

Results: Concentration-dependent anti-proliferative effects of PF-01367338 (AG-014699) were seen almost all ovarian cancer cell lines tested, but varied significantly between individual cell lines with up to a 2 log-fold difference in the IC50 values (IC50 range: 0.6–>10 μ M). However PF-01367338 (AG-014699) did not induce apoptosis or cell cycle arrest as a single agent. In contrast, when combined with chemotherapy PF-01367338 (AG-014699) significantly enhanced the apoptotic effects of chemotherapeutic agents. Synergistic drug interactions were observed for PF-01367338 (AG-014699) plus topotecan, doxorubicin, gemcitabine, carboplatin, and paclitaxel across multiple cell lines tested. Studies evaluating predictive markers are ongoing.

Conclusions: The PARP inhibitor PF-01367338 (AG-014699) has significant activity in human ovarian cancer cell lines. These pre-clinical data support the hypothesis that PARP inhibition may potentiate the effects of chemotherapy induced DNA damage and provide a clear biological rationale to test PF-01367338 (AG-014699) in combination with chemotherapy in patients with ovarian cancer.

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POSTER

A phase 1 dose-escalation study to examine the safety and tolerability of LY2603618 administered 1 day after pemetrexed 500 mg/m² every 21 days in patients with cancer

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Background: LY2603618 is a potent and selective inhibitor of Chk1, a protein kinase that plays a key role in the DNA damage checkpoint. Inhibition of Chk1 is predicted to enhance the effects of S-phase cytotoxic agents, such as pemetrexed. The objectives of this Phase 1 study were to assess the safety and tolerability and determine the MTD of LY2603618 in combination with pemetrexed and to assess PK and antitumor activity.

Methods: Study I2I-MC-JMMB was an open-label, multicenter, dose-escalation study in patients with solid tumors. Increasing doses of LY2603618 (40–195 mg/m²) were combined with 500 mg/m² of pemetrexed. In Cohort 1, LY2603618 was administered over 4.5 hrs. Based on PK data from Cohort 1, LY2603618 was reduced to a 1 hr infusion beginning in Cohort 2. During Cycle 1, LY2603618 was administered on Days 1 and 9 and pemetrexed on Day 8 in a 28 day cycle. For all other cycles, pemetrexed was administered on Day 1 followed by LY2603618 on Day 2 in a 21 day cycle. Patients were assessed every 2 cycles per RECIST criteria.

Results: A total of 31 patients were enrolled into 6 cohorts (3 at 40 mg/m² over 4.5 hours; 3 at 40 mg/m²; 3 at 70 mg/m²; 13 at 105 mg/m²; 6 at 150 mg/m²; and 3 at 195 mg/m²). The most frequent AEs reported included nausea, vomiting, diarrhea, hypokalemia, fatigue, constipation, and anemia. Most AEs were CTCAE Grade 1 and 2. Eleven patients experienced SAEs that were attributed to study treatment (diarrhea, fever, pancytopenia, infusion related reaction, pneumonia, anemia, fatigue, leucopenia, and neutropenia). Four patients experienced a DLT: diarrhea (105 mg/m²), reversible infusion-related reaction (150 mg/m²), and pancytopenia (n = 2, 195 mg/m²). The MTD was defined at 150 mg/m². A total of 9/31 patients had stable disease. One patient has an ongoing confirmed partial response in pancreatic cancer. The PK data demonstrated that the exposure of LY2603618 increased in a dose-dependent manner, with a relative minor amount of intracycle accumulation. In nonclinical models, the maximal PD effect correlated with a predicted human AUC >21,000 ng*hr/ml and/or C_{max} > 2000 ng/ml. Both these criteria were met at dose of 105 mg/m².

Conclusions: LY2603618 administered approximately 24 hours after pemetrexed demonstrated an acceptable safety profile; the MTD for this regimen was defined at 150 mg/m². Based on nonclinical predictions, exposures achieved at a dose of 150 mg/m² LY2603618 exceed those required for a biological effect.

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POSTER

Combining pemetrexed with temozolomide and TRC102 (methoxyamine) causes synergistic cytotoxicity in melanoma cells

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Pemetrexed, a folate antagonist, primarily inhibits the conversion of uridine to thymidine by thymidylate synthase (TS), resulting in decreased thymidine availability for DNA synthesis. The imbalance in nucleotide precursors results in the incorporation of uridine into DNA. Aberrant uracil residues are removed by uracil-DNA glycosylases to produce apurinic/aprimidinic (AP) sites, the first step in the processing of abnormal bases by base excision repair (BER). TRC102 (methoxyamine) rapidly and covalently binds AP sites to form lethal DNA lesions that are recognized by topoisomerase IIa (topo II) and can trap topo II in a DNA-cleavable complex. We hypothesized that TRC102 would block the repair of AP sites generated by both temozolomide (TMZ) and pemetrexed and would synergistically potentiate the cytotoxicity of both chemotherapeutics. In the present work, we examined the antitumor activity of the combination of TMZ, TRC102, and pemetrexed in melanoma cell lines. We found that both TMZ and pemetrexed were capable of inducing AP sites that bound TRC102 to form structure-modified AP sites that were refractory to repair by BER proteins. Importantly, the kinetics of AP site formation by TMZ and pemetrexed were different, with TMZ-formed AP sites peaking at 4–6 h and pemetrexed-induced AP sites peaking at 24 h following drug exposure. Thus, the combination of TMZ and pemetrexed increased the total number of AP sites and prolonged the TRC102 reaction with AP sites in cancer cells. Cytotoxicity was analyzed by MTT assay after continuous treatment with increasing combinations of TMZ, TRC102 (3 mM) and pemetrexed (50 μ M) for 3 days. The combination of the three drugs significantly enhanced cytotoxicity by 7-fold versus TMZ alone. The TMZ IC50 value was 30 μ M in combination with TRC102 and pemetrexed, 70 and 80 μ M in combination with pemetrexed or TRC102, respectively, compared to >350 μ M for TMZ alone. For *in vivo* studies, the therapeutic regimen was initiated when WM9 tumor xenografts reached ~100 mm³ in nude mice and treatment was continued for 5 days. At termination, we found 40–50% reduction in tumor volume following treatment with the combination of TMZ with TRC102 or with pemetrexed, and 77% reduction in tumor volume by combining the three drugs relative to TMZ alone, which resulted in no significant reduction in tumor growth compared to the untreated group. Data indicate that combining pemetrexed with TMZ and TRC102 is an attractive therapeutic option for the treatment of melanoma.

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POSTER

CX-4945, an inhibitor of protein kinase CK2, disrupts DNA damage repair, potentiates apoptosis and enhances antitumor activity of gemcitabine in a model of ovarian cancer

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Background: Cancer treatments that combine conventional chemotherapies and molecular targeted agents represent a multifaceted approach to treat cancer that may overcome the shortfalls of monotherapy. Protein kinase CK2 is a serine/threonine protein kinase that has emerged as an attractive molecular target due to its prevalent overexpression in cancer and regulatory role in key cellular processes including cell cycle control, DNA damage repair and apoptosis. CX-4945 is a first-in-class, selective, oral inhibitor of CK2 under investigation in Phase 1 clinical trials. DNA damaging chemotherapeutics like gemcitabine are commonly used to treat solid tumors but are limited in their application by side effects and resistance. Given that CK2 controls multiple pathways that regulate the sensitivity of cancer cells to gemcitabine, in particular DNA damage repair, replication recovery and apoptosis; we investigated the effects of CX-4945 in combination with gemcitabine in a model of ovarian cancer.

Methods: A2780 ovarian cells were used for cell cycle analysis, western blot analysis of proteins involved in DNA damage repair and apoptosis, COMET assays and xenograft studies.

Results: CX-4945 in combination with gemcitabine enhanced DNA damage as evidenced by increased phosphorylation of γ H2AX (S139) and prominent tails visualized in the comet assay. The combination delayed replication recovery and caused accelerated induction of apoptosis. Western blot analysis showed that the gemcitabine + CX-4945 combination diminished phosphorylation of DNA repair protein XRCC1 at the CK2-specific Ser519 site and depleted levels of the mediator of DNA damage checkpoint protein 1 (MDC1). Further, the combination reduced

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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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.

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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 IC₅₀ 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 ADME 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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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 (DNAPKs). We investigated the role of EGFR nuclear import, and its association with DNAPKs, 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, LNS123). 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 DNAPKs, 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 DNAPKs 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-DNAPKs 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.

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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 trabectedin 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 trabectedin toward two cisplatin-resistant ovarian cancer cell lines and two oxaliplatin-resistant colorectal cancer cell 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 tryptamycin and trabectedin. Neither compound was repaired by NER as measured by UDS and by adduct excision.