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Pre-clinical evaluation of the novel alkylating agent RH1 against paediatric tumour cell lines

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Despite dramatic improvements in survival from childhood cancer there remain many tumour types in which drug resistance is a major problem. There is thus an urgent need for access to novel agents for this group of patients. RH1 (2,5-diaziridinyl-3-[hydroxymethyl]-6-methyl-1,4-benzoquinone) is a novel alkylating agent which is currently in phase I trial in adults. The bioreductive pro-drug is activated to a more potent DNA interstrand cross-linking moiety by the obligate two-electron reductase DT-diaphorase (DTD), which is widely expressed in tumour cells. In keeping with its mechanism of action human colon and lung carcinoma cells that over-express DTD are more sensitive to RH1.

We evaluated the efficacy of RH1 against a range of paediatric tumour cells lines in vitro. DTD protein expression in a panel of childhood cancer cell lines was measured by Western blotting. A high and a low DTD expressing cell line from each of neuroblastoma, osteosarcoma, and Ewing's sarcoma was chosen to investigate further. Comparison of the cytotoxicity of RH-1 to that of cisplatin and doxorubicin using long term clonogenic assays showed IC50 values ranging from 1.5–7.5 nM, even in cell lines relatively resistant to cisplatin and doxorubicin. There was no correlation between sensitivity and DTD expression. However in short term SRB assays IC50 doses ranged from 1–200 nM and, as expected, sensitivity correlated with DTD expression. Finally, the combination index equation was used to define synergistic interactions between RH1, cisplatin and doxorubicin. Preliminary results suggest that in at least three cell lines, RH1 is synergistic with doxorubicin or cisplatin.

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Metabolism and pharmacokinetics of PR-104, a hypoxia-activated nitrogen mustard prodrug in phase I clinical trial

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PR-104 is a soluble phosphate ester "pre-prodrug" designed to be converted *in vivo* to the corresponding alcohol PR-104A, which is a hypoxia-activated nitrogen mustard prodrug. PR-104 shows strong antitumour activity in human tumour xenograft models, and is currently in Phase I clinical trial through Proacta Inc. Here we report initial results of a drug metabolism/pharmacokinetics (PK) study of PR-104.

The plasma PK of PR-104 and PR-104A was evaluated in three preclinical species (CD-1 nude mice, Sprague Dawley rats, and beagle dogs) at a range of doses, and in the initial cohorts of the Phase I trial, by HPLC/mass spectrometry with tetra-deuterated internal standards of both analytes.

PR-104 was rapidly hydrolysed to PR-104A in all species. A two compartment PK model, in which PR-104 is converted to PR-104A in the central compartment, fitted the plasma PK of both analytes. The model parameters were similar across species, with the exception of faster clearance of PR-104A in dogs (terminal half life 10 vs 17, 19 and 22 min in mice, rats and humans). No difference was seen between genders, and PK was little changed on the last dose of a qwx4 schedule in rats and dogs. The plasma PK of both analytes showed slight non-linearity with dose, which could be accounted for by a minor saturable elimination pathway for PR-104 itself.

An excretion and tissue biodistribution study in CD-1 nude mice bearing SiHa tumors, using [3]H-PR-104, demonstrated almost quantitative elimination within 48 hr (urine 46.3±1.8% [SEM] of total dose, faeces 50.2±2.4%). Retained radioactivity at 48 hr was highest in tumour and lowest in brain. The metabolite profile in plasma and urine was characterized by capillary LC/ion trap MS. Products of the mercapturic acid pathway were prominent in all species (especially a cysteine adduct of PR-104A), along with oxidative debromoethylation to the mesylate half mustard (especially in rodents) and *O*-glucuronidation of the alcohol side chain in dogs and humans.

Overall, this study demonstrates that the plasma PK and metabolism of PR-104 is similar in humans and preclinical species. Rapid hydrolysis of the phosphate to the corresponding alcohol competes effectively with its urinary elimination. PR-104A is extensively metabolised by GSH conjugation and glucuronidation, and by oxidative metabolism of the mustard moiety. The reduced metabolites responsible for metabolic activation in hypoxic tumour cells were not detected in plasma or urine.

Cellular therapies and cytokines

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Denileukin diftitox depletes T regulatory cells and causes regression of melanoma metastases in humans

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Background: Cognate immunity targeted against neoplastic cells depends on a balance between activated antigen-specific T cells and suppressive or regulatory T cells. Recently, a subset of CD4+CD25^{high} regulatory T (Treg) cells has been found to directly suppress the activation of anti-tumor effector T cells in a contact-dependent manner. Depletion of these Treg cells using anti-CD25 monoclonal antibodies induces a CD8+ T cell dependent immune rejection of melanoma in mice. Recombinant interleukin 2/diphtheria toxin conjugate (DAB[389]IL2) previously has been developed as a treatment for cutaneous CD25+ T cell lymphoma. DAB[389]IL2 binds to surface CD25 and, after internalization causes cell death within hours. We hypothesize that DAB[389]IL2 will selectively deplete CD4+CD25^{high} Tregs in patients with melanoma and allow induction of melanoma-specific immunity.

Materials and Methods: The effect of DAB(389)IL2 on tumor growth was examined in seven patients with Stage IV melanoma. DAB(389)IL2 (9 or 12 mcg/kg) was administered daily × 4 days every three weeks for four cycles. FDG-PET and/or CT imaging was obtained just prior to DAB(389)IL2 administration and within two weeks after completion of the fourth cycle. In a subset of patients, peripheral blood Treg cells were quantitated by flow cytometry (CD4+/CD25+/foxp3+) before and after DAB[389]IL2 administration. Immunohistochemical analyses of subcutaneous melanoma metastases were also performed for the melanoma-specific protein S-100 and the T cell surface antigen CD8.

Results: Two patients received 9 mcg/kg DAB(389)IL2 and, after two cycles, experienced overt progression consisting of a combination of tumor growth and newly detectable tumors. Five patients received 12 mcg/kg DAB(389)IL2 and, after four cycles, experienced significant regression of several metastatic tumors, including subcutaneous tumors and metastases in the liver and axillary lymph nodes. Two subcutaneous tumors in the lower extremity of a single patient became necrotic and infected, requiring surgical resection. Immunohistochemical analysis of these tumors revealed an apoptotic tumor surrounded by CD8+ T lymphocytes. The peripheral blood Treg concentration in this patient decreased after the second DAB[389]IL2 administration (day 1, 36.59 Tregs/microliter; day 7, 17.48 Tregs/microliter).

Conclusions: We conclude that depletion of Treg cells in tumor-bearing humans may allow the activation of cognate immunity leading to CD8+ T cell-mediated death of neoplastic cells. A phase II clinical trial to examine the efficacy of DAB(389)IL2 in this patient population that incorporates immunocorrelative analyses of peripheral blood and tumor-associated Treg cells and CD8+ melanoma-specific effector T cells is now underway.

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Recombinant human Interleukin-21 (rIL-21), a new cytokine for immunotherapy: results of two phase 1 studies in patients with metastatic melanoma (MM) or renal cell carcinoma (RCC)

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Background: rIL-21 is a pleiotropic class I cytokine that activates CD8+ T cells and NK cells. Based on preclinical data suggesting rIL-21 will have anti-tumor effects, clinical trials to characterize the safety and activity of this cytokine were initiated.

Methods: rIL-21 was administered by i.v. bolus injection to patients (pts) with AJCC stage IV MM or RCC in two phase 1 studies conducted in Australia or US. In the AUS study, two 6-week treatment regimens were tested during dose escalation: 6 cycles of thrice weekly dosing (3/w) or 3 cycles of 5 daily doses each followed by 9 days of rest (5+9). In the US study, a regimen of 2 cycles of 5 daily doses followed by 9 or16 days of rest was tested in two parts: a dose escalation part followed by a dose expansion part. Objectives were to estimate the maximally tolerated dose (MTD) and to assess pharmacokinetics, immunogenicity, immunomodulatory and anti-tumor activity of rIL-21.

Results: rIL-21 was administered to 44 pts at doses from 1–100 μg/kg during dose escalation and to 28 pts at 30 μg/kg during dose expansion.