

The information generated with single gene mRNA expression levels provides a rationale to explore the impact of combining such expression levels to increase the clinical value of such signatures. Such an approach reinforces the selection of responding patients of these combined signatures. In fact, in the cohort of 140 samples analysed, both PFS and OS are statistically significantly better in patients with combined low *BRCA1* mRNA expression and high levels of *ERCC1* ($p = 0.0007$ and $p < 0.01$ respectively) (Supplementary data, Figure 1).

Also, a significantly superior PFS was noted in patients with low *BRCA1* mRNA expression levels along with high *XPG* expression levels ($p < 0.01$). Moreover, this signature showed a positive non-significant trend with survival ($p = 0.09$) (Supplementary data Figure 1).

An attempt to further refine the signature was carried out by analysing treatment outcomes in patients bearing low *BRCA1* expression levels and high levels of *XPG* and/or *ERCC1*. This composite profile was analysed in a set of 99 patient samples. We, therefore, correlated the clinical outcome of this highly sensitive group versus all other combination signatures. The data indicate clinically and statistically significant differences in tumour control (69% versus 30%, $p = 0.006$), and Kaplan–Meier estimates of median PFS (7.1 versus 2.4 months, $p < 0.002$), PFS6 rate (60% versus 26%, $p = 0.0001$) and OS (25.7 versus 9.3 months, 0.01) (Table 1, Fig. 1).

There were no significant differences in baseline parameters between this trabectedin sensitive subgroup and the remaining study population. The triple molecular profile (low *BRCA1* + high *ERCC1* or *XPG* mRNA expression) identified a subgroup highly sensitive to trabectedin treatment whose tumours are characterised by deficient HRR machinery and a functional NER. In our study, 32% of the sarcoma population analysed harboured this sensitive signature.

4. Discussion

This retrospective study confirms the efficacy of trabectedin in unselected patients with pre-treated sarcomas. Long-lasting responses and tumour control were noted across a variety of histological sub-types that are known to bear different molecular profiles.[16] Apart from the retrospective nature of our analysis, it should be highlighted that the patient population in this series was very heterogeneous. They had a variety of mesenchymal malignancies, their previous treatment was not standardised and they came from various sarcoma reference centres that participated in a compassionate use programme of trabectedin. Even considering these limitations of our research, our results sustain the hypothesis of a gene signature that may be linked to sensitivity to trabectedin independently of the sarcoma tumour type. The objective remissions, tumour control and survival data presented in this series are consistent with recent studies of advanced pre-treated sarcoma patients treated with this marine-derived compound.[3–5]

The anti-tumour activity of trabectedin is mediated by DNA binding at the minor groove and establishing a covalent bond of hydroxyl C21 with the exocyclic 2-amino group of a guanine in one strand and one or two hydrogen bonds with other DNA bases in the opposite strand, depending on a sequence context.[7,17] This pharmacodynamic event causes a helix distortion bending of the DNA molecule towards the minor groove,[18] a lesion that is recognised by the NER

pathway. In fact, the binding of trabectedin to DNA is necessary but not sufficient for its anti-proliferative effects, since it requires the presence of specific groups in its C-subunit for interaction with non-DNA targets as NER proteins.[19]

It has also been proposed that the extrahelical protrusion of the C-subunit of trabectedin provides unique characteristics which traps an intermediate in NER processing of trabectedin-DNA adducts. In fact, it has been recently shown[20] that Rad13, the *Saccharomyces pombe* orthologue of the human NER endonuclease *XPG*, covalently binds to the C-subunit of trabectedin bound to the minor groove of DNA by means of arginine-961 residue located in the COOH terminus forming a ternary complex. Therefore, it appears that the interaction between trabectedin and *XPG* confers increased sensitivity to this compound.[20]

It has been shown that other NER-related proteins are also recruited to repair the trabectedin-induced DNA damage, forming larger, more toxic complexes that are able to produce lethal single strand brakes[21] flanking the trabectedin-DNA damage but unable to repair the damage.[22] This trapped intermediate protein-trabectedin-DNA adduct complex can be considered analogous to a topoisomerase I or topoisomerase II-DNA complex.[11,23]

The initial observation[20] made in *S. pombe* suggested crosstalk between the NER and HRR pathways to deal with trabectedin-induced lesions. A high sensitivity to trabectedin in yeast strains lacking Rad51, a gene involved in the repair of double strand breaks in the DNA by HRR, has been reported.

In summary, the antiproliferative effects of trabectedin in the tumour cells are mediated by the NER pathway and the HRR pathway acts as a survival mechanism converting the death-inducing ternary complex NER-trabectedin-DNA into double strand breaks that could be repaired by HRR, resulting in cell survival. A recent study[13] has demonstrated the capability of clinically relevant concentrations of trabectedin to induce DNA double strand breaks in human fibroblastic cell lines. Of interest, a high proportion of phosphorylated histone γ-H2AX foci were noted at an early S phase and was even observed at low concentrations (3 nM) of trabectedin. Moreover, the identification of γ-H2AX foci was followed by the generation of co-localised RAD51 foci, a clear indicator of functionality of the HRR machinery. In the same study, Fanconi anaemia cells (lacking BRCA2 protein function) were also highly sensitive to trabectedin. The significant impact of *BRCA1* mRNA expression levels in patient outcomes has been demonstrated in this study, thus confirming that HRR functionality plays a crucial role in the cellular response to trabectedin.

Overall, a number of laboratory models anticipated that clinical sensitivity to trabectedin in patients might occur in tumours having an efficient NER along with a non-functional HRR. Of note, high expression of NER related genes, such *ERCC1* and *XPG* are known to induce resistance in tumours to classical DNA interacting agents such as cisplatin[24–26] whereas high mRNA expression levels of *BRCA1* and RAP80 cluster sensitivity to taxanes.[27] The information generated in this retrospective translational study confirms the hypothesis suggested in experimental models and proposes a unique signature of sensitivity/resistance to trabectedin based on DNA repair pathways functionality. These data also suggest a mechanism for synergy of agents with different *ERCC1* and *XPG* resistance profiles, e.g. cisplatin and trabectedin, which will have to be examined formally in phase I–II clinical trials.

The characterisation of the NER functionality in tumour samples from this sarcoma population provides information of major clinical relevance. In contrast to the pattern applicable to other DNA interacting agents, neither high levels of mRNA expression of *ERCC1* nor *XPG* confers with poor outcome to trabectedin therapy in sarcoma patients. Of note, high levels of *XPG* mRNA expression are associated with a significant increase in objective response and tumour control with a positive non-significant trend in median survival; the 19% objective remission rate noted in high *XPG* patients along with a median PFS of 7 months reinforces a profile of sensitivity to trabectedin. The pharmacodynamics of trabectedin within the NER pathway have been further characterised in an elegant study conducted in an yeast model,[20] showing that the DNA damage induced by trabectedin is mostly mediated by the NER protein Rad13, the yeast equivalent to human *XPG*. Such a lesion is further repaired by homologous recombination, thus presenting a new framework of interaction of DNA repair machineries behind trabectedin exposure that has also been explored in this study.

We have also analysed the impact of a combined signature of both HRR and NER DNA repair pathways. Consistent with laboratory models, patients with low *BRCA1* and high *ERCC1* and/or *XPG* mRNA expression levels have a pattern of sensitivity and optimal outcome to trabectedin. A rate of 21% response rate with a projected 60% PFS6 and median survival of 25.7 months can be considered parameters of clinical impact in the setting of advanced pre-treated sarcomas. As a result of this retrospective study, *BRCA1*, *ERCC1* and *XPG* emerge as predictive indicators of sensitivity to trabectedin.

The multivariate analysis captures mRNA expression levels of DNA repair genes as parameters of significant impact in tumour control, PFS and survival. The model adds myxoid liposarcoma as a variable correlated to objective response, as well as PS as a variable of reference in survival.

Prospective studies are needed to confirm the current findings and the first trials based on these results have been initiated in sarcoma (EudraCT: 2008-008922-55, translocation related sarcoma Eudra CT: 2008-002326-11, myxoid liposarcoma EudraCT: 2007-000035-25). The proposed signature establishes a predictive, easily implemented gene signature for trabectedin sensitivity in sarcoma patients. The application of this model to other trabectedin-sensitive malignancies, such as ovarian and prostate cancer, is also warranted and currently ongoing. (breast cancer, EudraCT: 2007-000794-31, lung cancer EudraCT: 2007-006681-15 and prostate adenocarcinoma).

The molecular characterisation of sarcomas regarding their DNA repair machinery will likely have a major impact on strategies to further individualise treatment of these rare diseases. In addition, our study underlines the important contributions of research in the orphan setting of sarcoma to our understanding of cancer treatment in general.

Conflict of interest statement

None declared.

Acknowledgements

This work has primarily been financed by PharmaMar and has been partially supported by CONTICANET, Network of Excellence of the European Union (Contract number: FP6-018806). Robert Maki is also supported in part by NCI grants CA47179, CA148260, NCI-ASCO Cancer Foundation Clinical Investigator Team Leadership Supplemental Award, Cycle for Survival and the Shuman Fund for GIST Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.01.016.

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