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.

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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.

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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.

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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.