phosphorylation of the Hsp90 co-chaperone, Cdc37 at the CK2-specific Ser13 site that was accompanied by a reduction in levels of Chk1. Upon combination with gemcitabine, CX-4945 down regulated the expression of Mcl-1 and survivin, two anti-apoptotic proteins known to regulate sensitivity to gemcitabine. CX-4945 + gemcitabine administered to A2780 xenografts was well tolerated, showed an increase in PARP cleavage and significantly enhanced the antitumor activity compared to gemcitabine alone (6/10 regressions versus no regressions).
Conclusions: Inhibition of CK2 by CX-4945 enhances the antitumor activity of gemcitabine by disrupting DNA damage repair and down regulating anti-apoptotic mediators. These data provide preclinical support for combining CX-4945 with gemcitabine in solid tumors.

519 POSTER

Are the methylation of MGMT (O6-methylguanine-DNA methyltransferase) and the DNA mismatch repair (MMR) mechanism frequently involved in pediatric cancers?

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In pediatric oncology more and more protocols are including now temozolomide (TMZ) as a drug in the first line treatment or at relapse time. MGMT activity is known to circumvent the toxicity of alkylating agents such as TMZ. The defect of MMR mechanism is also one of the cellular way for cancers to resist to TMZ. Only few studies have been already done in the pediatric field around these markers of TMZ resistance. Based on the new therapeutic indications of this drug in pediatric cancers, we performed a study in 100 malignant tumors including: sarcomas, neuroblastomas (NB), high grade brain tumors and acute lymphoblastic leukemia (ALL) to evaluate the MGMT methylation and the MMR mechanism.
Material and Methods: 10 high grade gliomas (HGG), 12 medulloblastomas (MB), 16 osteosarcomas (OS), 15 Ewing sarcomas (EWS), 16 rhabdomyosarcomas (RMS), 14 NBs and 17 ALLs were included retrospectively in the study. DNA was extracted from tumors at diagnosis. In all tumors, the methylated status of MGMT was analyzed by methylation specific PCR. Its expression was studied by immunohistochemistry. An allelotyping method was performed in these tumors, using the NIH reference microsatellites, to analyze the MMR status in each tumor.
Results: 3 out of these 100 tumors (2 HGGs and 1 MB) were presenting a MSI (microsatellite instability), witness of a MMR deficiency. Only 4 tumors had a methylated MGMT: 2 EWSs, 2 HGGs. One of the HGG was presenting at the same time both abnormalities. For the tumors treated with TMZ (12 HGGs, 4 MBs, 2 EWSs and 4 NBs), there were no differences on survival between the unmethylated and methylated tumors.
Discussion: The methylation of MGMT and the MMR deficiency seem not to be the major mechanisms involved in the sensitivity to TMZ. These results suggest also that additional mechanisms of TMZ sensitivity and resistance are probably operational.

520 POSTER

NMS-P118, a Parp-1 selective inhibitor with efficacy in DNA repair deficient tumor models

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Poly (ADP-ribose) polymerase 1 and 2 (PARP-1 and PARP-2) are key nuclear enzymes that are activated by DNA damage and play a critical role in the base excision repair pathway. Previous studies have indicated that inhibitors of PARP-1 and PARP-2 could enhance the tumor toxicity of DNA damaging agents by preventing cancer cells from repairing DNA damage. More recently, PARP inhibitors have been shown to be efficacious as single agents in particular tumor settings, such as those deficient in BRCA or pTEN functions. PARP inhibitors in clinical development generally inhibit more than one PARP isoform. Through RNA interference we demonstrated that, in line with the data deriving from knock out mice, PARP-1 inhibition is sufficient to kill tumor cells deficient in DNA repair without affecting viability of DNA repair proficient cells while double PARP-1 and PARP-2 depletion also affect normal cells. Hence a selective PARP-1 inhibitor might have better safety profile, especially in light of a chronic treatment.
Here we show the *in vitro* and *in vivo* characterization of NMS-P118, a low nanomolar PARP-1 small-molecule inhibitor, which is highly selective versus other PARP family members. In cells, NMS-P118 inhibits DNA damage induced PARP-1 activity with an IC_{50} of 10 nM and displays single agent cytotoxicity in BRCA and pTEN deficient tumor cell lines by inducing double strand breaks, that are not properly repaired. NMS-P118 shows almost complete oral bioavailability and excellent ADMET properties across species. In xenografts, daily oral dosing of NMS-P118 is well tolerated and results in dose-dependent tumor growth inhibition in DNA repair deficient models with a confirmed mechanism of action.

521 POSTER

EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment

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Overexpression of the epidermal growth factor receptor (EGFR) is associated with resistance to chemotherapy and radiotherapy. EGFR involvement, in repair of radiation-induced DNA damage, is mediated by association with the catalytic subunit of DNA protein kinase (DNAPKcs). We investigated the role of EGFR nuclear import, and its association with DNAPKcs, on DNA repair following treatment either with cisplatin or ionizing radiation (IR).
EGFR- null murine NIH3T3 cells were transfected with wild type or with mutated EGFR (mutations found in human cancers L858R, EGFRvIII and mutations in the EGFR nuclear localization signal (NLS) sequence NLS123, LNLS123). Comet assay analysis, which measures unhooking of cisplatin crosslinks and repair of IR induced strand breaks, demonstrated that wtEGFR and EGFRvIII completely repair cisplatin and IR induced DNA damage. Immunoprecipitation studies show that repair is associated with the binding of both wtEGFR and EGFRvIII to DNAPKcs, which increases by 2-fold 18 hours following cisplatin treatment. Confocal analysis and proximity ligation assay indicated that this association takes place both in the cytoplasm and in the nucleus resulting in a significant increase of DNA-PK kinase activity.
Intermediate levels of repair as shown by the L858R construct with impaired nuclear localization demonstrated that EGFR kinase activity is partially involved in repair but is not sufficient to determine EGFR nuclear expression. EGFR-NLS mutants showed impaired nuclear localization and impaired DNAPKcs association resulting in significant inhibition of DNA repair and down-regulation of DNA-PK kinase activity.
Our data suggest that EGFR nuclear localization is required for the modulation of cisplatin and IR induced DNA damage repair. The EGFR-DNAPKcs binding is triggered by cisplatin or IR and not by EGFR nuclear translocation *per se*.
Understanding mechanisms regulating EGFR subcellular distribution in relation to DNA repair kinetics will be a critical determinant of improved molecular targeting and response to therapy.

522 POSTER

The marine-derived product PM01183 shows activity toward platinum-resistant cells and attenuates nucleotide excision repair

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Background: PM01183 is a novel ecteinascidin (ET) derivative with structural similarity to trabectedin (yondelis, ET-743), but with different pharmacokinetics as indicated by early clinical studies. Both compounds form bulky monofunctional adducts in the minor groove of DNA. Cells deficient in nucleotide excision repair (NER) proteins are highly sensitive to classical alkylating agents like cisplatin, but show unchanged or even increased resistance to ecteinascidins, probably due to formation of abortive repair complexes. Cells with acquired platinum resistance often have increased NER activity. We here characterize the repair of PM01183 and trabectidin and determine the activity of the two ETs toward cells with acquired resistance to cisplatin and oxaliplatin.
Material and Methods: NER was determined by unscheduled DNA synthesis (UDS) and by quantification of adduct removal by immunocytochemistry. The activity of PM01183 and trabectidin toward two cisplatin-resistant ovarian cancer cell lines and two oxaliplatin-resistant colorectal cancer cells lines as well as the respective parental cell lines was determined by the MTT viability assay. The effect of drug combinations was evaluated by Chou and Talalay analysis.
Results: All platinum-resistant cell lines show either unchanged or enhanced sensitivity to tryptamicidin and trabectedin. Neither compound was repaired by NER as measured by UDS and by adduct excision.

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.

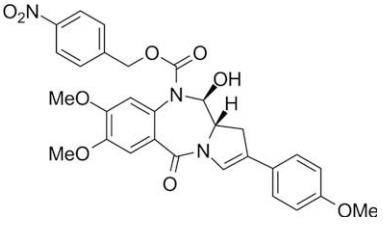

Figure 1: Structure of Nitroreductase PBD Prodrug

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