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

clinical results. Finally, we simulated putative treatment schedules for identifying a possible effective treatment for GBM.
Results: Our simulation results successfully reproduce the experimental results of Kruse *et al*, (2001) for BTs grade III. Our model suggests several alternative schedules and dosages that manage to destroy the tumor. For a patient who died from a recurrence of BT grade III tumor, our model predicts that a longer treatment course may have been required to prevent tumor resurgence. The model interprets the failure of immunotherapy in the case of BT grade IV and predicts that a more intensive treatment protocol could eradicate GBM. We suggest alternative treatment courses for the eradication of GBM.
Conclusions: CTL immunotherapy is an effective therapy for BT grade III and IV. It can be optimized to prevent tumor recurrence. We believe that the experimental failure of Kruse *et al.* (2001) to treat GBM patients originated from immunotherapy not sufficiently intensive to overcome such a highly aggressive tumor (for example, using the same CTL dose we suggest daily infusions instead of every 4–5 days).

References

Kruse CA, Rubestein D. 2001. In Liau LM, Becker DP, Cloughesy TF, Bigner DD (Eds), *Brain Tumor Immunotherapy.* Humana Press, Totowa, NJ. pp149–170.

273 POSTER

Candidate tumor suppressor gene DLEC1 on 3p21.3 is hypermethylated in hepatocellular carcinoma

G.H. Qiu[1], N. Wheelhouse[2], D. Harrison[2], G.G. Chen[3], S. Manuel[1], P. Lai[3], J.A. Ross[2], S.C. Hooi[1]. *[1]National University of Singapore, Singapore, Singapore; [2]University of Edinburgh, Edinburgh, UK; [3]The Chinese University of Hong Kong, Hong Kong, China*

Background: DLEC1 (previously also known as DLC1) is located at 3p21.3, which is one of the most frequent LOH regions in human chromosomes. It encodes a 1755-amino acid polypeptide and is localized only in the cytoplasm, with no homology to any known protein or domain. DLEC1 has been shown to have tumor suppressor function in cancer cell lines by colony formation assay. However, no alteration of the gene has been detected to cause dysfunction of its product in any of the cancers examined. Nevertheless, a CpG island has been found in the region of its promoter and first exon. Therefore, we tested the hypothesis that methylation of DLEC1 might suppress its expression to inactivate this tumor suppressor gene in hepatocellular carcinoma (HCC).
Material and Methods: HCC cell lines Hep3B, HepG2, Chang Liver, PLC/PRF/5 and SK-Hep-1, and 57 pairs of HCC primary tumors and matched adjacent normal samples were used. DNA methylation was detected by MSP and expression level by RT-PCR. Transfection of cell lines was mediated by Lipofectamine 2000 and transfected cells were selected by G418.
Results: DLEC1 is methylated in HCC cell lines Hep3B, HepG2, Chang Liver, PLC/PRF/5 and SK-Hep-1. The treatment of these cell lines with 5-aza-2'-deoxycytidine restored its expression. Using real-time RT-PCR and HCC primary tissues, we found that the expression level of DLEC1 in tumor samples was significantly lower than that in matched adjacent normal samples (t test, $p < 0.05$). Similarly, expression of DLEC1 in methylated samples was also significantly lower than that in unmethylated samples (t test, $p < 0.05$). Moreover, hypermethylation of DLEC1 was detected in 40 of 59 (67.8 %) primary tumors, while only 6 in 57 (10.5 %) nonmalignant specimens ($p < 0.001$, chi-square). We examined the relationship between DLEC1 methylation status and clinicopathological features, including age, gender, alpha-fetoprotein (AFP) levels, tumor size, ALTSG stage, AJCC stage, differentiation status, cirrhosis, encapsulation, vascular and capsule invasion in 49 samples with their tumor stages identified. The DLEC1 methylation status was associated with AJCC stages of tumors ($p = 0.036$, chi-square). Colony formation assay of exogenous expression of DLEC1 in cell lines showed that DLEC1 significantly inhibited cancer cells growth.
Conclusions: Our data showed that *DLEC1* is hypermethylated in the majority of hepatocellular carcinoma and able to suppress the growth of liver cancer cell lines, supporting its role as a tumor suppressor.

274 POSTER

Effect of IFN-α on gene expression: cDNA microarray analysis in human epidermoid cancer cells

G. Giuberti[1], M. Marra[1], A.M. D'Alessandro[1], A. Lombardi[1], D. Vozzi[2], P. Gasparini[2], A. Abbruzzese[1], A. Baldi[1], M. Caraglia[1]. *[1]Second University of Naples, Department of Biochemistry and Biophysics, Naples, Italy; [2]Second University of Naples, Department of Pathology, Naples, Italy*

Introduction: Interferon-alpha a cytokine commonly used in the human cancer therapy, our research group is deeply involved in the characterization of the effects of IFNalpha activity on tumor cells. In details, we have found that IFN induces into human epidermoid cancer cells KB apoptosis and upregulates the expression of the Epidermal Growth Factor Receptor (EGFR) [1,2].
Materials and Methods: RNA Extraction; Probe Synthesis; Hybridization on cDNA Arrays; Statistical Analysis; Northern blot analysis; Quantitative Real Time PCR, Western blotting.
Results: In order to better characterize the molecular pathways that are elicited or suppressed by the action of IFN. A human 1.7k microarray (Microarray centre U.H.N., Canada) array was used for this experiment, which allows the simultaneous analysis of more than 1.5 thousand genes. Analysis of the hybridization signals through the use of a dedicated software (SAM and processed and analysed with MIDAS), has identified 25 differentially expressed genes: 19 down-regulated and 6 up-regulated in KB cells treated with IFN: prenylcysteine lyase:PCL, tissue inhibitor of met-alloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory):TIMP3, proteasome (prosome, macropain) activator subunit 1 (PA28 alpha), guanine monophosphate synthetase:GMPS. The differential expression of these genes in the two cell lines is being confirmed by northern blot analysis and quantitative RT-PCR as like the proteins expression.
Conclusions: This study represents a useful basis to define an extended molecular database of potential relevance both in basic cell biology studies and in therapeutic options.

References

[1] M. Caraglia et al., (1999) Cell Death Differ 6: 773–780
[2] M. Caraglia et al., (2003) Cell Death Differ. 10(2):218−29.
[3] M. Boccellino et al., (2004) J Cell Physiol, in press
[4] C. Esposito et al., (2003) Biochem. J. 370, 205–212
[5] A. Baldi et al., (2002) J Cell Physiol.;193(2):145−53. Review.

275 POSTER

Tumour cells from stage III melanoma patients are often resistant to growth inhibition by Oncostatin M

A. Lacreusette[1], J. Nguyen[2], M. Pandolfino[1], A. Khammari[3], B. Dreno[3], Y. Jacques[1], A. Godard[1], F. Blanchard[4]. *[1]INSERM U601, Cancerology department, Nantes, France; [2]PIMESP, CHU, Nantes, France; [3]Unit of Skin Cancer, CHU, Nantes, France; [4]INSERM ERI7, Nantes, France*

Oncostatin M (OSM) is an Interleukin-6 (IL-6) type cytokine originally described by its capacity to inhibit melanoma proliferation in vitro. However, OSM responsiveness is often lost in advanced stages melanoma cells. Here, the mechanisms involved in resistance to growth inhibition by OSM and IL-6 were analyzed for the first time on a large panel of metastatic melanoma cell lines (35). For 28% of the cell lines, OSM resistance correlated with the epigenetic loss of the OSM receptor beta (OSMRb) subunit. Treatment of these cells with the histone deacetylase inhibitor Trichostatin A re-established histone acetylation in the OSMRb promoter, expression of OSMRb and growth inhibition by OSM. Other defects linked to OSM resistance were identified, for 31% of the cell lines, on specific signal transduction pathways such as STAT3 (Ser727 phosphorylation), PKCa/b/d and/or AKT, explaining their co-resistance to OSM and IL-6. The use of PKCa/b/d inhibitors indicated that these serine kinases, together with STAT3, have a crucial role in growth inhibition by OSM. In nude mice injected with sensitive melanoma cell lines, OSM notably reduced tumour growth. Moreover, the patients whose melanoma cells were sensitive to growth inhibition by OSM and/or IL-6, and who were treated with tumor-infiltrating lymphocytes (as a potent source for these cytokines; $n = 13$), have a mean relapse-free survival of 8 years. Those whose melanoma cells were resistant to these cytokines ($n = 6$), have a mean relapse-free of only 15 months. Altogether, our results suggest a role for OSM in the prevention of melanoma progression in vitro and in vivo, and that metastatic melanoma cells could escape this growth control by the loss of OSMRb or defects on specific signal transduction pathways. We are currently validating on larger cohorts of patients, the involvement of IL-6 type cytokines in the response to immunotherapy and looking for a specific inflammatory state that could induce this cytokine resistance.

276 POSTER

The frizzled 8-related antiproliferative factor from IC patients inhibits bladder and kidney carcinoma cell proliferation in vitro

S. Keay[1], G. Tocci[2], K. Koch[1], C. Zhang[3], D. Grkovic[1], C. Michejda[2]. *[1]University of Maryland School of Medicine and VA Medical Center, Medicine, Baltimore, MD, USA; [2]National Cancer Institute, Center for Cancer Research, Frederick, MD, USA; [3]University of Maryland School of Medicine and VA Medical Center, Pathology, Baltimore, MD, USA*

Background: Antiproliferative factor (APF) is a potent sialoglycopeptide inhibitor of epithelial cell proliferation made by bladder cells from patients