22 pts received up to 11 cycles of extended treatment. The safety profile of rIL-21 was similar in the 2 studies and 2 patient populations. rIL-21 was well tolerated at doses of 1–10 μg/kg. Overall, the most commonly reported adverse events were fatigue, pyrexia, chills, nausea, and rash. In the 3/w regimen 30 μg/kg was declared the MTD based on DLTs in 3/7 pts and no higher doses were studied. The MTD for the 5+9 regimen was estimated to be 30 μg/kg in both studies though higher doses of 50 and 100 μg/kg were tolerated by some pts. Immunomodulatory effects were observed at all dose levels with increased levels of phosphorylated STAT3 even at the 1 μg/kg dose level; increased soluble CD25; increases in NK, CD8+ and CD4+ cells; and upregulation of perforin and granzyme-A & -B mRNA at doses ⩾3 μg/kg. Three pts (all RCC) achieved confirmed PRs and two pts (both MM, one previously treated in a vaccine study) achieved CRs according to RECIST after up to 11 cycles of treatment.
Conclusions: Based on data from 72 pts with MM or RCC exposed to rIL-21 in two phase 1 studies, rIL-21 was generally well tolerated. Relevant biological activity was observed at doses as low as 1 μg/kg. Four responses of 50 evaluable pts treated at doses ⩾30 μg/kg provide encouragement for future studies of rIL-21.

266 POSTER

Preclinical evaluation of IL-21 combination therapy with sorafenib and sunitinib in renal cell carcinoma

P.V. Sivakumar[1], B. Johnson[1], K. Brasel[1], M. Anderson[1], S. Hughes[1], C. Clegg[1], D.M. Miller[1], A. Hey[2], P.E.G. Kristjansen[2], M. Kragh[2]. *[1]Zymogenetics Inc, Hematology and Oncology, Seattle, USA; [2]Novo Nordisk Corp, Copenhagen, Denmark*

Background: Sorafenib and sunitinib are tyrosine kinase inhibtors (TKIs) recently approved for the treatment of advanced RCC. Mechanisms of TKI-mediated tumor inhibition include direct inhibition of tumor cell proliferation and inhibition of angiogenesis via VEGF and PDGF pathways. While both drugs have demonstrated meaningful clinical benefit in RCC, most patients will relapse. The efficacy of TKIs may be improved in combination with other agents. IL-21 is a novel cytokine that has shown potent efficacy in preclinical models of RCC through mechanisms involving activation of NK cells and tumor-specific CD8 T cells. A Phase 1 study showed IL-21 to be tolerated as an outpatient regimen in RCC and pharmacologically active. Combining IL-21 with TKIs may result in greater clinical benefit by influencing multiple independent pathways. A series of preclinical pharmacology studies were designed with the objective of characterizing the potential pharmacologic interactions of TKIs and IL-21.
Methods: The effects of sorafenib and sunitinib on IL-21-mediated effector functions were tested under conditions of concurrent or sequential exposure using a range of concentrations of TKIs including steady state and maximal levels reported in patients. Assays employed measured NK cell cytotoxicity and IFNγ production, and IL-21 co-stimulation of CTL proliferation. Furthermore, effects of TKIs on IL-21R expression and STAT3 phosphorylation on PBMCs were evaluated. In vivo studies with murine IL-21 and TKIs were performed in subcutaneous B16 melanoma and RenCa RCC in mice.
Results: At steady-state concentrations of drug reported in serum of human patients, neither sorafenib nor sunitinib inhibited IL-21R expression or IL-21-induced STAT3 phosphorylation in human PBMCs, human or mouse CD4 and CD8 T cell proliferation, human NK cell granzyme B expression and ADCC activity. IL-21 treatment did not affect sunitinib or sorafenib-mediated anti-tumor effects in the syngeneic tumor models at maximal doses of TKIs. Additive anti-tumor effects were observed with IL-21 in combination with sub-maximal sorafenib.
Conclusions: Preclinical evaluation of the combination of TKIs and IL-21 suggests that the TKIs, when used at concentrations simulating therapeutic exposure, do not inhibit IL-21 or immune effector functions in vitro. Further, IL-21, in combination with TKI has additive effects in preclinical models, suggesting that testing of IL-21 and TKIs clinically is warranted.

267 POSTER

A chimera of interleukin-2 and a variant of the channel-forming protein aerolysin is selectively toxic to cells displaying the interleukin-2 receptor

T. Buckley, M. Osusky, L. Teschke, F. Merchant, X. Wang. *Protox Therapeutics, Vancouver, Canada*

Background: Proaerolysin is the inactive precursor of the bacterial toxin aerolysin. The protoxin binds to GPI-anchored proteins (GPI-AP) on mammalian cells and is converted to aerolysin by proteolytic nicking. Aerolysin then spontaneously forms a stable oligomer that inserts into the plasma membrane and forms channels that cause cell death. Once inserted into the membrane, the toxin cannot leave, so that bystander cells cannot be affected: thus aerolysin may provide an advantage over enzyme toxins such as diphtheria toxin and exotoxin A, as a component of hybrid molecules that can target specific cell types.
Methods: To prevent binding of proaerolysin to normal cells, we made the variant R336A-PA. The R336 residue was identified based on our previous studies of proaerolysin binding to GPI-anchored proteins and the known structure of the protoxin. We also made a hybrid (IL-2-PA) of IL-2 fused to native proaerolysin and a second hybrid (IL-2-R336A-PA), by fusing IL-2 to R336A-PA. A six amino acid spacer separated the IL-2 and the proaerolysin. We determined whether the proaerolysin forms of these molecules could be converted to the aerolysin forms by proteolytic nicking, as well as the ability of the aerolysin forms to produce stable oligomers. Flow cytometry was used to compare binding of R336A-PA and the two hybrid molecules to cells displaying the IL-2 receptor and to cells that do not. Cell killing was studied using a variety of cell lines.
Results: We showed that all of the molecules could be converted to the aerolysin form by proteolytic nicking and that this led to the production of stable oligomers. The R336A variant of proaerolysin did not bind and was only very weakly active against all cell types tested. The IL-2-PA hybrid was active against all cell types, as it could bind to GPI-AP and form functional oligomers. The Il-2-R336A-PA hybrid could not bind to normal GPI-AP positive cells and it had little or no activity against them. Remarkably, this hybrid could bind to cells that display the IL-2 receptor and it was nearly as toxic to these cells as native PA.
Conclusions: The channel-forming protein aerolysin can be targeted to cells displaying the IL-2 receptor. Targeted aerolysin molecules such as IL-2-R336A-PA may have advantages over targeted enzyme toxin molecules in cancer therapy.

268 POSTER

Cancer immunotherapy by Interleukin-21: theoretical evaluation of potential treatment strategies

M. Elishmereni, A. Cappuccio, Z. Agur. *Institute for Medical BioMathematics, Bene-Ataroth, Israel*

Background: The newly-characterized Interleukin-21 (IL-21), a natural derivative of T-helper cells, plays a central role in the transition from innate immunity to adaptive immunity. Murine studies show substantial elimination of various tumors in response to IL-21 application, thereby encouraging its addition into the growing cancer immunotherapeutic arsenal. Still, conditions for efficacious IL-21-therapy, and its conflicting immunostimulatory and immunoinhibitory influence on anticancer cellular responses, are yet to be fully defined.
Methods: We have studied the effects of IL-21 on tumor eradication in a mathematical model focusing on the NK-cell and CD8+ T cell-mediated lysis of tumor cells. To estimate model parameters we used studies in mice inoculated with poorly immunogenic (PI) melanomas, highly immunogenic (HI) fibrosarcomas, or thymoma, and treated with cytokine gene therapy (CGT), hydrodynamics-based gene delivery (HGD), or standard interval dosing (SID) of IL-21. Model accuracy in retrieving tumor growth curves has been validated in independent experiments of melanoma and fibrosarcoma progression in mice treated by IL-21. Putative immunotherapy strategies were simulated and their efficacy was estimated.
Results: Computer simulations accurately retrieved experimental growth dynamics in B16 melanoma, MethA and MCA205 fibrosarcomas, showing a strong dependence of the NK-cell/CD8+ T-cell balance on tumor immunogenicity. Efficient tumor elimination was achieved in melanoma, when simulating an IL-21 dosing regimen that was dynamically-determined according to changes in tumor mass, as in CGT. In contrast, in fibrosarcoma, such a strategy did not prove superior to that of constant dosing protocols, HGD or SID.
Conclusions: Our model analysis supports clinical use of IL-21 as a potent stimulator of cellular immunity against cancer, and suggests selecting the immunotherapy strategy according to tumor immunogenicity. In PI malignancies, but not in HI, IL-21 dosing, at any time, should depend on tumor mass at that time. This method imitates, yet amplifies, the natural anticancer immune response, rather than accelerating only one of the response arms, in an unbalanced manner.

269 POSTER

Targeting brain tumor stem cells with oncolytic virus in combination with temozolomide

M. Alonso[1], C. Gomez-Manzano[1], Y. Piao[1], O. Lee[1], H. Jiang[1], R. Alemany[2], F. Lang[1], A. Yung[1], J. Fueyo[1]. *[1]M.D. Anderson Cancer Center, Brain tumor Center, Houston, USA; [2]Institute Catala D'Oncologia, Barcelona, Spain*

Background: Recently, several groups have described the existence of a cancer stem cell population in human brain tumors. These population is a preferred therapeutic target since has been proposed to be a possible

source of cancer resilience to conventional anti-cancer therapies. Currently, temozolomide (TMZ) constitutes standard treatment for many patients with malignant glioma. Because TMZ generates only partial responses, due to overexpression of MGMT, glioblastoma treatment requires multimodal therapy. Therefore, new therapeutic approaches are desperately needed. Oncolytic adenoviruses designed to replicate in and destroy tumor cells selectively represent a promising new therapeutic strategy that could improve the outcome of this malignancy. We hypothesize that TMZ can be successfully combined with ICOVIR-5, an oncolytic adenovirus, resulting in an enhanced cytotoxic effect against the brain tumor stem cell population (BTSCs).
Methods: NSC-2 and NSC-11 brain tumor stem cell lines were isolated and cultured from brain tumor specimens. MTT assays were carried out to evaluate the cytotoxicity of ICOVIR-5 and TMZ alone or in combination in BTSCs. TCID50 assays were used to evaluate the replication of the virus in BTSCs when administered alone or in combination with TMZ. Cell cycle profiles were analyzed by flow cytometry. RT-PCR and western blot were performed to assess the expression levels of viral (E1A, E3, E4orf3, E4orf6-E1B55k) and cellular (MGMT, MRN complex) transcripts and proteins.
Results: Our data showed that ICOVIR-5 induced a robust cytotoxic effect on BTSCs that was further enhanced when combined with TMZ. This cytotoxic effect was greater in NSC-2 cells. Interestingly, examination of basal MGMT expression in these cell lines showed high levels of MGMT in NSC-2 versus no expression in NSC-11 cell line. Importantly, treatment with temozolomide further increased MGMT levels in NSC-2 and triggered the expression of the enzyme in NSC-11 indicating a possible resistance to the treatment. However, infection with ICOVIR-5 abrogated MGMT expression levels in both cell lines. Cell cycle profile of cells treated with TMZ showed G2/M arrest (more than 70% of the cells), importantly infection with ICOVIR -5 abrogated G2/M arrest rendering BTSCs more sensitive to cell death (apotosis or autophagia).
Conclusions: This work represents the first evidence of successfully targeting BTSCs with an oncolytic virus alone or in combination with chemotherapy. Combination treatment of ICOVIR-5 with temozolomide resulted in enhanced antitumor effect in BTSCs through abrogation of DNA repair mechanism. These data deserved further in vivo testing since might constitute important criteria for the selection of patients for future clinical trials involving the combination of ICOVIR-5 and TMZ.

270 POSTER

Recombinant human IL-18 (iboctadekin) PKPD and clinical activity in phase I-II

K.M. Koch[1], D. Jaworski[1], L. Kirby[1], S. Kathman[1], B. Bell[1], M. Robertson[2], J. Mier[3], T. Logan[2], J. Kirkwood[4], M.M. Dar[1]. *[1]GlaxoSmithKline, Clinical Pharmacology & Discovery Medicine, Research Triangle Park, USA; [2]Indiana Univ Med Center, Indianapolis, USA; [3]Beth Israel Hospital, Boston, USA; [4]Hillman Cancer Center, University of Pittsburgh, Pittsburgh, USA*

Background: IL-18 is a cytokine that stimulates immune cell mediated anti-tumor activity in syngeneic murine models. The recombinant human form (rhIL-18) is in early clinical trials. Pharmacokinetic-pharmacodynamic (PKPD) relationships characterizing biological and clinical activity in Phase I were used to guide dose and regimen selection for Phase II efficacy evaluation. Biological and clinical activity appeared to follow similar relationships to dose or plasma concentration.
Methods: Phase I studies were conducted in patients with metastatic melanoma (MM) and renal cell carcinoma (RCC) receiving intravenous (IV) doses ranging from 3 to 2000 mcg/kg. Regimens included either 5 daily doses given monthly or a single dose given weekly for up to 6 months. Treatment was extended for patients receiving clinical benefit. Tolerability, pharmacokinetics (ELISA), biomarkers including plasma cytokines (ELISA, multiplex biochip) and immune cell activation (flow cytometry), and clinical activity (reduction in tumor size, duration of stable disease), were assessed to define an optimal dose range to evaluate in Phase II.
Results: rhIL-18 administration produced 1) rapid increases in inflammatory cytokines and chemokines detectable in circulation and 2) activation of NK cells and CTLs leading to their rapid nadir and subsequent recovery or rebound. These PD effects along with the PK of rhIL-18 were concentration- and time-dependent due in part to interactions with the IL-18 binding protein (BP), an inducible, high-affinity circulating modulator of rhIL-18 activity. The relationships of nearly all biological responses to dose and concentration suggested bell-shaped curves, consistent with both the regulatory effects of BP and pharmacological models based on inhibition due to excess substrate. Time-dependent attenuation of biological responses, also consistent with the effects of BP, was in part related to dosing schedule. PD relationships characterized by composite Emax models to describe these bell-shaped curves were used to predict an optimally active dose range in contrast to simple Emax models for predicting a maximum tolerated dose. Limited clinical activity observed in Phase I patients, consisting mainly of prolonged ($\geqslant$ 6 months) stable disease, appeared to be related to biological responses. Phase II efficacy results were consistent with these relationships.
Conclusion: Clinical efficacy results affirmed the PKPD relationships developed in Phase I.

271 POSTER

Murine interleukin 21 (mIL-21) protein therapy increases the density of tumor infiltrating CD8+ T cells and inhibits the growth of subcutaneous syngeneic tumors

H. Søndergaard[1], K.S. Frederiksen[1], P. Thygesen[1], E.D. Galsgaard[1], K. Skak[1], N.P.H. Møller[2], P.E.G. Kristjansen[2], M. Kragh[1]. *[1]Novo Nordisk A/S, Biopharmaceuticals Research Unit, Måløv, Denmark; [2]Novo Nordisk A/S, Development Projects, Bagsværd, Denmark*

IL-21 is a recently discovered cytokine in early clinical development. IL-21 has shown encouraging anti-tumor activity in various animal models. In the present study, we examine the anti-tumor activity of mIL-21 protein therapy in two syngeneic tumor models, and its effect on the density of tumor infiltrating $CD4^+$ and $CD8^+$ T cells. Subcutaneous tumors were established by inoculation of B16 melanomas or RenCa renal cell carcinomas into the right flank of C57BL/6 or BALB/c mice, respectively. When the tumors reached a size of ~5 mm^3 (early treatment) or ~50 mm^3 (late treatment), intraperitoneal (IP) or subcutaneous (SC) daily treatment with mIL-21 protein (50 μg) was initiated. The effect of NK cells and T cells on the anti-tumor activity was examined in mice specifically depleted by monoclonal antibodies. All experiments were terminated when the mean tumor sizes reached ~1000 mm^3; tumors were taken out and immunohistochemically stained for CD4 and CD8. Subsequently, the densities of tumor infiltrating $CD4^+$ and $CD8^+$ T cells were scored as the number of cells in intratumoral areas. Early treatment (IP and SC) inhibited tumor growth in both cancer models, whereas only SC administration produced a significant growth inhibition when the treatment was started later. We found no signs of discomfort or weight loss in any of the treated animals, indicating that the mIL-21 therapy was well tolerated. ^{125}I-labelled mIL-21 showed a slow release of mIL-21 from the subcutaneous site. Together with increased lymph drainage this might account for the increased activity of SC administration. The observed anti-tumor activity was not a direct anti-tumor effect, since the tumor cells did not express mIL-21 receptor mRNA and there was no anti-proliferative effect of mIL-21 *in vitro*. Specific depletion of $CD8^+$ T cells completely abrogated the anti-tumor activity whereas $NK1.1^+$ cell depletion revealed no decrease in activity. In accordance, our immunohistochemical analysis of tumor infiltrating $CD8^+$ T cells showed a 7–10 fold increase in the density of $CD8^+$ T cells in mIL-21 treated B16 tumors ($p < 0.05$) and a 3–8 fold increase in the density of $CD8^+$ T cells in mIL-21 treated RenCa tumors ($p < 0.05$). Furthermore, we found a significantly higher density of tumor infiltrating $CD8^+$ T cells in SC treated RenCa tumors compared to IP treated ($p < 0.05$). In both models, the densities of $CD4^+$ T cells were unchanged following IP and SC administration. Taken together, our data demonstrate anti-tumor activity of mIL-21 in established tumors and suggest that SC administration of IL-21 could be advantageous. Furthermore, we show that mIL-21 therapy strongly increases the density of tumor infiltrating $CD8^+$ T cells, and that $CD8^+$ T cells are essential for the anti-tumor activity.

272 POSTER

Effective immunotherapy treatment for glioblastoma multiforme: predictions of a mathematical model

N. Kalev-Kronik, Y. Kogan, V. Vainstein, Z. Agur. *Institute for Medical BioMathematics, Bene Atarot, Israel*

Background: Glioblastoma multiforme (GBM) is a highly aggressive grade IV brain tumor (BT grade IV). GBM is refractive to conventional treatments. Life expectancy of GBM patients stands at up to eighteen months. Clinical trials suggest immunotherapy is a promising avenue for treatment of GBM, as it is target specific, has relatively mild side-effects, and is applicable in cases where all other treatments have failed. To provide physicians with optimized bedside treatments (schedule and dosage) per patient we have constructed a mathematical model of BT grade III and IV (GBM), which describes brain tumor-immune system interactions. Ours is the first mathematical model to consider the direct use of alloreactive cytotoxic-T-cells (CTL) infusions to the tumor site.
Materials and Methods: Our model consists of six coupled differential equations describing the rate of change of key players in tumor-immune relationship: tumor cells, CTLs, TGFβ, IFNγ, MHC class I, and MHC class II receptors. Parameter values were calculated from current literature. Verification of the model was performed by comparing the results of computer simulations (using published treatment scenarios), to published