510 POSTER

5-azacitidine restores and amplifies the response to bicalutamide and radiotherapy on preclinical models of androgen receptor expressing or deficient prostate tumors

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Background: Epigenetic modifications play a key role in the in prostate cancer (Pca) progression to a hormone refractory state (HRPC) and the current use of agents targeting epigenetic changes has become a topic of intense interest in cancer research. In this regard, 5-Azacitine (5-Aza) represents a promising epigenetic modulator. This study tested the hypothesis that 5-Aza may restore and enhance the responsiveness of HRPC cells to Bicalutamide (BCLT) on AR expressing (22rv1) and AR deficient (PC3) Pca cells. Additionally, the radiosensitizing effect of 5-Aza was also studied.
Materials and Methods: The effects of 5-Aza and BCLT were studied *in vitro* and *in vivo* models. Two aggressive 22rv1 (AR+) and PC3 (AR−) cell lines were used for these experiments. The effect of treatments on in vitro models was evaluated measuring: (1) Thymidine incoprporation, (2) apoptosis machinary and (3) caspase activity. In in vivo models the different treatments were evaluated measuring: (1) Tumour volume, (2) tumor weight, (3) Complete response (CR) defined as the disappearance of a measurable lesion, (4) Partial response (PR) defined as a reduction of greater than 50% of tumor volume, (5) Stable disease (SD) defined as a reduction of less than 50% or an increase of less than 50% of tumor volume; (6) Tumor progression (TP) defined as an increase of greater than 50% of tumor volume; (7) Time to progression. The radiosensitivity of tumor cell lines was made by clonogenic survival assay and by in vivo studies.
Results: The combined treatment between 5-Aza and BCLT upregulated the expression of FasL, phospho-FADD, $p16^{INKA}$, Bax, Bak and $p21^{WAF1}$ and inhibited FLIP, Bcl-2 and Bcl-XL expression. The re-activation of hormonal response of AR negative PC3 cell line was partially due to the AR re-expression mediated by 5-Aza treatment. In contrast, the increase in the response to anti-androgenic therapy in 22rv1 did not correlate with AR expression levels. Furthermore xenograft studies revealed that the combined treatment of 5-Aza with BCLT had additive/synergistic effects in repressing tumor growth in vivo and the underlying mechanisms responsible for these effects seemed to be in part mediated by the induction of apoptosis. Finally, our results show that 5Aza treatment resulting in G2/M cell cycle significnatly improve the radiotherapy efficacy both *in vitro* and *in vivo*.
Conclusions: This study suggests a therapeutic potential of 5-Aza in combination with BCLT and radiotherapy in patients with in AR expressing and AR deficient prostate tumors. These preclinical results may have a significant clinical impact on the management of men with prostate cancer.

511 POSTER

MEK/ERK inhibitor U0126 increases the radiosensitivity of rhabdomyosarcoma cells in vitro and in vivo by down regulating growth and DNA repair signals

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Background: Multimodal treatment has improved the outcome of many solid tumors, and in some measure the use of radiosensitizers is responsible for this gain. Activation of the extracellular signal-regulated kinase (MEK/ERK) pathway generally results in stimulation of cell growth and confers a survival advantage playing the major role in human cancer. The potential involvement of this pathway in cellular radiosensitivity remains unclear. We reported previously that the disruption of c-Myc through MEK/ERK inhibition blocks the expression of the transformed phenotype, affects in vitro and in vivo growth and angiogenic signals, and induces myogenic differentiation in the embryonal rhabdomyosarcoma cell lines (RD). The present study was designed to examine whether the ERK pathway affects intrinsic radiosensitivity of rhabdomyosarcoma cancer cells.
Materials and Methods: Exponentially growing human rhabdomyosarcoma RD, xenograft derived RD-M1 and TE671 cell lines were used. For *in vivo* experiments, 45-day-old female nude CD1 mice were xenotransplanted by s.c. injection in the leg using a 21-gauge needle on a tuberculin syringe (1×10^6 cells in 200 ml). Treatments started when tumors reached a volume of 0.2 to 0.5 cm3. U0126 was administered 3 times/week, the day before of RT treatment. Mice were irradiated at room temperature using an Elekta 6-MV photon linear accelerator. Five fractions of 2 Gy were delivered over 5 consecutive days for a total dose of 10 Gy. A dose rate of of 1.5 Gy/min will be used with a source-to-surface distance (SSD) of 100 cm. Prior to irradiation mice were anesthetized and were protected from off-target radiation by a 3 mm. lead shield. Before tumor inoculation mice were randomly assigned to 4 experimental groups. Each group was composed of 8 mice. One control group received intraperitoneal (i.p.) injection of 200 ml carrier solution; one group received i.p. injection of 200 ml U0126 solution at the dose of 25mmol/Kg; one group received RT (6 fractions of 2 Gy delivered 3 times/week to a total dose of 12 Gy); one group received 200 ml U0126 solution at the dose of 25 mmol/Kg coupled with RT (6 fractions of 2 Gy delivered 3 times/week to a total dose of 12 Gy) delivered 24 hrs after the beginning of treatment with U0126. Experiments were stopped 12 days after the last RT treatment and mice were sacrificed by carbon dioxide inhalation. Tumours was directly frozen in liquid nitrogen for protein analysis and biochemical evaluation.
Results: The specific MEK/ERK inhibitor, U0126, reduced the clonogenic potential of the three cell lines and when combined with radiation it strongly potentiated the decrease in clones formation. U0126 besides to inhibit phospho/active ERK1/2 and c-Myc level significantly reduced DNA-PKCs and Cyclin D1 levels leaving unchanged f phospho- inactive GSK3-beta. These results together with the biochemical data suggest that ERKs and DNA-PKCs cooperate in the radioprotection of rhabdomyosarcoma cells. Radiosensitivity was also evaluated in TE671 cell line-xenotransplanted mice. The reduction of tumor masses and the increase in the time of tumor progression (TTP) following U0126 treatment was accompanied by down regulation of DNA-PKcs and c-Myc. These phenomena were greatly improved under combined U0126 treatment with radiotherapy.
Conclusion: Our data indicate a tight correlation between MEK/ERK inhibition and pathways sustaining radioresistance in rhabdomyosarcoma cells configuring the pharmacological targeting of MEK/ERK pathway as biologically plausible in combination with radiotherapy.

DNA repair and inhibitors

512 POSTER

NCIC CTG IND.181: Phase I study of AT9283 given as a weekly 24 hour infusion

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Background: AT9283 is a small molecule inhibitor of aurora kinases A and B.
Material and Methods: Patients with refractory solid tumours (up to 2 prior regimens for advanced disease) or lymphoma were eligible and received escalating doses of AT9283 (given as 24 hour infusion) on days 1,8 every 3 weeks. Pharmacokinetics (PK) were planned for all patients. Serial tumour and tissue acquisition to examine pharmacodynamic (PD) effects were planned at the recommended phase II dose level (RP2D), using Immunohistochemistry (IHC) evaluation of histone H3 phosphorylation, upregulation of p53 and the proliferation marker PCNA.
Results: 35 patients were treated at 9 dose levels from 1.5 mg/m^2 to 47 mg/m^2. RP2D was 40 mg/m^2. Dose limiting toxicity was febrile neutropenia. Other than myelosuppression, all other toxicities were mild and included fatigue (31%), alopecia (11%), anorexia (14%), and nausea (17%). Myelosuppression was dose proportional. One partial response was reported in a patient with anal cancer, while 4 patients had stable disease (r; 2.1−3.5 months). PK are dose proportional and neutropenia correlated with AUC, Cmax and clearance. Four patients had serial tumor and skin biopsies taken at the RP2D. Immunohistochemistry (IHC) was performed on these sections for evidence of biological activity of AT9283 in the tissue. Pharmacological evidence of Aurora inhibitory activity was noted, including reduction in PCNA in 3 out of 4 tumour samples following AT9283 administration. Multi-nucleated cells, a consequence of Aurora inhibition, were also observed.

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.

513 POSTER

Chemo-sensitization using cancer targeted Spermidine–CoA based compound

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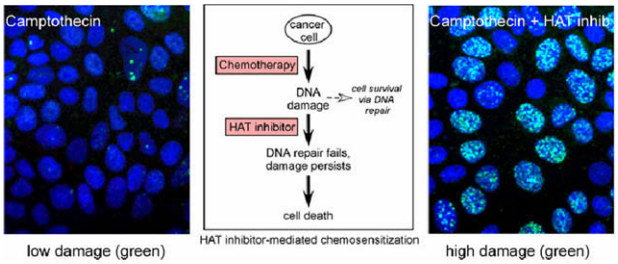
The efficacy of cancer chemotherapy and radiotherapy relies on generation of DNA damage. Since intrinsic DNA repair pathways enable cancer cells to survive by repairing these damaged lesions, inactivation of DNA repair coupled with chemotherapy or radiotherapy has a potential to enhance the effect of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

515 POSTER

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.