Trabectedin and Its C Subunit Modified Analogue PM01183 Attenuate Nucleotide Excision Repair and Show Activity toward Platinum-Resistant Cells

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Abstract

PM01183 is a novel marine-derived covalent DNA binder in clinical development. PM01183 is structurally similar to trabectedin (yondelis, ecteinascidin-743) except for the C subunit, and this modification is accompanied by different pharmacokinetics in cancer patients. We here characterize the interaction of PM01183 with the nucleotide excision repair (NER) pathway in comparison with trabectedin. Our results show for the first time that although neither PM01183 nor trabectedin is repaired by NER, both compounds are able to interfere with the NER machinery thereby attenuating the repair of specific NER substrates. We further show that the NER activity is increased in 3 of 4 cellular models with acquired resistance to cisplatin or oxaliplatin, confirming the involvement of NER in the resistance to platinum derivatives. Importantly, both PM01183 and trabectedin show unchanged or even enhanced activity toward all 4 cisplatin- and oxaliplatin-resistant cell lines. We finally show that combinations of PM01183 and cisplatin were mostly synergistic toward both parental and cisplatin-resistant ovarian carcinoma cells as indicated by Chou and Talalay analysis. These data show that the C subunit of trabectedin can be subjected to at least some structural modifications without loss of activity or NER interaction. While PM01183 and trabectedin appear functionally similar in cellular models, it is likely that the differences in pharmacokinetics may allow different dosing and scheduling of PM01183 in the clinic that could lead to novel and/or increased antitumor activity. Taken together, our results provide a mechanistic basis to support clinical trials of PM01183 alone or in combination with cisplatin. Mol Cancer Ther; 10(8); 1–9. ©2011 AACR.

Introduction

PM01183 is a novel marine-derived ecteinascidin derivative in clinical development (1). It is a covalent DNA minor groove binder and is structurally similar to trabectedin (yondelis, ecteinascidin-743) except for the C subunit, where the tetrahydroisoquinoline present in trabectedin is replaced by a tetrahydro-β-carboline in PM01183 (2) as outlined in Fig. 1A. This structural variation is accompanied by important modifications of the pharmacokinetic and pharmacodynamic properties in cancer patients (1).

Trabectedin has an unusual interaction with the nucleotide excision repair (NER) machinery, which distinguishes it from other covalent DNA binders. It is currently unclear if the same will be the case for PM01183 considering that the interaction of the C subunit with NER proteins is controversial. In particular, one study indicated that the C subunit was essential for interaction with NER proteins (3), whereas another study reported that the C subunit had no influence (4).

NER-deficient mammalian cells show at least 2-fold resistance to trabectedin (5, 6) in clear contrast to what is observed for other covalent DNA binding agents where NER deficiency is accompanied by increased sensitivity (7–9). Elegant studies in yeast indicate that the trabectedin-DNA adducts interact with the Rad13 endonuclease, the fission yeast orthologue of the NER endonuclease XPG (10). This finding suggests that trabectedin exposure is accompanied by the accumulation of ternary DNA–trabectedin–endonuclease complexes on the DNA (5, 6, 11), which upon collision with the replication fork leads to formation of DNA double-strand breaks (DSB). In agreement, we have previously shown that the levels...
of trabectedin-induced DSBs are less for XPG-deficient than for XPG-proficient human cells (12). By extension, one could speculate that cells with increased NER activity might show increased sensitivity to trabectedin. However, this issue has, to the best of our knowledge, not been addressed so far.

NER is one of our most versatile repair pathways in terms of lesion recognition and influences the response to many DNA-binding anticancer agents (13). In particular, NER is considered as a key pathway for platinum compounds, a widely used class of anticancer agents (14) and acquired resistance to platinum-based therapies has classically been associated with increased NER activity (14–16). Therefore, an attractive strategy for second-line treatment would be to treat patients with relapsed tumors previously exposed to platinum regimens with drugs that are not subject to NER repair (17). Based on the results of the phase III OVA301 study (18), trabectedin was approved by the European Union in 2009 for treatment of patients with relapsed platinum-sensitive ovarian cancer in combination with pegylated liposomal doxorubicin.

More recently, the relative contribution of NER to platinum resistance has been reassessed. The determination of NER activity in tissues from cancer patients is typically based on the expression of the ERCC1 endonuclease, the last step in the NER pathway. However, the demonstration that ERCC1 also contributes to other types of DNA repair including recombination repair (19–23), the lack of a straight-forward relationship between ERCC1 protein levels and NER activity in cellular models (9, 24) as well as technical issues (25) raise the question to which degree acquired platinum resistance is accompanied by upregulation of NER activity.

We here characterize the repair of PM01183 and trabectedin adducts in living cells. Our results show for the first time that although neither compound is repaired by NER, both are able to interfere with the NER machinery thereby attenuating the repair of specific NER substrates. We further show that the NER activity is increased in 3 of 4 cellular models with acquired resistance to cisplatin or oxaliplatin confirming the involvement of NER in the resistance to platinum derivatives. Importantly, trabectedin and PM01183 show either unchanged or enhanced activity toward the cisplatin- or oxaliplatin-resistant cell lines coherent with previous findings for trabectedin in NER-deficient mutants. The data show that the C subunit can be subjected to at least some structural modifications without loss of the NER interaction. Taken together, our results provide a mechanistic basis to support clinical trials of PM01183 alone or in combination with cisplatin.

Materials and Methods

Drugs and cell lines
PM01183, trabectedin and biotinylated trabectedin (PM01118) were provided by PharmaMar. The acronycine derivative S23906 was obtained from Institut de Recherches Servier, whereas cisplatin was purchased from Merck Génériques. Oxaliplatin (Eloxatin) was obtained from Sanofi-Aventis. NER-proficient (198VI/AS198) and XPA-deficient (XP162VI/AS162) primary fibroblasts were derived from unexposed skin specimens of control individuals and a patient with Xeroderma pigmentosum complementation group A, respectively, in the laboratory of Alain Sarasin. HT-29 colon carcinoma cells were obtained from Richard Camalier (National Cancer Institute), whereas the HCT-116 colon carcinoma cells were a gift from Bert Vogelstein. Oxaliplatin-resistant HT-29 (HT-29/oxa) and HCT-116 (HCT-116/oxa) cells were developed in the laboratory of Annette K. Larsen. Parental A2780 and cisplatin-resistant A2780/CP70 ovarian carcinoma cells were kindly provided by Robert Brown, whereas parental IGROV1 and cisplatin-resistant IGROV1/CDDP ovarian carcinoma cells were provided by Alain Pierre. The cells were maintained in MEM (AS198, AS162), McCoy’s 5A (HCT-116,
HCT-116/oxa), RPMI 1640 (IGROV1, IGROV1/ CDPP), or Dulbecco’s Modified Eagle’s Medium (DMEM; HT-29, HT-29/oxa) supplemented with 5% fetal calf serum (FAS), 2 mmol/L L-glutamine (FAS), 100 units/mL penicillin, and 100 μg/mL streptomycin (PanPharma). All cell lines were regularly tested for Mycoplasma contamination using the Mycoplasma Detection Kit Myco Alert (# LT07-318).

**Unscheduled DNA synthesis**

DNA repair synthesis associated with NER was measured as described previously (9, 26). Briefly, cells were seeded on glass coverslips and grown for 24 hours. The concentration of fetal calf serum was reduced to 0.5% in the presence of 1 μmol/L fluoro-deoxyuridine. The following day, the cells were exposed for 1 hour to PM01183, trabectedin, or S23906 or irradiated with the indicated doses of UV light (254 nm). The cells were then labeled with [3H]thymidine (10 μCi/mL) for 3 hours, postincubated with cold thymidine for 1 hour, and subjected to autoradiography. After 1 week, the number of grains per nucleus was determined. At least 30 nuclei per sample were analyzed for primary cells and at least 100 nuclei for transformed cells. To determine the influence of PM01183 or trabectedin on the repair of UV-induced lesions, cells were preincubated for 1 hour at 37°C with 1 of the 2 drugs before irradiation. The cells were then analyzed as described above.

**Immunofluorescence and microscopy**

Immunofluorescence labeling was carried out as previously described (27). When indicated, cells were washed twice in PBS, resuspended in cold CSK-lysis buffer (150 mmol/L NaCl, 3 mmol/L MgCl2, 1% Triton X-100, 50 mmol/L HEPES pH 7.4, and 30 mmol/L sucrose and protease inhibitors) and kept at 4°C for 5 minutes before fixation in 4% paraformaldehyde (Electron Microscopy Sciences). The cells were then permeabilized with PBS-Triton (0.5%) for 20 minutes. Coverslips were saturated in PBS-Triton (0.5%) for 20 minutes. Coverslips were treated with the primary antibodies (clone E3 or 64M-2, Cosmo Bio Co). The secondary antibody used was a Cy3-conjugated donkey antiserum (1:100, 50 mmol/L NaCl, 3 mmol/L MgCl2, 1% Triton X-100, 100 units/mL penicillin, and 100 μg/mL streptomycin (PanPharma). All cell lines were regularly tested for Mycoplasma contamination using the Mycoplasma Detection Kit Myco Alert (# LT07-318).

**Viability assays**

Cellular viability was determined by the MTT assay as described previously (28). Cells were exposed to PM01183, trabectedin or S23906 for 1 hour and postincubated in drug-free media for 4 to 5 doubling times. Alternatively, the cells were exposed to cisplatin or oxaliplatin throughout the entire incubation period. All values are averages of at least 3 independent experiments each done in duplicate. The combination studies were carried out by simultaneous exposure of the cells to increasing concentrations PM01183 and fixed concentrations of cisplatin (IC10, IC20, IC50, and IC70) values as described previously (29). Similar experiments were carried out with fixed concentrations of PM01183 and increasing concentrations of cisplatin.

**Statistical analysis**

Drug combination effects were determined using the Chou and Talalay method based on the median-effect equation (30), and are indicated in terms of combination index (CI). CI values of less than 0.8 indicate synergy, values between 0.8 and 1.2 indicate additive effects, and values more than 1.2 indicate antagonism. Data were analyzed using the concentration effect analysis software (Biosoft). Statistical analysis and graphs were accomplished by GraphPad Prism version 5.00 (GraphPad Software).

**Results**

**PM01183 and trabectedin-induced DNA lesions are not repaired by NER**

Previous studies with NER-deficient cells indicate that trabectedin adducts are not repaired by NER (5, 6). For direct confirmation, we used the unscheduled DNA synthesis (UDS) assay, which measures DNA repair synthesis, the last step of the NER pathway, in living cells. Exposure of human fibroblasts to UV irradiation (Fig. 1B left) or to S23906, another covalent DNA minor groove binder with similar binding specificity as the ecteinascidins (31, 32; Fig. 1B right), was accompanied by a dose-dependent increase in UDS that was NER specific because no influence on UDS was observed for the NER-deficient XPA cells. In clear contrast, no UDS was observed for cells exposed to PM01183 or trabectedin, whatever the dose (Fig. 1C).

**Chromatin-associated trabectedin is slowly removed**

To characterize DNA repair in proliferating cells we used biotinylated trabectedin, which enabled us to follow the removal of the ecteinascidin adducts. Proliferating cells were exposed to biotinylated trabectedin for 1 hour followed by postincubation in drug-free medium for various times. The results show that trabectedin exposure leads to the formation of biotinylated nuclear foci, which persists throughout the 4-hour wash-out period (Fig. 2A). Image analysis of the labeling intensity showed no
Significant differences between NER-proficient and -deficient cells (Fig. 2B), confirming the incapacity of the NER process to remove the ecteinascidin adducts. The decrease in DNA-associated ecteinascidin adducts observed for both NER-proficient and -deficient cells after 4-hour wash-out is most likely because of homologous recombination repair (12).

**PM01183 and trabectedin interferes with the repair of UV-induced DNA lesions**

The results presented in Figs. 1 and 2 indicate that (i) either the PM01183- and trabectedin-induced DNA lesions are not recognized by the NER machinery, or (ii) that the lesions are initially recognized by the NER machinery but that the NER process is arrested at one of the subsequent steps as previously suggested (5, 6, 11, 12). If the adducts induced by PM01183 and trabectedin are recognized by the NER machinery, we would expect them to be able to compete with other NER substrates. Therefore, NER-proficient cells were exposed to different doses of UV radiation in the absence or presence of PM01183 or trabectedin and the NER activity was measured by UDS. The results show that both compounds are able to slow down the repair of UV adducts in a dose-dependent manner (Fig. 3A and B). At 15 J/m², the UDS activity in cells pretreated with PM01183 or trabectedin was significantly different ($P < 0.001$) from the UDS in control cells as determined by Student’s $t$ test.

For further confirmation, we determined the influence of PM01183 and trabectedin on a different step of the NER pathway, the excision of the UV adducts. For this, we used specific antibodies directed against UV-induced 6-4 PPs. Proliferating cells were preincubated in the absence or presence of PM01183 and trabectedin (100 nmol/L) for 1 hour followed by UV irradiation at 40 J/m² and post-incubation for the indicated times. The 6-4 PP lesions were efficiently repaired and could no longer be detected in repair-proficient cells 4 hours after UV irradiation (Fig. 3C, open circles) as classically found, whereas the levels of 6-4 PPs remained elevated for the XPA-mutant cells (Fig. 4C, black circles). Although the 6-4 PPs were still repaired by 4 hours in the presence of PM01183 and trabectedin (100 nmol/L), the initial repair process was slower (Fig. 3C, open triangles and open squares) with a half-life of 48 minutes in the absence of PM01183 or trabectedin and 110 minutes in their presence. Both PM01183 and trabectedin adducts modify the local structure of DNA (2, 33), which could possibly influence the formation of UV adducts. Therefore, cellular levels of 6-4 PP adducts were compared immediately after UV irradiation for the different treatment conditions. The results show that 6-4 PP formation corresponded to 14.5 ± 3.9 arbitrary units) for the vehicle control, 13.8 ± 5.2 for the cells pretreated with trabectedin, and 14.8 ± 4.5 for the cells pretreated with PM. Therefore, preincubation with trabectedin or PM01183 had no detectable influence on the formation of UV-induced 6-4 PP adducts.

**NER activity in cells with acquired platinum resistance**

To establish if acquired resistance to cisplatin and oxaliplatin was accompanied by increased NER activity, we selected 2 pairs of cisplatin-resistant ovarian carcinoma cells and 2 pairs of oxaliplatin-resistant colon...
carcinoma cells and determined the repair kinetics by UDS after UV irradiation. UV was the treatment of choice because the formation of UV adducts is not influenced by possible resistance mechanisms linked to altered drug uptake, intracellular distribution or metabolism, and leads to an immediate formation of well-characterized DNA adducts that are selectively removed by NER. The results show an important increase in NER activity in 3 of the 4 platinum-resistant cell lines (Fig. 4A–C), which is particularly pronounced for the oxaliplatin-resistant HCT-116 cells (Fig. 4C). In contrast, the NER activity had not changed for the oxaliplatin-resistant HT-29 cells (Fig. 4D) suggesting that the resistance of HT-29/oxa cells is mediated by other mechanism(s).

PM01183 and trabectedin show activity toward cells with acquired platinum resistance

Decreased NER activity is typically associated with increased resistance to trabectedin in repair-deficient mutants. By extension, we speculated that the increased NER activity observed in most of our platinum-resistant cell lines (Fig. 4) might be accompanied by increased sensitivity to PM01183 and trabectedin. For comparison, we also determined the cytotoxic activity of S23906, cisplatin, and oxaliplatin toward the 4 pairs of platinum-resistant cells. The results show that the 2 cisplatin-selected cell lines were approximately 15-fold more resistant to cisplatin, compared with the parental cells, whereas the oxaliplatin-selected cell lines were 9- to 12-fold resistant to oxaliplatin (Fig. 4E and Supplementary Figs. S1 and S2).

Three of the 4 platinum-resistant cell lines showed increased resistance to cisplatin, oxaliplatin, and S23906 (Fig. 4E and Supplementary Figs. S1 and S2). The exception was the oxaliplatin-resistant HT-29 cells that showed unchanged sensitivity to cisplatin, S23906, PM01183, and trabectedin (Fig. 4E and Supplementary Fig. S2). This is also the only of the 4-resistant cell lines with unchanged NER activity (Fig. 4D).

In comparison, 3 of 4 platinum-resistant cells showed no cross-resistance to PM01183 or trabectedin, whereas the fourth cell line, HCT-116/oxa showed 2- to 3-fold increased sensitivity to the 2 ecteinascidins (Fig. 4E and Supplementary Figs. S1 and S2). Interestingly, it is the same cells that displayed the highest increase in NER activity (Fig. 4C).

Combinations of PM01183 and cisplatin are synergistic toward cisplatin-sensitive and -resistant cells

Combinations of cisplatin and trabectedin have shown synergistic activity in both cellular and xenografts models (34, 35) including the ovarian cancer cell lines used in the present work. More recently, the combination of the 2 drugs has showed initial activity in ovarian cancer patients in a phase I study although the optimal
We here characterize the activity of PM01183, a novel covalent DNA minor groove binder in clinical development, in comparison with trabectedin. The 2 compounds are structurally similar except for the C subunit. We therefore wished to establish if combinations of PM01183 and cisplatin were also active.

Ovarian A2780 carcinoma cells were exposed to different concentrations of PM01183 in the presence of fixed concentrations of cisplatin corresponding to the IC_{10}, IC_{20}, IC_{30}, and IC_{50} concentrations followed by the MTT viability assay (Fig. 5A and Supplementary Table S3A). Alternatively, A2780 cells were exposed to different concentrations of cisplatin in the presence of fixed concentrations of PM01183 corresponding to the IC_{10}, IC_{20}, IC_{30}, and IC_{50} concentrations (Fig. 5B and Supplementary Table S3B). To obtain the CI, the functional interactions between drugs were determined using the median-effect plot analysis according to Chou and Talalay (30). A CI value below 0.8 indicates synergy, above 1.2 antagonism, whereas a CI between 0.8 and 1.2 corresponds to an additive effect (29). For both protocols, most combinations were synergistic with average CI values between 0.43 and 0.85. The only exceptions were observed for low concentrations (IC_{10}) of cisplatin (Supplementary Table S3A) with an average CI of 1.62 and for high concentrations (IC_{50}) of PM01183 (Supplementary Table S3B) with an average CI of 1.23.

Because PM01183 is active toward platinum-resistant cells, combinations of PM01183 and cisplatin were also tested for cisplatin-resistant A2780/CP70 cells. The results show that most combinations were synergistic with CI values between 0.42 and 0.89 (Fig. 5C and Supplementary Table S3C). The exception was observed for low concentrations (IC_{10}) of cisplatin, which resulted in an average CI value of 1.28. Similar results were obtained for the cisplatin-resistant IGROV1 cells (data not shown). These findings show that combinations of PM01183 and cisplatin are synergistic under most conditions in agreement with previous results for combinations of trabectedin and cisplatin (34, 35).

Discussion

Because PM01183 is active toward platinum-resistant cells, combinations of PM01183 and cisplatin were also tested for cisplatin-resistant A2780/CP70 cells. The results show that most combinations were synergistic with CI values between 0.42 and 0.89 (Fig. 5C and Supplementary Table S3C). The exception was observed for low concentrations (IC_{10}) of cisplatin, which resulted in an average CI value of 1.28. Similar results were obtained for the cisplatin-resistant IGROV1 cells (data not shown). These findings show that combinations of PM01183 and cisplatin are synergistic under most conditions in agreement with previous results for combinations of trabectedin and cisplatin (34, 35).

Similar controversy surrounds the impact of the C subunit for the interaction with NER proteins. The A and B subunits of trabectedin are primarily responsible for the binding to the minor groove of DNA, whereas the C subunit that protrudes outside of the DNA minor groove is capable of interaction with nuclear proteins (3). The C subunit of trabectedin is essential for interaction with NER proteins (29), whereas another study found that the C subunit had no influence (4). To clarify this important question, we determined the NER activity by the UDS assay, which is the established assay for diagnosis of the NER-deficient syndrome, Xeroderma pigmentosum (42) and that measures DNA repair synthesis, the last step of the NER pathway. Our results clearly indicate that neither PM01183 nor trabectedin is repaired by NER. Specifically, the results show the absence of UDS following exposure to PM01183 or trabectedin, in clear contrast to what is observed after treatment with UV or S23906, another covalent DNA minor groove binder with similar binding specificity as trabectedin.
A2780/CP70 cells were exposed to increasing concentrations of PM01183 in combination with cisplatin at the IC10 (circles), IC20 (triangles), IC30 (inverted triangles), and IC50 (diamonds) values. B, cisplatin-sensitive A2780 exposed to increasing concentrations of cisplatin and fixed concentrations of PM01183 at the IC10 (circles), IC20 (triangles), IC30 (inverted triangles), and IC50 (diamonds) values. C, cisplatin-resistant A2780/CP70 cells were exposed to increasing concentrations of PM01183 in combination with fixed concentrations of cisplatin at the IC10 (circles), IC20 (triangles), IC30 (inverted triangles), and IC50 (diamonds) values.

Figure 5. Cytotoxic activity of PM01183 and cisplatin combinations in ovarian carcinoma cells as determined by Chou and Talalay. A, exposure of cisplatin-sensitive A2780 cells to increasing concentrations of PM01183 in combination with cisplatin at concentrations corresponding to the IC10 (circles), IC20 (triangles), IC30 (inverted triangles), and IC50 (diamonds) values. B, cisplatin-sensitive A2780 exposed to increasing concentrations of cisplatin and fixed concentrations of PM01183 at the IC10 (circles), IC20 (triangles), IC30 (inverted triangles), and IC50 (diamonds) values. C, cisplatin-resistant A2780/CP70 cells were exposed to increasing concentrations of PM01183 in combination with fixed concentrations of cisplatin at the IC10 (circles), IC20 (triangles), IC30 (inverted triangles), and IC50 (diamonds) values.

The absence of NER repair could be because the PM01183- and trabectedin-induced DNA lesions are not recognized by the NER machinery. Alternatively, the lesions might be recognized initially by NER proteins but not then arrested at one of the subsequent steps as previously suggested. To distinguish between these 2 possibilities, we assumed that if PM01183- or trabectedin adducts were indeed recognized by NER proteins, they would act as decoys able to sequester NER components thereby attenuating the repair of true NER substrates such as UV adducts. A similar model has previously been proposed for RNA Pol II, where the need for common factors (e.g., TFIH) during the initial step of both repair and transcription decreases the ability of the initiating Pol II to proceed to elongation (27). Our results show that PM01183 and trabectedin have comparable ability to inhibit the removal and repair of UV-induced lesions. Interestingly, the fact that we observe a delayed repair rather than complete inhibition of the NER process suggest that the sequestration of NER components is not an irreversible phenomenon, but follows an equilibrium between bound and free forms. This hypothesis is enforced by the fact that even at high concentration of PM01183, the level of NER inhibition does not exceed 50%. Thus, although our results do not exclude that some modifications of the C subunit might lead to loss of recognition by NER proteins, this is clearly not a universal rule, because PM01183 and trabectedin show comparable activity with respect to their capacity to interfere with NER despite the differences in their C subunit.

Decreased NER activity in repair-deficient cells is accompanied by increased resistance to trabectedin. By extension, we speculated that cells with increased NER activity might show increased sensitivity to trabectedin. However, this issue has, to the best of our knowledge, not been addressed so far. We first characterized the NER activity in 2 pairs of cisplatin-resistant and 2 pairs of oxaliplatin-resistant cells in comparison with their corresponding parental cells. The results show that acquired platinum resistance was accompanied by increased NER activity in 3 of the 4 cell lines, which was particular pronounced for the oxaliplatin-resistant HCT-116 cells. These findings confirm the role of NER in the resistance to platinum derivatives.

We then determined the sensitivity of the 4 platinum-resistant cell lines to PM01183 and trabectedin in comparison with the corresponding parental cells. Three of 4 platinum-resistant cell lines showed no cross-resistance to PM01183 or trabectedin, whereas the fourth cell line, HCT-116/oxa, showed 2- to 3-fold increased sensitivity to the 2 ecteinascidins. Interestingly, it is also the HCT-116/oxa cells that displayed the highest increase in NER activity, suggesting that collateral sensitivity to PM01183 or trabectedin may only be observed when comparing cells with important difference in NER activity. These findings were specific for the 2 ecteinascidins, because S23906 displayed a sensitivity profile that was comparable with cisplatin.

Combinations of cisplatin and trabectedin have shown synergistic activity in both cellular and xenografts models including the ovarian cancer cell lines used in the present work (34, 35) and with initial indications of activity in the clinical setting (36). We therefore wished to establish if
combinations of PM01183 and cisplatin were also active. Most of the combinations were synergistic, except for low concentrations (IC10) of cisplatin or high concentrations (IC50) of PM01183. In the latter case, it is likely that the lack of additivity/synergy is due the potent cytotoxic activity of PM01183, which induces rapid cell death rather than modulating the NER activity. These findings suggest that moderate concentrations of PM01183 might be more synergistic than high doses when combined with cisplatin. Interestingly, most combinations of PM01183 and cisplatin were also synergistic when tested on platinum-resistant cells.

It should be noted that our observations for the platinum-resistant cells have a potential caveat, because these cells were selected for resistance to platinum rather than for resistance to platinum-containing drug combinations, in contrast to the clinical situation. Therefore, it can not be excluded that agents administered in combination with platinum compounds as first-line treatment could select for resistance mechanisms that might influence the activity of the ecteinascidins. This is particularly true with respect to drug-efflux pumps, because trabectedin is a substrate for ABCB1/P-glycoprotein (43).

In conclusion, we here show for the first time that although neither trabectedin nor PM01183 is repaired by NER, both compounds are able to interfere with the NER machinery thereby attenuating the repair of specific NER substrates. We further show that the NER activity is increased in 3 of 4 cellular models with acquired resistance to cisplatin or oxaliplatin confirming the involvement of NER in the resistance to platinum derivatives. Importantly, trabectedin and PM01183 show either unchanged or enhanced activity toward all cisplatin- or oxaliplatin-resistant cell lines in extension of previous findings with trabectedin in NER-deficient mutants. Our data clearly show that the C subunit can be subjected to at least some structural modifications without loss of activity or interaction with NER proteins. While PM01183 and trabectedin show comparable activity in cellular models, it is likely that the differences in pharmacokinetics will allow different dosing and scheduling of PM01183 in the clinic that may lead to novel and/or increased antitumor activity. Taken together, our results provide a mechanistic basis to support clinical trials of PM01183 alone or in combination with cisplatin.

Disclosure of Potential Conflicts of Interest

A.K. Larsen has received partial research funding from PharmaMar.

Grant Support

This work was supported in part by Conicet (EU Network of connective tissue cancers), CAPES/COFECUB (French-Brazilian collaborative research grant No. 583/07) and PharmaMar. D.G. Soares was supported by the Association pour la Recherche sur le Cancer (ARC), Villejuif, France. M.S. Machado was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

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Received April 4, 2011; accepted May 18, 2011; published OnlineFirst May 27, 2011.

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Molecular Cancer Therapeutics

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Mol Cancer Ther Published OnlineFirst May 27, 2011.

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