However, both compounds were able to interfere with the NER process as shown by attenuated repair of UV-induced DNA lesions that are specific NER substrates. Accordingly, combinations of PM01183 and cisplatin were at least additive toward both parental and cisplatin-resistant ovarian cancer cells.
Conclusion: We here show that PM01183 and trabectedin are not repaired by NER, but are able to interfere with the NER process, probably by acting as decoys for NER proteins. Cells with acquired resistance to cisplatin and oxaliplatin show unchanged or even increased sensitivity to the two ETs. Combinations of PM01183 and cisplatin are at least additive toward both parental and cisplatin-resistant ovarian carcinoma cells. Our data provide a mechanistic basis to support clinical trials of PM01183 in combination with cisplatin toward both platinum-sensitive and -resistant tumors.
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523 POSTER

The XRCC1 Arg280His polymorphism is associated with high-grade radiation-induced late toxicity in prostate cancer patients

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Background: Polymorphisms in genes responsible for DNA damage signaling and repair might modulate DNA repair capacity and therefore affect cell and tissue response to radiation and influence individual radiosensitivity. The purpose of the present investigation was to evaluate the role of single nucleotide polymorphisms in genes involved in DNA repair for the development of radiation-induced late side effects in prostate cancer patients treated with radiotherapy.
Patients and Methods: To analyze the role of polymorphisms in DNA repair genes for late toxicity 603 participants from the Austrian PROCAGENE study were included in the present investigation. Eligible for inclusion in the present analysis were male patients with histologically confirmed prostate cancers who underwent three-dimensional conformal radiation therapy. High energy photons (18 MV) were generally delivered in a three-field technique using an anterior and two lateral fields. All patients underwent three-dimensional conformal radiotherapy. Six functional candidate polymorphisms in XRCC1 (Arg194Trp, Arg280His, Arg399Gln), XRCC3 (Thr241Met) and ERCC2 (Asp312Asn, Lys751Gln) were selected and determined by 5′-nuclease (TaqMan) assays.
Results: Within a median follow-up time of 35 months, 91 patients (15.7%) developed high-grade late toxicities (defined as genitourinary and/or gastrointestinal late toxicity RTOG $\geqslant$2). In a Kaplan–Meier analysis, carriers of the XRCC1 Arg280His polymorphism were at decreased risk of high-grade late toxicity ($p = 0.022$). Univariate Cox proportional hazard analyses showed a lower risk of high-grade late toxicity for carriers of the XRCC1 280His allele (HR = 0.28, 95% CI 0.09–0.90; $p = 0.032$), in multivariate analysis the XRCC1 Arg280His polymorphism remained a significant predictor for high-grade late toxicity (HR = 0.27, 95% CI 0.09–0.86; $p = 0.025$). No significant associations were found for the remaining polymorphisms.
Conclusion: We conclude that the XRCC1 Arg280His polymorphism may be protective against the development of high-grade late toxicity after radiotherapy in prostate cancer patients.

524 POSTER

Potent DNA alkylating agents against human prostate cancer in xenograft model

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Prostate cancer is the most common type of cancer in men in the United States. It is the second leading cause of cancer death in U.S. men after lung cancer. The treatment of this disease includes radiotherapy, proton therapy, chemotherapy, immunotherapy and hormone therapy. The cancer cells may metastasize to other parts of body (such as bones, lymph nodes, rectum, and bladder). We have recently designed and synthesized a series of water-soluble phenyl N-mustards by linking phenyl N-mustard pharmacophore to water-soluble benzenes via a urea linker. These compounds possess potent cytotoxicity in vitro and significant therapeutic efficacy in animal model against various human tumor xenografs. Among these compounds, we found that BO-1055 exhibited potent antitumor activity against human prostate cancer in xenograft model. Human prostatic adenocarcinoma cell lines (LNCaP, 22RV-1 and PC-3 cell lines) were used for evaluating the antitumor activity of the newly synthesized compounds. Significant tumor inhibition (>99%) was achieved when nude mice bearing human prostate adenocarcinoma PC-3 (subcutaneous implantation) were treated with BO-1055 [30 mg/kg, Q2D$\times$4 and then 40 mg/kg, Q2D$\times$3, intravenous injection (iv inj.)]. Moreover, we found that this agent possessed potent therapeutic efficacy in nude mice bearing prostate adenocarcinoma 22RV-1 (derived from a human prostatic carcinoma xenograft, CWR22R, an androgen-responsive human PC cell line) via orthotopic implantation. We have also investigated the mechanism of action of BO-1055 and found that this compound is able to induce DNA interstrand cross-linking. This suggests that DNA cross-linking is probably the main mechanism of action of this compound. The early ADME study reveals this derivative is stable in rat plasma with long half-life in rat. The current studies suggest that this agent may have high potential for clinical application.

Gene therapy and antisense approaches

525 POSTER

Design and synthesis of N10-protected pyrrolobenzodiazepine (PBD) prodrugs for use in nitroreductase-mediated GDEPT therapies

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The over-expression of telomerase in cancer cells has been previously exploited for gene therapy strategies. One approach involves the use of a plasmid containing a telomerase promoter to control the expression of an exogenous nitroreductase enzyme capable of activating bioreductively-sensitive prodrugs. CB1954 is the most commonly studied prodrug for use in bioreductive GDEPT approaches, although it has a number of drawbacks including relatively low potency, inherent toxicities and a lack of patent protection. Therefore, we have designed some novel bioreductive prodrugs based on the sequence-selective DNA-interactive pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumour agents.
The PBDs interact covalently with DNA through formation of a covalent aminal bond between their electrophilic N10-C11 position and the nucleophilic C2-NH_2 of guanine bases. The prodrug design concept involves the introduction of a bulky bioreductively-sensitive protecting group at the N10-position which effectively blocks interaction with DNA thus reducing potency. However, release of the N10-protecting group under bioreductive conditions restores the ability to interact with DNA along with the original biological activity.

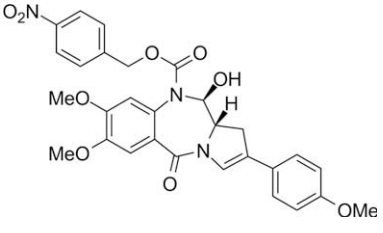

Figure 1: Structure of Nitroreductase PBD Prodrug

As proof-of-principle, we installed a *p*-nitrobenzylcarbamate group at the N10-position of a PBD (Figure 1). We found that upon reduction to the N10-(*p*-aminobenzylcarbamate), this grouping self-immolated to afford the biologically-active parent PBD, *p*-nitrobenzyl alcohol and carbon dioxide. Control molecules including non-reducible N10-benzyl- and N10-SEM-protected analogues incapable of self-immolation were also synthesized. Along with the parent N10-unsubstituted PBD, these molecules were all evaluated in matched *in vitro* panels of A2780 (ovarian), A549 (lung), C33a (cervical) and 5637 (bladder) human tumour cells, one panel being transfected with plasmids containing the Nitroreductase (NTR) gene under the control of a CMV promoter ("NTR+"), a surrogate for the telomerase promoter. The CMV NTR+ panel was found to be more sensitive to the prodrug than the non-CMV NTR panel, with an order of sensitisation of 18.4 > 8.1 > 2.6 and 1.5 for the A2780, A549, C33a and 5637 cell lines, respectively. Crucially, the prodrug was significantly less cytotoxic in all cell lines (*e.g.,* IC_{50} = 0.29 and 0.015 μM in NTR+ and NTR- A2780 cells, respectively) compared to the parent non-N10-substituted PBD (*e.g.,* IC_{50} = 0.000151 μM and 0.00028 μM in NTR+ and NTR- A2780 cells, respectively). Thus, in A2780 NTR+ cells, the prodrug is 1,920-times less

cytotoxic than the parent PBD it releases. The N10-benzyl and SEM control molecules were significantly less cytotoxic in both NTR+ and NTR- A2780 cells, with IC_{50} values ranging from 1.9 to 3.3 mM. In preliminary *in vivo* experiments, the N10-(*p*-nitrobenzylcarbamate) prodrug was evaluated in a nude mouse human tumour xenograft model implanted with A2780 CMV-NTR cells. A clear response to the prodrug was observed at non-toxic doses.

526 POSTER

A potent PBD-heterocyclic polyamide conjugate targeting an ICB2 transcription factor binding site

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The binding of Nuclear Factor Y (NF-Y), a ubiquitous CCAAT-binding transcription factor, to five inverted CCAAT boxes (ICBs) within the promoter region of DNA topoisomerase IIa (topo IIa) results in control of cell proliferation. The regulation of NF-Y/topo IIa interactions by small molecules is of interest in relation to both the development of novel anticancer agents and also chemical tools and probes for use in cancer biology experiments. In this context, we have recently demonstrated that the pyrrolo[2,1-*c*][1,4]benzodiazepines (PBD) C8-*bis*-pyrrole conjugate, GWL-78, can interact specifically at CCAAT-box sites and block NF-Y binding (Kotecha, M. *et al*, *Molecular Cancer Therapeutics*, 7, 1319–1328, 2008).

To further explore this property of GWL-78, and to try to improve both selectivity and potency, a third heterocycle has now been added to the GWL-78 C8-side chain to produce examples of pyrrolo[2,1-*c*]-[1,4]benzodiazepines (PBD) C8-*tris*-heterocyclic conjugates which should span a slightly longer region of DNA upon interaction within the minor groove and offer additional molecular interactions (*e.g.,* hydrogen bonds, electrostatic interactions etc) that may further stabilize the adduct and modify sequence selectivity. On this basis, a library of fourteen PBD C8-*tris*-heterocyclic conjugates was synthesized by attaching a PBD capping unit to pre-constructed tri-heterocyclic polyamides comprising of a combination of pyrrole, imidazole and thiazole heterocycles assembled in a combinatorial fashion. The effect of the composition and length of the C8-heterocyclic polyamide side-chains on DNA binding was evaluated using a number of biophysical and cellular methods. Binding affinity was measured using a calf thymus DNA thermal denaturation assay, and sequence selectivity was evaluated using DNase I footprinting. Their ability to disrupt interaction of the NF-Y transcription factor with its cognate binding site was measured using an EMSA assay, and cytotoxicity was evaluated in the NCI 60 cell line panel.

One conjugate, RMH-41 (Py-Py-Im-PBD; Figure 1), which had the highest DNA binding affinity, also exhibited the ability to inhibit NF-Y transcription factor binding and had significant selective cytotoxicity in human tumour cells. Interestingly, the results of the footprinting experiments showed that, of all the novel conjugates evaluated, RMH-41 appeared to discriminate between two of the ICBs studied, binding to the ICB2 site at a lower concentration compared to that required to bind to ICB1.

In conclusion, the ability of low molecular weight ligands such as RMH-41 to recognize predetermined DNA sequences and prevent endogenous transcription factors from binding could be successfully exploited to modulate transcription and block cancer cell proliferation as part of a therapeutic strategy.

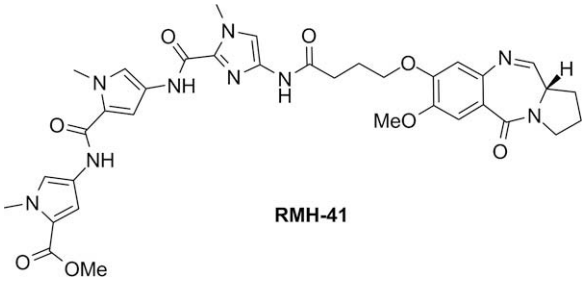

Figure 1. Structure of RMH-41

527 POSTER

siRNA targeting of thymidylate synthase and thymidine kinase for anti-cancer therapy

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Thymidylate synthase (TS) is the only *de novo* source of thymidylate (dTMP) for DNA synthesis and repair. Cytosolic thymidine kinase 1 (TK1) and mitochondrial TK2 are salvage pathways for producing dTMP. TS and TKs are often upregulated in human tumors, suggesting a role for both in malignancy. We have previously shown that antisense oligodeoxynucleotides (ODNs) targeting TS, as single agents, inhibit human tumor cell growth *in vitro* and *in vivo*. In addition, anti-TS ODNs and small interfering RNAs (siRNAs) enhance tumor cell growth inhibition by TS-targeting drugs. TS mRNA is a good target for development of antisense anticancer drugs. However, we hypothesize that when TS enzyme activity is inhibited, the ability of TKs to generate dTMP may mediate resistance to TS-protein targeting drugs.

To determine whether antisense to TK has potential therapeutic benefit, siRNAs targeting TK1 or TK2 were used *in* vitro in 3 different protocols against cultured tumor cell lines: (1) on their own; (2) in combination with TS siRNA; (3) in combination with TS siRNA and the anti-TS drugs 5-fluorodeoxyuridine (5FUdR) or pemetrexed. siRNAs targeting TS or TK1 or TK2, as single agents and in combination with each other, decreased TS or TK mRNA by more than 85% in human cervical carcinoma (HeLa) and human breast carcinoma (MCF7) cell lines (5 nM siRNA, 24 h post-transfection) and decreased TS and TK1 protein. siRNAs targeting TS, TK1 or TK2 did not independently decrease HeLa cell proliferation but did decrease TS, TK1 and TK2 mRNA and TS and TK1 protein levels (5 nM siRNA, 24 and 96 h post-transfection). The capacity of each siRNA to downregulate its target mRNA was unaffected by combination treatment with other siRNAs. HeLa cell TK2 protein after TK2 siRNA treatment was not measured. Treatment with TS siRNA sensitized HeLa cells to 5FUdR by approximately 50% and to pemetrexed by approximately 34% compared to non-targeting control siRNA. siRNA targeting TK1 or TK2 alone did not enhance tumor cell sensitivity to 5FUdR. In support of the concept that TK activity can reduce the ability of antisense TS to sensitize human tumor cells to TS-targeting drugs, we report that simultaneous treatment with TK2 siRNA and TS siRNA enhanced sensitivity to 5FUdR by approximately 25%, and adding TK1 siRNA to TS siRNA enhanced sensitivity to pemetrexed by approximately 20%, beyond the sensitization induced by TS siRNA alone. Enhanced sensitization to 5FUdR and pemetrexed by targeting both TS and TK with siRNAs suggests that the TK salvage pathways are potential targets for anticancer therapies. These data support the hypothesis that combined antisense targeting of TS and TK1/TK2 is more effective than either siRNA used alone to sensitize tumor cells to the effects of TS-targeting chemotherapeutic drugs.

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528 POSTER

Observation of the reversibility of formation of a pyrrolobenzodiazepine (PBD) covalent DNA adduct using HPLC/MS and CD spectroscopy

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It has been previously demonstrated that exposure of a pyrrolobenzodiazepine (PBD) DNA adduct to heat and/or acidic conditions leads to the loss of up to 70% of covalently-bound ligand. For example, Hurley and co-workers have reported that anthramycin cleaves from DNA after heating in the presence of TFA, with simultaneous oxidative formation of a C11a-C1-dehydro product which is non-electrophilic at the N10-C11 position and so cannot re-react with DNA. Therefore, in principle, given the relatively labile nature of the aminal bond formed between PBDs and DNA, an adduct formed from a non-oxidizable PBD should be reversible upon exposure to conditions such as heat or low pH, although this has not been previously demonstrated. The PBD conjugate GWL-78, which is not prone to such oxidation, comprises a C-ring-unsubstituted PBD attached to a methyl ester terminated *bis*-(*N*-methylpyrrole) unit *via* a four-carbon linker between the C8-oxygen of the PBD A-ring and the N-terminus of one pyrrole unit. Using HPLC/MS and CD methodologies to monitor the interaction of GWL-78 with short oligonucleotides, we have now demonstrated for the first time that such reversibility occurs.

A GWL-78/DNA adduct was initially formed and characterized by HPLC/MS and CD. It was then heated to 90ºC at a rate of 10ºC/min which resulted