We isolated two HUV-EC-C/ntr$^+$ clones presenting a sensitivity to CB1954 that was 15- to 30-folds higher with respect to untransfected HUV-EC-C cells (HUV-EC-C/ntr$^-$). These clones were injected subcutaneously, in association with the murine melanoma cell line B16-F10, into nude mice that were then treated with CB1954. Animal survival, as well as histological analysis of tumors, lung, spleen and liver were evaluated.
After the treatment of the animals with CB1954, we observed a prolonged survival of animals carrying the HUV-EC-C/ntr$^+$ clones with respect to control animals injected with HUV-EC-C/ntr$^-$ cells, but no significant differences in tumor growth. However, histological analysis of tumors showed large areas of necrosis, likely due to tumor ischemia, in the presence of HUV-EC-C/ntr$^+$ clones with respect to control animals. Histological analysis of lung and spleen did not show the presence of tumor metastasis, as well as histological analysis of liver showed neither tumor metastasis nor animal toxicity.
To our knowledge, this is the first report showing the efficacy of the GDEPT-based approach to prolong tumor-bearing animal survival after the delivery of the *ntr* gene to tumor vasculature and the treatment with CB1954, without inducing animal toxicity. Altogether our data indicate that the targeting of the tumor vasculature by this GDEPT strategy may be an effective approach for cancer treatment *in vivo*, relied on one possible bystander mechanism based on tumor ischemia.

184 POSTER

Pharmacokinetics of CNTO 328 in a three month intravenous dose toxicity study in cynomolgus monkeys with concomitant IL-2 therapy

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Background: CNTO 328 is a chimeric (murine-human) monoclonal antibody (mAb) specific for IL-6, which is in involved in tumor growth, invasion and metastasis as well as tumor angiogenesis. IL-6 is also considered to be a causative factor in cancer-related morbidity from afflictions such as asthenia/cachexia and bone resorption. The goal of this study was to determine the pharmacokinetics of CNTO 328 when administered once weekly via an intravenous (IV) infusion to cynomolgus monkeys for 13 weeks, in combination with IL-2, followed by a one-month recovery period.
Material and Methods: Cynomolgus monkeys, 32 male and 32 female, were randomly assigned to 4 groups (16 monkeys per group) and were infused either with saline and IL-2 therapy alone, or a combination of weekly 10 and 50 mg/kg CNTO 328 with IL-2 therapy. Pharmacokinetic calculations were conducted using WinNonlin. These data were then compared with the PK parameter estimates from another study where CNTO 328 was administered alone.
Results: Animals treated with CNTO 328, in combination with IL-2 therapy, received extensive exposure to CNTO 328 over the three-month dosing and one month recovery periods. A five-fold increase in dose from 10 to 50 mg/kg resulted in an approximately 3.49 and 3.37 fold increase in Cmax and AUC (0–96hr), respectively. Steady state serum CNTO 328 serum concentrations were achieved by Week 11 (Day 71) with mean steady state trough concentrations of approximately 446.39 μg/mL and 3162.88 μg/mL (on Day 85) for the 10 and 50 mg/kg dose groups, respectively. The mean apparent terminal half-life was approximately 19.89 days.
Conclusion: CNTO 328 PK estimates in this combinatory study was comparable with the results obtained in previous study in which CNTO 328 was administered alone. This indicated that the intravenous infusions of CNTO 328 with or without combination of IL-2 do not appear to influence CNTO 328 exposure.

185 POSTER

Phage display-derived peptides specific to the galectin-3 carbohydrate recognition domain inhibit metastasis-mediated cancer cell adhesion

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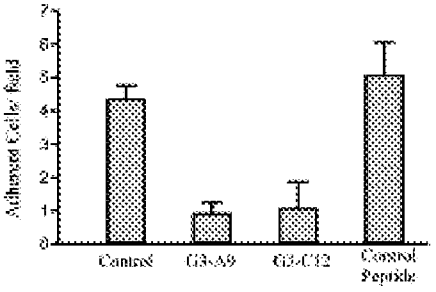
Background: Interactions between circulating cancer cells and the endothelial cells of blood vessels have a significant influence on metastasis. Studies indicate that galectin-3 (gal-3), a member of the galectin family of carbohydrate-binding lectins, is involved in carbohydrate-mediated metastatic events, and interacts with the tumor specific Thomsen-Friedenreich glycoantigen (TFA). Our laboratory is actively pursuing TFA and gal-3 as potential targets for breast, prostate, and colon cancer detection and therapy (Glinsky, Can. Res. 60:2584 and 61:4851). Because increased carcinoma cell adhesion is associated with increased metastatic potential, we hypothesized that inhibition of the galectin-3-TFA interaction would reduce homotypic (between carcinoma cells) and heterotypic (between carcinoma cells and endothelium) adhesion. To test this, we identified peptide antagonists of gal-3 using combinatorial bacteriophage (phage) display.
Methods: Two random phage display libraries, f88/15 and f88/Cys6 encoding for random 15- and 6-amino acid peptides respectively, were used for affinity selections against purified recombinant gal-3. After 4 rounds of selection, eighty individual phage clones were analyzed. ELISA, immunoblot, and fluorescence quenching were employed to analyze peptide affinity and specificity. The ability of the peptides to functionally modulate adhesion was tested using MDA-435 human breast carcinoma cell homotypic adhesion, and heterotypic adhesion to human bone marrow endothelium in a parallel plate laminar flow chamber system, which reconstructs *in vitro, in vivo* microvascular blood flow.
Results: Peptides bound to purified gal-3 protein with high affinity (K_d ≅17–80 nM), but did not bind other galectins or lectins tested. Experiments using truncated gal-3 proteins indicated that the selected peptides bound to the carbohydrate-recognition domain (CRD) of gal-3. Two such peptides, G3-A9 and G3-C12, blocked the interaction between gal-3 and TFA, and inhibited MDA-MB-435 breast carcinoma cell homotypic and heterotypic adhesion by greater than 60% (Figure 1).
Conclusions: Our results have physiological importance because they demonstrate that carbohydrate-mediated metastatic cell adhesion can be inhibited efficiently with peptides that bind to the CRD of gal-3. Small galectin-3-specific peptides, offer advantages over antibody or carbohydrate gal-3 modulators, and have the potential to affect the entire metastatic process and control hematogenous cancer spread.



MDA-435 cells were infused across human endothelial cell monolayers in the presence or absence of 25 μM G3-A9, G3-C12, control peptide or no peptide (control). After stabilization, the number of rolling and adherent tumor cells was recorded. Gal3 peptides inhibit human breast cancer cell adhesion to endothelium under flow conditions.

186 POSTER

Potent analogues of the antiangiogenic agent NM-3 have enhanced antiproliferative activity in vitro

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NM-3 (8-hydroxy-6methoxy-3-(2-methylcarboxymethyl)-isocoumrarin) is an orally active antiangiogenic agent that is being evaluated in clinical trials. In preclinical studies, NM-3 inhibits the proliferation of human umbilical vein endothelial cells (HUVEC) as well as enhances anti-tumor effects of standard chemotherapy and radiotherapy in mice xenografts without noticeable toxicity. NM-3 has also been shown to induce lethality in some human carcinoma cell lines in vitro, however, at concentrations above 100 μM. In this project, NM-3 analogues have been synthesized which displayed enhanced antiproliferative activity on HUVEC (200 times NM-3), as well as on different tumor cell lines that are not sensitive to NM-3. We investigated the mechanism whereby these compounds, in comparison with NM-3, exert their activity on HUVEC and on tumor cell lines. Immunoblotting analysis of different cell cycle-associated proteins in cells treated with these novel compounds indicated that they are at least 5 fold more potent in increasing p53 and p21/WAF levels than NM-3 and that cells are blocked in G2.
In summary, NM-3 analogues have been synthesized and shown to have significantly more potent antiangiogenic and antiproliferative activities which might contribute to their antitumor activity in vivo.