Conclusions: AT9283 given as a weekly (day 1,8 every 21 days) 24 hour infusion has clinical activity and has a tolerable toxicity profile. NCIC CTG has activated a phase II trial in refractory multiple myeloma using this dose schedule.

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Chemo-sensitization using cancer targeted Spermidine–CoA based compound

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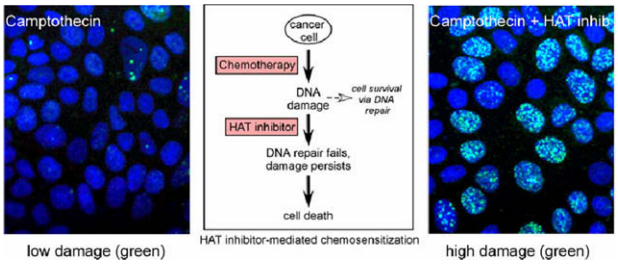
The efficacy of cancer chemotherapy and radiotherapy relies on generation of DNA damage. Since intrinsic DNA repair pathways enable cancer cells to survive by repairing these damaged lesions, inactivation of DNA repair coupled with chemotherapy or radiotherapy has a potential to enhance the effect of these therapies. We have used an S-substituted coenzyme A (CoA) inhibitor of histone acetylation, consisting of spermidine (Spd) linked to the S-terminus of CoA through a thioglycolic acid linkage (adduct abbreviated as Spd–CoA), as well as truncated version of the Spd–CoA structure in which the negatively charged portion of the CoA moiety is removed. While exposure of cancer cells to the Spd–CoA compounds has little effect on cell viability, it causes a rapid inhibition of acetylated lysines, including H3-K9 and H3-K56. That inhibition correlates with a transient arrest of DNA synthesis, a transient delay in S-phase progression, and an inhibition of nucleotide excision repair and DNA double strand break repair. The Spd–CoA inhibitor is synergistic at inducing cell killing when used in combination with DNA-damaging chemotherapeutic drugs such as cisplatin (Platinol™), 5-fluorouracil, and camptothecin, as well as UV-C radiation. However, a synergistic sensitization effect is not observed with the chemotherapeutic agent, Taxotere, which targets microtubules. This further supports the notion that a common mechanism, relevant to DNA damage, underlies the ability of histone acetylation inhibition to synergize with drugs and radiation. After the treatment with Spd–CoA and the DNA damaging drug, camptothecin, DU145 prostate cells were tested for persistence of accumulated gamma-H2AX, a histone variant that accumulates at sites of DNA double strand breaks. Both Western analysis and immunofluorescence staining show the presence of enhanced gamma H2AX accumulation after the combined treatment, under conditions where neither the HAT inhibitor nor camptothecin are effective as single agents, indicating impairment of DNA repair response. Normal human fibroblasts and epithelial cells are not sensitized to DNA damage by Spd–CoA due to a barrier to uptake, indicating that this differential uptake can be exploited to achieve cancer cell specific sensitization. Therefore, this apparently nontoxic compound could significantly improve the therapeutic index of established chemotherapeutic agents in vivo, thereby reducing toxicity to normal tissues. Furthermore, therapy sensitization occurs in both p53-null in cancer cells expressing wild-type p53, indicating that p53-mediated apoptosis is not required. The truncated Spd–CoA derivative displays similar but enhanced chemosensitization effects, suggesting that this class of inhibitors may be amenable to further refinement and have considerable clinical potential as a novel class of potent therapy sensitizers applicable to a broad range of conventional cancer treatments, particularly to reduce therapy toxicity and reverse therapy resistance.



514 POSTER

Sensitisation of paediatric solid tumours to DNA-damaging chemotherapy by inhibition of DNA-dependent protein kinase (PRKDC)

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Background: DNA-dependent protein kinase (DNA-PK or PRKDC) plays an essential role in the repair of DNA double strand breaks which are typically induced by ionizing radiation (IR) and topoisomerase II poisons. For the DNA-PK inhibitor NU7441, chemo- and radiosensitization in vitro and in vivo has been demonstrated for a variety of drugs in various adult cancers.
Material and Methods: To evaluate whether the modulation of DNA-PK activity could potentiate the effect of DNA-damaging chemo-/radiotherapy regimes used for the therapy of paediatric cancers, an initial series of experiments was undertaken to identify chemotherapeutic drug classes whose activity is dependent upon DNA-PK status. A pair of engineered isogenic cell lines was used, which are deficient (V3) or proficient (V3-YAC) for DNA-PK. Both cell lines were treated in parallel with a panel of the most clinically relevant DNA-damaging chemotherapeutics currently used in paediatric oncology (cisplatin, cyclophosphamide, doxorubicin, etoposide, temozolomide, topotecan), or IR, and differences in cell survival/colony formation were assessed using clonogenic assays. The drugs displaying the greatest DNA-PK dependent difference in cell survival, and thus the most specific effects of DNA-PK modulation, were the topoisomerase II inhibitors (doxorubicin, etoposide) and IR. The Ewing sarcoma family of tumours was selected to extend our investigations, since doxorubicin, etoposide and localised radiotherapy are routinely used in their initial treatment. The disease-representative cell lines TC-71 and VH-64, which carry the translocation t(11;22) (q24;q12) (EWS/FLI-1 fusion transcript) were selected for initial experiments.
Results: Using Ewing tumour cell lines VH-64 and TC-71 in clonogenic assays, the specific and selective DNA-PK inhibitor, NU7441, itself showed no significant cytotoxic effect when used alone at concentrations $\leqslant 5\,\mu M$. Co-treatment of TC-71 or VH-64 with NU7441 (of 1 μM) sensitized the cells towards the effects of doxorubicin, etoposide and IR in a dose-dependent fashion with a reduction of the median lethal dose (LD_{50}) by factors of 2−2.5 (doxorubicin), 3.3−3.8 (IR) and 3.7−5.7 (etoposide).
Preliminary data suggest that medulloblastoma cell lines (D425, D283) can also be sensitized towards IR when co-incubated with NU7441 and tested by XTT/survival assays.
Conclusions: In summary, we report here first evidence of *in vitro* chemosensitization of Ewing tumour cells to the effects of doxorubicin, etoposide and IR by co-treatment with the DNA-PK inhibitor NU7441. We are now planning to evaluate NU7441 in combination with Etoposide and Doxorubicin in an orthotopic mouse model for Ewing's sarcoma using serial imaging (MRI). These data strongly support the comprehensive assessment of DNA-PK inhibitors for the improved therapy of paediatric solid tumours.

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Therapeutic advantage of chemotherapy drugs in combination with PARP inhibitor PF-01367338 (AG-014699) in human ovarian cancer cells

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Background: Targeting the nuclear enzyme Poly (ADP-ribose) Polymerase (PARP) represents a novel approach to the treatment of ovarian cancer (OC) and appears to be particularly promising for those patients carrying mutations in BRCA1 and -2 genes, but also in sporadic OC its role is emerging.
Material and Methods: We examined the effects of PF-01367338 (AG-014699) on proliferation, apoptosis, and cell-cycle using a panel of 40 established human ovarian cancer cell lines representing the known molecular heterogeneity of human OC. Growth inhibition was studied using a short term 2-D growth assay and a long term anchorage independent clonogenic assay. Molecular markers for response prediction were studied using gene expression profiling, Western blot analysis, and mutational analysis. Cell lines were also analyzed for BRCA1/2 methylation status. Multiple drug effect/combination index (CI) isobologram analysis was used to study the interactions between PF-01367338 (AG-014699) and carboplatin, doxorubicin, gemcitabine, paclitaxel or topotecan.The effects of PF-01367338 (AG-014699) on apoptosis were compared when using it as a single agent or in combination with chemotherapy.

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.

518 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 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