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.

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

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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.

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

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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.

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

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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