ara-U in patients receiving the recommended phase II dose (RP2D) by continuous infusion (CIV).
Material and Methods: Eight patients with relapsed/refractory acute myeloid leukaemia (AML) receiving 2000 mg/m^2/day CP-4055 CIV (24 hours) in a day 1−5 q3w schedule, were included. Blood samples for plasma pharmacokinetics were taken at time intervals on day 1 through day 6.
Results: Steady state concentrations (Css) of ara-C and ara-U were reached within day 1, while Css for CP-4055 apparently was not reached until 96 h after start of infusion. A threefold variation was seen in area under the curve (AUC) of CP-4055 and a five fold variation in AUC of ara-U. Smaller variation was seen in AUC of ara-C. There seemed to be an inverse relationship between AUC of CP-4055 and ara-U. CP-4055 ($t0.5 = 2 \pm 0.58$ h) was detected in plasma up to 24 hours after end of infusion in three out of seven patients. CP-4055 was well tolerated at RP2D. Complete remission was attained in a 24 year old female patient receiving CP-4055. The induction course was discontinued after for only 3 days due to elevation of liver function tests.
Conclusions: CP-4055 is well tolerated and activity has been reported in patients with acute myeloid leukaemia (AML) at the RP2D. Variations were seen in AUC of CP-4055 (three fold) and ara-U (five fold). Smaller variations were seen in AUC of ara-C. The study has continued into the phase II part.

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The IMPDH inhibitor AVN944 synergizes with Clofarabine to induce cell death in myeloid cancer cell lines

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AVN944 is an orally bioavailable inhibitor of inosine monophosphate dehydrogenase 1 and 2 (IMPDH). IMPDH2 is highly upregulated, and required for the de novo production of guanine nucleotides in many malignancies, including hematologic cancers. AVN944 is currently in a repeat dose escalation trial in patients with advanced hematologic cancers as well as a Phase II trial in pancreatic cancer in combination with gemcitabine. Over the last several years molecularly targeted agents have gained approval from the Food and Drug Administration as standard of care for specific tumor types. These agents, however, are not curative as single agents, as even the best example, imatinib, does not eradicate BCR-ABL positive cells in most patients. In order to identify drugs that would synergize with AVN944 we designed combination treatments with drugs approved for treatment of leukemia and lymphoma that would complement the nucleotide metabolism and energy pathway inhibition activity of AVN944. Compounds tested included Ara-c, 5FU, Clofarabine and hydroxyurea. One agent, Clofarabine, inhibits DNA synthesis by a direct inhibition of ribonucleotide reductase, DNA polymerase and induction of DNA strand breaks whereas AVN944 reduces the pool of available GTP nucleotides required for synthesis of DNA and progression through S phase of the cell cycle. To test the activity of these agents in combination, we treated HL-60 and KG-1 cells for 48 and 72 hours with a course of 4 doses each agent alone, and in combination. Apoptotic cell death, using flow cytometry on Annexin V and staining for propidium iodide. Calculations of synergy were carried out using the method developed by Chou and Talay, (1998) whereby combination indices (C.I) are calculated and values <1.0 is indicative of a synergistic combination. The C.I. for HL-60 and KG-1 at 72 hours treatment using 50% cell death as the endpoint was 0.20 and 0.5 respectively. These values clearly indicate the combination of AVN944 with Clofarabine is strongly synergistic. Upon confirmation of these data in in vivo xenograft experiments a clinical trial would be designed to test the combined activity of these agents in man.

Bioreductive agents

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The bioreductive prodrug PR-104 is activated under aerobic conditions by human aldo-keto reductase 1C3 (prostaglandin F synthase)

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PR-104, a hypoxia-activated prodrug currently in clinical trial, is a water-soluble phosphate ester which is converted *in vivo* to the corresponding alcohol, PR-104A. This 3,5-dinitrobenzamide-2-nitrogen mustard is activated by reduction to the corresponding 5-hydroxylamine (PR-104H) and 5-amine (PR-104M) (Patterson et al., Clin Can Res 2007, 13:3922). We have observed that certain neoplastic cell lines display atypical aerobic cell sensitivity that correlates with NQO1 expression in vitro but NQO1 cDNA expression did not increase aerobic cytotoxicity or PR-104A metabolism (Guise et al., Biochem Pharmacol, 2007, 74:810). This suggested the hypothesis that the aerobic PR-104A reductase is co-ordinately regulated with NQO-1. To identify the reductase, a panel of 12 cell lines were evaluated for aerobic PR-104A sensitivity and were shown to cover a 78-fold range (IC50 = 2–157 uM). In parallel, aerobic PR-104A metabolism was monitored using LC/MS/MS and compared with the gene expression profile (Affymetrix U122Plus2.0 chips) by unsupervised hierarchical clustering analysis (Cluster 3.0). Probes associated with gene ontology term 'oxidoreductase activity' and descendents separated the 12 cell lines into three distinguishable groups, indicating that the resulting expression profiles were cell-specific. Two-way ANOVA and false discovery rate correction (FDR, Limma's empirical Bayes adjusted p value <0.1) identified 260 probes that were positively correlated with the rate of PR-104H & M formation across the 12 cell lines. Intriguingly, a cluster of four aldo-keto reductases genes (AKR 1B10, 1C1, 1C2 and 1C3) featured in the seven most highly up-regulated messages in PR-104A metabolism-proficient cells (65, 41, 31 and 42-fold, respectively; adj. p values <0.02). The NQO1 transcript was also co-ordinately expressed (9.3-fold range; adj. p = 0.054), independently supporting the relationship we have previously observed.
We expressed these candidate aldo-keto reductases in aerobic metabolism-null HCT116 cells and showed by LC/MS/MS that only AKR1C3 (NM_003739) expression results in activation of PR-104A to its active metabolites. Cytotoxicity assays confirmed AKR1C3 as a major determinant of aerobic PR-104A sensitivity, and aerobic metabolism of PR-104A was shown to be highly correlated with AKR1C3 expression by western blot. To our knowledge this is the first report that AKR1C3 is capable of nitroreduction. The clinical implications of this finding will be discussed.

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Relationships between reductive metabolism, DNA crosslinks and antitumor activity of the hypoxia-activated prodrug PR-104 in preclinical models

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Background: PR-104, currently in clinical trial, is a phosphate ester that is converted systemically to the dinitrobenzamide nitrogen mustard PR-104A which is reduced selectively in hypoxic cells to DNA inter-strand crosslinking (ICL) hydroxylamine (PR-104H) and amine (PR-104M) metabolites (Patterson et al., Clin Cancer Res 2007; 13: 3922–3932). Here we evaluate the role of ICL in the hypoxic and aerobic cytotoxicity of PR-104, and the utility of ICL as a surrogate marker for clonogenic cell killing.
Materials and Methods: Using a panel of 9 human tumor cell lines, cytotoxicity was determined by clonogenic assay following a 2 hr exposure to PR-104A, at a range of concentrations, under aerobic or hypoxic conditions. PR-104H and PR-104M concentrations were determined by HPLC/mass spectrometry (LC/MS/MS) during this exposure and ICL by the alkaline comet assay after growth for a further 24 hr in fresh medium under aerobic conditions. ICL and clonogenic cell killing were also assessed in the corresponding human tumor xenografts following i.p. dosing of CD-1 nude mice with PR-104 (0.6 mmol/kg).
Results and Conclusions: PR-104A was selectively toxic to all cell lines under hypoxia, but with widely differing hypoxic cytotoxicity ratios (from 6.6 for H460 to 53 for HCT116 cells), primarily reflecting large differences in aerobic cytotoxicity. Under hypoxia, the relationship between ICL and cell killing was indistinguishable across the cell line panel, suggesting that ICL are responsible for hypoxic cytotoxicity in all cases. Under aerobic conditions there was a similar relationship between ICL and cytotoxicity in most cell lines, but cells with very low rates of PR-104H and PR-104M formation (A2780, C33A, H1299) did not show ICL even after highly cytotoxic PR-104A exposures. These same 3 cell lines have low levels of AKR1C3 expression, which is the major enzyme responsible for aerobic PR-104A reduction in human tumor cell lines (Guise et al., this meeting). Thus there is an ICL-unrelated mechanism of PR-104A cytotoxicity in cells with very low reductase activity. Despite this, in xenografts ICL frequency was highly correlated with clonogenic cell killing ($r^2 = 0.743$), and A2780, C33A and H1299 followed the same pattern. Therefore the ICL-independent cytotoxicity observed in culture is not significant in the pharmacologically relevant dose range in mice, and ICL frequency appears to be a broadly applicable pharmacodynamic biomarker for tumor cell killing by PR-104.