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 μmol). 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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A phase 1 dose-escalation study to examine the safety and tolerability of LY2603618 administered 1 day after pemetrexed 500 mg/m^2 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/m^2) were combined with 500 mg/m^2 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/m^2 over 4.5 hours: 3 at 40 mg/m^2: 3 at 70 mg/m^2; 13 at 105 mg/m^2; 6 at 150 mg/m^2; and 3 at 195 mg/m^2). 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/m^2), reversible infusion-related reaction (150 mg/m^2), and pancytopenia (n = 2, 195 mg/m^2). The MTD was defined at 150 mg/m^2. 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/m^2.
Conclusions: LY2603618 administered approximately 24 hours after pemetrexed demonstrated an acceptable safety profile; the MTD for this regimen was defined at 150 mg/m^2. Based on nonclinical predictions, exposures achieved at a dose of 150 mg/m^2 LY2603618 exceed those required for a biological effect.

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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/apyrimidinic (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 μM) for 3 days. The combination of the three drugs significantly enhanced cytotoxicity by 7-fold versus TMZ alone. The TMZ IC50 value was 30 μM in combination with TRC102 and pemetrexed, 70 and 80 μM in combination with pemetrexed or TRC102, respectively, compared to >350 μM for TMZ alone. For *in vivo* studies, the therapeutic regimen was initiated when WM9 tumor xenografts reached ~100 mm^3 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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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 γH2AX (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 XRCC1at the CK2-specific Ser519 site and depleted levels of the mediator of DNA damage checkpoint protein 1 (MDC1). Further, the combination reduced