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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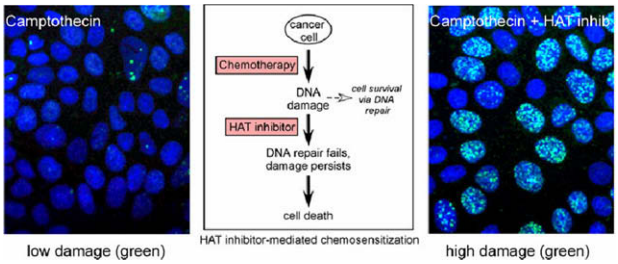
The efficacy of cancer chemotherapy and radiotherapy relies on generation of DNA damage. Since intrinsic DNA repair pathways enable cancer cells to survive by repairing these damaged lesions, inactivation of DNA repair coupled with chemotherapy or radiotherapy has a potential to enhance the effect of 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.



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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.