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Abstracts

LAB-EXPERIMENTAL (PRE-CLINICAL) THERAPEUTICS AND PHARMACOLOGY

ET-01. ANTI-CANCER MECHANISM OF RG3 NANOPATICLES IN GLIOBLASTOMA CELLS

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Ginsenoside Rg3 is a natural active ingredient that is extracted from Korean red ginseng root. It elevates the therapeutic effect of radiotherapy and chemotherapy, but previous studies found that the application of Rg3 is heavily limited by its low bioavailability and poor absorption via oral administration. To overcome these problems, Rg3-loaded PEG-PLGA-NPs (Rg3-NPs) were prepared by the modified spontaneous emulsification solvent diffusion (SESD) method, and the physicochemical characteristics of Rg3-NPs were investigated. We treated primary glioblastoma with 50 μM Rg3-NPs for 48h. We then used gene expression arrays (Illumina) for genome-wide expression analysis and validated the results for genes of interest by means of real-time PCR. Functional annotations were then performed using the DAVID and KEGG online tools. The results showed that the Rg3-NPs are slick and uniform, the average diameter of the nanoparticles is 75-90 nm, and their entrapment efficiency is $89.7 \pm 1.7\%$. MTT showed that the growth of cells can be significantly inhibited by Rg3-NPs in a dose-dependent manner. FCM testing showed Rg3-NPs can be released from the conjugate nanoparticle and react with the genes in the cell nuclei, causing changes in the gene molecules. We also found that cancer cells treated with Rg3-NPs undergo cell-cycle arrest at different checkpoints. This arrest was associated with a decrease in the mRNA levels of core regulatory genes BUB1, CDC20, TTK, and CENPE, as determined by microarray analysis and verified by real-time PCR. Furthermore, Rg3-NPs induced the expression of the apoptotic and anti-migratory protein p53 in cell lines. The results of the present study, together with the results of earlier studies, show that Rg3-NPs target genes involved in the progression of the M-phase of the cell cycle. It is associated with several important pathways, which include apoptosis (p53). Rg3-NPs may be a potent cell-cycle regulation drug targeting the M-phase in glioblastoma cell lines.

ET-03. NOVEL THERAPEUTIC APPROACHES USING NANOLIPOSOMAL CPT-11 FOR THE TREATMENT OF HUMAN BRAINSTEM GLIOMA

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INTRODUCTION: Children with diffuse intrinsic pontine gliomas (DIPGs) die within 2 years after initial diagnosis. The infiltrative nature and anatomic location of DIPGs in an eloquent area of the brain preclude surgical resection, and the blood-brain barrier (BBB) reduces the availability of systemically administered agents. In order to improve outcomes for patients with DIPG, new therapeutic strategies that circumvent the BBB are needed. Convection-enhanced delivery (CED) uses positive-pressure infusion to distribute drugs into the brain parenchyma through a surgical catheter. Intranasal delivery (IND) is a practical, noninvasive method to deliver therapeutic agents into the brain along with the olfactory and trigeminal nerves pathway. METHODS: Rats bearing human GS2 brainstem tumors were treated with nanoparticle liposomes containing CPT-11 (nanoliposomal CPT-11) by intravenous (IV) injection, CED, and IND. To determine the in vivo distribution, hydrophobic fluorophore (DiI)-labeled nanoliposomal CPT-11 was administrated into the tumor bearing rats. In a therapy-response experiment, rats were randomly assigned to empty nanoliposomes, free-CPT-11, and nanoliposomal CPT-11 treatments. Tumor growth and response to therapy were quantitatively measured by bioluminescence imaging, and efficacy was assessed by survival analysis. RESULTS: Fluorescence optical imaging detected a fluorescence signal in the brainstem 6 h following CED, with persistence of the signal for >14 days. DiI-fluorophore deposits were found dispersed from the olfactory bulb throughout the different brain regions and lateral edge of the brainstem tumor at 6 h after IND. CED of nanoliposomal CPT-11 showed a dose-dependent inhibition of tumor growth and increased animal survival when compared with rats treated with empty liposome (0.01mg: $p = 0.0304$, 0.1mg: $p = 0.01$, and 1.0 mg: $p = 0.01$). IND and IV of nanoliposmal CPT-11 inhibited tumor growth and significantly prolonged animal survival (IND: $p = 0.0220$, IV: $p = 0.0246$). CONCLUSION: CED and IND could be an alternative to systemic drug delivery for brainstem tumors and are well suited for liposomal formulations of therapeutics.

ET-04. HIGH-THROUGHPUT SMALL MOLECULE SCREENING OF GENETICALLY DIVERSE GLIOBLASTOMA STEM CELLS

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Survival statistics for glioblastoma multiforme (GBM) have remained static despite years of research. Mounting evidence suggests that glioblastoma stem cells (GSCs) play critical roles in GBM's growth, spread, vascularity, chemotherapeutic resistance, and near-universal recurrence. Increasing survival in GBM, therefore, demands a therapeutic strategy with efficacy against GSCs. To address this, we have conducted a high-throughput screen of over 3,000 small molecule compounds for activity against three independently derived GSC lines. These cell lines were isolated from patient samples and vary in their expression of subtype-related genes identified by The Cancer Genome Atlas. Our fluorescence-based assay is sensitive, reliable, and largely free of row- and column-based systematic errors. We identified novel compounds that impair the viability of all three of these genetically diverse GSC lines but have comparably limited toxicity against a normal human glial cell line. Many of these compounds are small and lipophilic as well, making them ideal candidates for follow-up in vivo evaluation in GSC-based xenograft models either through intravenous delivery or through encapsulation in highly penetrative polymeric nanoparticles.

ET-05. TARGETING THE MET SIGNALING PATHWAY IN GLIOBLASTOMA MULTIFORME: HEPATOCYTE GROWTH FACTOR-AUTOCRINE ACTIVATION INDICATES SENSITIVITY TO MET INHIBITORS

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Our goal is to develop novel therapeutic strategies for treating glioblastoma (GBM) patients by identifying and targeting the molecular mechanisms of GBM invasiveness, which underlies mortality. Genomic analysis of primary glioblastoma has shown that MET pathway activation correlates with a "mesenchymal signature," which indicates invasive tumor growth and short survival time for patients. Epidermal growth factor receptor (EGFR) is frequently amplified in GBM and is often associated with MET pathway activation. Both MET and EGFR predominantly signal through the RAS-MAPK and Akt pathways, which are aberrant in 88% of primary GBM tumors. Because MET and EGFR inhibitors are both in clinical development against several types of cancer including GBM, it is therefore important to find determinants that accurately identify patient subgroups suitable for these specific therapies. Our previous study showed that hepatocyte growth factor (HGF)-autocrine expression correlated with high p-MET levels in GBM and were significantly sensitive to MET inhibitors. Here we report that V-4084, a novel selective MET inhibitor, can specifically inhibit HGF-autocrine GBM growth in vivo. Preclinically, V-4084 inhibition of the MET-mediated MAPK pathway was observed as early as 24 h after the first dose was administered. Tumor regression was observed within a week. Microarray analysis comparing sensitive and insensitive tumors revealed 301 genes that were significantly up- or downregulated in sensitive tumors, providing a molecular signature indicating the sensitivity to MET inhibitors. For tumors that did not respond to V-4084 alone, tumor growth was reduced by a combination with erlotinib. We conclude that HGF-autocrine activation can result in oncogene addiction to the MET signaling pathway in GBM via a unique panel of genes. While V-4084 showed promising anti-tumor activity against HGF-autocrine tumors, our preclinical results also suggest that

a combination of MET and EGFR inhibitors be considered as a strategy for treating glioblastoma.

ET-06. ANTI-TUMOR ACTIVITY OF DUAL PI3K/MTOR INHIBITORS PF-04691502 AND PF-05212384 IN GLIOBLASTOMA STEM-LIKE CELLS WITH DIFFERENTIAL PI3K PATHWAY ACTIVATION

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Recent evidence highlights the PI3K/AKT pathway as a core signal transduction pathway often dysregulated in glioblastoma. Epidermal growth factor receptor (EGFR) overexpression, amplification, or mutation; PIK3CA amplification or mutation; or PTEN loss or homozygous deletion are all mechanisms by which glioblastoma tumors may be "addicted" to this pathway. Thus, PI3K pathway inhibitors have gained much attention as potential therapies for glioblastoma. PF-0491502 and PF-05212384 are potent ATP-competitive dual inhibitors of Class I PI3K and mTOR. IC50 values for PI3K are <10 nM for both drugs, and 40 nM and 0.4 nM for mTORC1/2 for PF-0491502 and PF-05212384, respectively. PF-0491502 has the advantages of oral bioavailability and good CSF penetration ($>30\%$ of serum levels). We have created a library of glioblastoma stem-like cell (GSC) lines of different genotypes harvested from more than 100 patients. Xenografts from these GSC-enriched neurospheres have been shown to recapitulate the distinct phenotype and genotype of the corresponding patient-derived tumors and are therefore ideal models for preclinical testing. We sought to investigate the efficacy of PF-0491502 and PF-05212384 on GSCs in vitro and in vivo. Four lines with different PTEN and Akt status were investigated: MGG8 (PTEN expression and low Akt activity), MGG18 (no PTEN expression and high Akt), MGG65 (PTEN mutant and high Akt), and MGG24 (EGFRvIII mutant, PTEN expression, and low Akt). Both inhibitors potently inhibited cell proliferation with IC50 values of 90-170 nM for PF-04691502 and 30-60 nM for PF-05212384. Western blotting showed dose-dependent abrogation of Akt phosphorylation (T308/S473), p-S6, and p-4EBP1 at 4 hours. Inhibition of Akt and mTOR activities at 50 nM could be seen as early as 2 hours and was sustained for more than 48 hours. In vivo studies with mouse orthotopic xenografts are ongoing. In conclusion, PF-0491502 and PF-05212384 demonstrated potent anti-proliferative activity and PI3K/Akt pathway inhibition in vitro in GSC models regardless of PI3K/Akt pathway activation status. Further results including those of in vivo studies will be presented.

ET-07. RECEPTOR TARGETED LIPOSOMES FOR THE TREATMENT OF PERIPHERAL NERVE TUMORS

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Neurofibromatosis type-1 (NF-1) is an autosomal dominant disorder often leading to neurocutaneous tumors. NF-1 tumors are benign soft tissue tumors with the ability to transform to malignant peripheral nerve sheath tumors (MPNST). Currently, NF-1 tumors are treated either by surgery, anti-angiogenic therapy, or farnesyl transferase inhibitor therapy. Chemotherapy is an option for malignant metastatic peripheral nerve sheath tumors. In our current investigation we found that IL13Rα2, a receptor for interleukin-13, is expressed in several malignant and benign peripheral nerve sheath tumors and corresponding cell lines. By utilizing IL-13 conjugated liposomes, we could deliver doxorubicin or Ras inhibitors to the tumors selectively and enhance their therapeutic potential. We demonstrated the specific receptor-mediated binding and delivery of the doxorubicin to the nucleus of MPNST cells and the cytotoxic effect of the IL-13-conjugated liposomal doxorubicin (IL13LIPDXR) in both in vitro monolayer and spheroid MPNST cell culture models. We also investigated the cytotoxic effect of farnesyl thiosalicylic acid (FTS), a Ras inhibitor, and IL13LIPDXR when added concomitantly to established MPNST cell lines. At a 25-100 μM concentration of FTS and 1 μg/mL concentration of IL13LIPDXR, a higher cytotoxic effect was observed compared to the treatment with liposomal doxorubicin alone, indicating a synergistic cytotoxic effect on the MPNST cells. Based on those observations, we modified the lipid bilayer of the doxorubicin liposomes with FTS, studied their cytotoxic effect in cell culture, and demonstrated an enhanced cytotoxic effect. Thus when Ras inhibitor is delivered through a lipid bilayer, the hydrophobic environment will facilitate the competitive binding of the FTS for the galectin site on the plasma membrane of MPNST cells, thus inhibiting Ras-GTP nanocluster formation and dislodging Ras from the membrane. Development of a sciatic nerve tumor model in mice for therapeutic efficacy studies with IL-13 conjugated liposomes is in progress.

ET-08. PEPTIDE-MEDIATED NON-COVALENT DELIVERY OF CISPLATIN TO THE BRAIN VIA INDUCED ACTIVELY PASSIVE TRANSPORT AT THE BLOOD-BRAIN BARRIER

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We have recently demonstrated non-covalent delivery of beta-galactosidase, IgG, IgM, and antibodies against amyloid plaques to the brain upon mixing the proteins with a bivalent synthetic peptide followed by femoral vein injection in mice. The peptide (termed K16ApoE) comprises a moiety with sixteen lysine residues (K16) aimed at strong non-covalent binding to a target protein, and a 20-amino acid segment representing the low-density lipoprotein receptor (LDLR)-binding domain of apolipoprotein E (ApoE). Subsequent experiments indicated that K16ApoE temporarily but reversibly compromises the integrity of the blood-brain barrier (BBB) for up to $\sim$10 min. These results led us to hypothesize that when introduced in the bloodstream by itself, K16ApoE quickly binds to various proteins in the blood, which assumes ApoE-like structures, and then crosses the BBB via transcytosis through the LDLR. We further hypothesized that transport of such ApoE-like molecules creates transient pores in the BBB, which can enable the passage of small molecules. To test these hypotheses, we have, in separate experiments, injected K16ApoE first followed by injection of Evans Blue, Crocein Scarlet, and Light Green SF and evaluated transport of the dyes to the brain. All three dyes were transported to the brain when injected in this manner. These results provided a rationale for delivering anti-neoplastic drugs to the brain. To evaluate this premise, we delivered cisplatin to the brain aided by prior injection of K16ApoE. As a modification of the method, we have first mixed K16ApoE with cetuximab, injecting the mix followed by injection of cisplatin, an approach that allows delivery of both cetuximab and cisplatin to the brain. Presently, our approach enables delivery of $\sim$0.5% of the injected dose of cisplatin to the brain, which is $\sim$50-fold more than when K16ApoE is not used. Efficiency of cisplatin delivery may need to be improved to achieve therapeutic potential.

ET-09. MULTIPLE MECHANISMS OF CILENGITIDE TREATMENT FOR MALIGNANT GLIOMA

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OBJECTIVE: Malignant gliomas are highly lethal cancers that pose great therapeutic challenges. Current treatment involves a combination of surgery, radiation, and chemotherapy, yet these modalities rarely extend the life of patients to more than one year from diagnosis. Integrins are expressed in tumor cells and tumor endothelial cells. Cilengitide is the first integrin inhibitor in clinical phase III trials for glioblastoma. In this study, we investigated the multimodal anti-glioma effects of cilengitide by examining its cytotoxic, anti-angiogenic, anti-invasive, and synergetic effects. METHODS: Cilengitide was generously provided by Merck KgaA and the National Cancer Institute. We evaluated the apoptotic effect of cilengitide by quantitative PCR (Q-PCR) analysis in U87ΔEGFR (human malignant glioma cell line) in vitro and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining in vivo. Anti-angiogenic effects were assessed by a tube formation assay in human umbilical vein endothelial cells (HUVECs) and anti-invasive effects by scratch assay in U87ΔEGFR. We also evaluated its synergistic effect with oncolytic virus (OV) therapy. RESULTS: In Q-PCR analysis, caspase-8 mRNA in U87ΔEGFR treated with cilengitide was increased compared to that of control cells. In a U87ΔEGFR nude rat model, cilengitide-treated animals demonstrated significantly higher percentage of TUNEL-positive cells than control animals. Cilengitide inhibited tube formation in HUVECs in a concentration-dependent manner and attenuated tumor invasion in U87ΔEGFR cells. OV, combined with cilengitide, induced synergistic effects in the survival of mice treated with combination therapy. CONCLUSION: The results indicated cilengitide exerted its apoptotic, anti-angiogenic, and anti-invasive effects on malignant glioma and also showed a promising synergy with cilengitide and another new therapy.

ET-11. CXCR7 INHIBITION SIGNIFICANTLY PROLONGS SURVIVAL IN THE ENU RAT MODEL OF GLIOBLASTOMA

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Potent and selective small molecule antagonists of the chemokine receptor CXCR7 have been identified using [^{125}I]CXCL12 binding and trans-

endothelial cell migration assays. These antagonists retain potency against CXCR7 in the presence of 100% serum and have activity against the human, rat, and mouse forms of the receptor. In addition, these compounds inhibit CXCL12-dependent trans-endothelial migration of CXCR4 + /CXCR7+ cells with high potency. CCX662 is one such molecule that displays high potency (IC_{50}: 9 nM) and high selectivity for CXCR7. Glioblastoma (GBM) is the most common form of malignant brain cancer. Despite aggressive therapy, consisting of radiotherapy, surgical resection, and chemotherapeutic treatment, the prognosis remains dire. Using immunohistochemical analysis of tumor microarrays, we show high expression of CXCR7 by both human glioma cells and tumor-associated vasculature. Expression levels increase with glioma grade. CXCL12, one of two CXCR7 chemokine ligands, is also expressed by tumor-associated vasculature. In the ENU-induced GBM model in rats, CXCR7 expression is also seen on both tumor cells and tumor-associated vasculature. The therapeutic potential of CXCR7 inhibition was tested in this rat model, which has proved resistant to conventional therapy. Inhibition of CXCR7 in combination with radiotherapy resulted in a significant extension of survival time. Treatment of rats with CCX662 for 28 days following irradiation resulted in a median survival time of 370 days, compared to a mean survival time of 200 days seen with vehicle + irradiation. Extending the dosing of CCX662 to 56 days post-irradiation extended median survival beyond 418 days ($p < 0.01$ versus vehicle treated). These results indicate that CXCR7 inhibition is a promising strategy for the treatment of glioblastoma.

ET-12. SYNERGISTIC GROWTH SUPPRESSION OF MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)-EXPRESSING GLIOMA XENOGRAFT BY COMBINATION OF TEMOZOLOMIDE AND ANTI-EGFR MONOCLONAL ANTIBODY NIMOTUZUMAB

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A mutant form of epidermal growth factor receptor (EGFR), EGFRvIII or ΔEGFR, is common in glioblastoma (GBM) and confers enhanced tumorigenic activity as well as resistance to chemotherapeutic drugs. Nimotuzumab, a humanized monoclonal EGFR antibody, has been shown to have modest activity in patients with GBM, but its specific activity against ΔEGFR has not been fully investigated. We treated human glioma cells overexpressing either wild-type (wt) EGFR or ΔEGFR with nimotuzumab with or without temozolomide (TMZ). Nimotuzumab treatment resulted in reduction in tyrosine phosphorylation at the C-terminus of ΔEGFR more preferentially than that of wtEGFR, albeit to a lesser extent than tyrosine kinase inhibitor AG1478. Nimotuzumab-induced EGFR tyrosine dephosphorylation was associated with a decrease in AKT phosphorylation, suggesting suppression of downstream signaling pathways. In animal models, growth of subcutaneous xenografts was suppressed by TMZ but was not affected by nimotuzumab monotreatment. However, antitumor activity was synergistically enhanced when TMZ was combined with nimotuzumab in both U87MG.ΔEGFR and LNZ308.ΔEGFR xenografts, whereas such an effect was limited in wtEGFR-overexpressing xenografts. Similarly, compared with nimotuzumab or TMZ monotherapy, the combination treatment of TMZ with nimotuzumab significantly elongated survival of mice bearing intracerebral xenografts derived from U87MG.ΔEGFR ($p < 0.001$, log-rank test) and U87MG.wtEGFR (although to a lesser extent [$p = 0.006$]). The U87MG.ΔEGFR intracerebral tumors that had re-grown after the combination treatment with TMZ and nimotuzumab ("escapers") exhibited increased expression of O6-methylguanine-DNA methyltransferase (MGMT) as well as a decrease in mismatch repair (MMR) proteins MSH6 and MLH1. The reduction of MMR expression was further retained in subcultured escaper cells. These expression changes were not observed in xenografts treated with any of the studied vehicles or monotherapies. These results suggest that nimotuzumab has antitumor activity against mutant EGFR-expressing glioma in vivo when combined with TMZ, and the resistance to this combination treatment might involve expression change of both MGMT and MMR.

ET-13. MICRORNA-181B IS ASSOCIATED WITH CHEMOSENSITIVITY AND MAY SERVE AS A PROGNOSTIC BIOMARKER IN GLIOMAS

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Recent studies have reported that microRNA-181b (miR-181b) functions as a tumor suppressor in gliomas. However, whether miR-181b expression associates with prognosis in glioma patients and its relation to chemosensitivity remain unclear. The aim of this study was to investigate the roles of miR-181b expression and association with chemoresponse to temozolomide (TMZ) and cisplatin (DDP) in glioma cell lines as well as clinical glioma samples and survival of glioma patients. The miR-181b expression levels in 90 human glioma samples and 8 glioma cell lines were assessed by real-time PCR. TMZ and DDP cytotoxicity in glioma cell lines and human glioma specimens were evaluated by the WST-8 assay. The expression level of miR-181b was negatively associated with glioma grade and was much lower in human glioma specimens than in normal brain tissues. Kaplan-Meier survival analysis showed that low expression of miR-181b significantly correlated with short survival time in glioma patients ($p = 0.008$). Furthermore, the expression level of mir-181b was strongly associated with response to TMZ ($r_s = -0.576$, $p = 0.00$) and DPP ($r_s = -0.413$, $p = 0.00$) in clinical glioma specimens. Moreover, in glioma cell lines, we also have revealed that higher levels of miR-181b indicate higher sensitivity to TMZ ($r_s = -0.738$, $p = 0.037$) and DDP ($r_s = -0.762$, $p = 0.028$). Our results suggest that miR-181b expression is associated with chemoresponse to TMZ and DDP in human gliomas. It may also serve as a prognostic biomarker in gliomas.

ET-14. ANTITUMOR EFFICACY OF A SMALL MOLECULE MIR-21 INHIBITOR ON GLIOMAS

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MicroRNAs (miRNAs) belong to a family of small noncoding RNAs (~21 nucleotides) that are a key component of post-transcriptional gene regulation. Aberrant expression of miRNAs has been shown to be involved in tumorigenesis. Currently, small molecule intervention of microRNA misregulation has the potential to provide new therapeutic approaches to such diseases. Here, we report a small molecule inhibitor of miR-21, one of the most frequently overexpressed miRNAs in human glioblastoma, identified through a novel high-throughput screening method. In vitro, the small molecule significantly reduced mature miR-21 expression in a time- and dose-dependent manner in LN229 and U87glioblastoma cell lines, as assessed by real-time PCR. Meanwhile, Western blot and luciferase assay indicated that the downstream targets EGFR, AKT, STAT3, and β-catenin expression and transcriptional activity were also inhibited. However, potential miR-21 targets PDCD4, PTEN, and RECK were upregulated after the treatment. Additionally, the inhibitor effectively repressed the cell proliferation, invasion, and migration activity detected by cell cycle, colony formation, transwell, and apoptosis assays. Similar results were also observed in vivo in an intracranial model of U87 glioblastoma cells. Intraperitoneal injections of this small molecule downregulated the expression of miR-21, induced more apoptotic cells, and delayed intracranial tumor growth. Moreover, inhibitor-treated nude mice exhibited an increased body weight and a better prognosis in comparison to a dimethyl sulfoxide (DMSO) treatment group. Together, our findings provide the first evidence for this small molecule's antitumor efficacy both in vitro and in vivo, and the agent could have potential to improve treatment of malignant gliomas.

ET-15. CHEMOTHERAPEUTIC MODULATION OF GLIOMA DEVELOPMENT AND INVASION IN VIVO

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Malignant gliomas are neurologically devastating tumors that are rapidly fatal. Previously, signal transducer and activator of transcription 3 (STAT3) has been shown to drive many of the key components of malignancy. Using a genetically engineered murine model of malignant glioma potentiated by STAT3 in neural progenitor cells, we hypothesized that distinct chemotherapy approaches would differentially influence key malignant glioma features of invasion, "stemness," immune suppression, and the mesenchymal phenotype. Therefore, we treated mice with gliomas with the STAT3 inhibitor WP1066 or the standard of care, therapeutic temozolomide (TMZ). Both of these agents increased median survival time to 91 and 99 days, respectively, in comparison with 68 days in the control. Although both WP1066 and TMZ could reduce the propensity for the development of the high-grade gliomas, contralateral invasion via corpus callosum involvement was inhibited to a greater degree by WP1066. Although both agents could inhibit intratumoral p-STAT3, only the inhibition by WP1066 was statistically significant. Furthermore, both agents could inhibit tumor-supportive macrophages. Given the anti-tumor effects, WP1066 may provide an alternative treatment to TMZ in GBM.

ET-16. COMPARISON OF SYSTEMIC VERSUS DIRECT INTRATUMORAL ADMINISTRATION OF NANOLIPOSOMAL IRINOTECAN, WITH AND WITHOUT FRACTIONATED RADIOTHERAPY, FOR EFFICACY IN TREATING ORTHOTOPIC GLIOBLASTOMA XENOGRAFTS

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The effectiveness of traditional chemotherapeutic agents for glioblastoma is compromised in part by poor access to tumors, as well as by a short half-life in patients. Liposomal formulation of anticancer agents is known to extend the half-life of chemotherapeutics and to prolong the anti-tumor effect of therapy. In this study, we investigated the anti-tumor activity of MM-398 (Merrimack Pharmaceuticals, Cambridge, MA), a nanoliposomal irinotecan formulation. Luciferase-modified human GBM43 cells were injected into the right striatum of athymic mice that were randomized to six groups: 1) untreated; 2) radiation (RT) only, with 1.5 Gy for 5 fractions; 3) systemic administrations of MM-398 only (0.4 mg/administration) with treatments on days 7 and 11; 4) MM-398 only by convection-enhanced delivery (CED) at a dose of 0.4 mg on day 7; 5) regimens 2 and 3; and 6) regimens 2 and 4. All animals were monitored using bioluminescence imaging (BLI) to assess tumor growth and response to therapy. BLI data revealed substantially reduced tumor growth rates in mice receiving MM-398 monotherapy, regardless of administration route, and in relation to untreated or RT only groups. When animals were treated with MM-398 in combination with RT, tumor growth was further delayed. Consistent with the BLI results, mice treated with combination therapy, either systemically or by CED, survived significantly longer than mice receiving no treatment or treated with RT only: 14 and 17 days for the latter two groups, respectively, versus 24 days for intravascular administration and a yet-to-be determined median survival for CED. We are currently examining intracerebral tumors for molecular indicators of proliferative and apoptotic response to the various treatments, and we are investigating the intracerebral PK of MM-398, to determine whether radiation improves agent access to intracerebral tumors, with associated results to be presented at the meeting.

ET-17. INHIBITION OF BET BROMODOMAIN TARGETS GENETICALLY DIVERSE GLIOBLASTOMA

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Glioblastoma is highly refractory to the conventional chemoradiotherapy. Newly developed molecular-targeted agents, mainly inhibitors of receptor tyrosine kinases, have yet to show significant clinical efficacy. The Myc oncoprotein family, particularly c-Myc, plays instrumental roles in the pathogenesis of many human cancers, including glioblastoma. We and other groups have further shown that c-Myc is critically implicated in self-renewal and survival of glioblastoma stem cells, highlighting the potential of c-Myc as a therapeutic target in glioblastoma. However, pharmaceutical inhibition of c-Myc has proven challenging. Recently, a small molecule inhibitor, JQ1, was shown to decrease the expression and activity of c-Myc through targeting the bromodomain and extra-terminal (BET) family of proteins. Here we report that JQ1 dose-dependently reduces the proliferation and survival of genetically diverse glioblastoma cells with an IC50 in the submicromolar range. JQ1 treatment also decreases protein and mRNA levels of c-Myc. Downregulation of c-Myc by JQ1 induces G1 cell cycle arrest and apoptosis associated with induction of p21. In an orthotopic glioblastoma tumor model, JQ1 significantly extends the median survival of tumor-bearing mice. Taken together, our study establishes BET bromodomain inhibition as a novel therapeutic strategy for glioblastoma treatment that may not be limited to specific genotypes.

ET-18. THE INTEGRIN ANTAGONIST CILENGITIDE AUGUMENTS ANTI-TUMOR EFFECT OF VASCULOSTATIN-EXPRESSING ONCOLYTIC VIRUS

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INTRODUCTION: Oncolytic viral (OV) therapy has been considered as a promising treatment modality for malignant glioma. Previously, vasculostatin (the fragment of brain-specific angiogenesis inhibitor-1; BAI-1)-expressing oncolytic HSV-1 (rapid antiangiogenesis mediated by oncolytic virus; RAMBO) has been reported to have a potent antitumor effect against malignant glioma. Cilengitide (EMD121974), an inhibitor of integrins, has also demonstrated preclinical efficacy against malignant glioma. In this study, we investigated the therapeutic efficacy of a combination of RAMBO and cilengitide for malignant glioma and the mechanism of the anti-glioma effect of combination of RAMBO and cilengitide. METHODS: Cilengitide was generously provided by Merck KgaA and the National Cancer Institute. In vitro, we conducted scratch wound and cell viability assays to assess the combined treatment with RAMBO and cilengitide by using human microvascular endothelial cells (HMVEC) and U87ΔEGFR human glioma cells, respectively, and gene expression analysis with DNA microarray. In vivo, seven days after implantation of U87ΔEGFR glioma cells into nude mouse brain, RAMBO or solvent (control) was injected stereotactically into the brain tumor and treated with either cilengitide or solvent intraperitoneally 3 times a week. The survival of mice in each group was analyzed by the Kaplan-Meier method. RESULTS: In vitro, cilengitide treatment reduced the rate of wound closure of HMVEC. Combining cilengitide with CM of RAMBO significantly decreased the wound closure. Combining cilengitide with RAMBO induced synergistic cytotoxicity in U87ΔEGFR glioma cells in vitro. In gene expression analysis, genes that were associated with induction of apoptosis by intracellular signals were over-represented. In vivo, there was a statistically significant increase in survival of mice treated with combination therapy compared to RAMBO or cilengitide monotherapy. CONCLUSIONS: These results indicate that cilengitide enhaces new vasculostatin-expressing OV therapy for malingnat glioma and provide a rationale for designing future clinical trials combining the two agents.

ET-19. IDENTIFICATION OF IMMUNOGENIC EPITOPES FROM CD133 AND THEIR POTENTIAL FOR USE TO IMMUNOLOGICALLY TARGET GLIOBLASTOMA CANCER STEM CELLS

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CD133 is a marker that identifies cancer stem cells (CSC) on many solid tumors, and its expression in glioblastoma (GBM) tumors has been correlated with shortened survival. Although expressed on many normal tissues and stem cells, CD133 is overexpressed on CSC, whereas normal SC are negative or express low levels of MHC making them less sensitive to recognition by CD8+ cytotoxic T lymphocytes (CTL). Potential human CTL epitopes were identified by algorithms to predict binding to HLA-A2. Binding studies showed high affinity binding and low off rates for four candidate epitopes. CTL induction studies using dendritic cells to stimulate CD8+ T cells evaluated the immunogenicity of the candidate peptides after 3-4 weekly in vitro stimulations. Immune responses were observed to the CD133-753, 405, and 708 epitopes with multimers and interferon (IFN)-gamma ELISpots. Subsequent studies demonstrated killing of T2 cells loaded with peptide. To evaluate potential autoimmunity, mouse homolog peptides of the 753 and 405 epitopes shown to have high-affinity binding to human HLA-A2 were used to immunize HLA-A2 transgenic mice (9CB6F1-Tg (HLA-A*0201/H2-Kb) A*0201). Mice were immunized 3 times at 3-week intervals using IFA + HBV helper peptide. Spleens were harvested 2 weeks after the last immunization and stimulated in vitro for one week with peptide-pulsed LPS stimulated- APC. IFN-gamma ELISpot assays showed immune responses to the 753 and 405 peptides in 30% and 33% of mice, respectively. Organs, including heart, lung, liver, kidney, stomach, intestine, brain, bone marrow, gonads, and eyes from mice with immune responses were found to be negative for lymphocytic infiltrations, supporting a lack of autoimmunity related to the immune response to these peptides. Together these studies support the safety and immunogenicity of these peptides as a potential vaccine to target CD133 cancer stem cells in GBM.

ET-20. SPICING UP GLIOBLASTOMA RESEARCH: TURMERIC AS A NOVEL PRIMARY AND ADJUVANT THERAPEUTIC AGENT

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Modern medicine has begun to look to the ancient world for "new" ideas and anti-neoplastic agents. Curcuma longa (turmeric) has long been used

primarily as a spice but also for its medicinal properties. Curcumin, the active ingredient in turmeric, has gained the attention of tumor biologists and, to date, 68 National Institutes of Health (NIH) clinical trials are underway to study its effects in a variety of diseases, including cancer; however, glioma is notably absent from this list. The typically poor prognosis and few effective therapies make glioma an ideal candidate for further study. Previous studies have identified molecular targets for curcumin and shown its efficacy in both cell cultures and murine models using glioblastoma (GBM) cell lines. However, studies have yet to clearly demonstrate a true survival benefit, dose-response curves, and tumor suppressive effects using patient-derived GBM progenitor-like cells. Preliminary data obtained by our lab demonstrate that exposure to curcumin results in decreased tumor proliferation using standard XTT assays (Roche) and decreased cell viability using patient-derived GBM cells as well as standard GBM cell lines (U87MG and U251MG). Exposure to 5 uM curcumin inhibited cell proliferation 55-100%, depending on the cell line, at 96 hours, whereas 10 uM curcumin resulted in complete growth suppression of all cells tested. After 48 hours, in vitro scratch assays demonstrate a 40% reduction in cell migration using similar concentrations of curcumin using both patient-derived cells and standard GBM cell lines. Ongoing research in our lab is confirming the effects of curcumin on brain tumor progenitor-like cells, as well as normal neuronal, astrocytic, and endothelial lines to facilitate in vivo testing, specifically direct parenchymal implantation in a murine host. Curcumin has been shown to be an effective agent against glioblastoma cells in vitro, and our aim is to carry this research forward to demonstrate its efficacy in vivo and in clinical applications.

ET-21. MIBEFRADIL ENHANCES THE TUMORICIDAL EFFECT OF RADIATION THERAPY IN AN INTRACRANIAL GLIOMA MODEL

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The survival of patients with malignant gliomas remains unfavorable. Mibefradil, a calcium channel T-type specific inhibitor capable of synchronizing dividing cells at G1 phase, has demonstrated potential benefit in conjunction with chemotherapeutic agents for gliomas in in vitro studies. In vivo study of mibefradil and radiation therapy (RT) is lacking. We utilized an intracranial rat C6 glioma model to study mibefradil and RT. Beginning two weeks after implantation of the C6 cells into the animals, each rat underwent a biweekly MRI. Then, the rats were randomly assigned to one of the experimental groups based upon magnetic resonance imaging (MRI) confirmation of tumor. Tumor volumes were measured on MRIs. Experimental group 1 received 30 mg/kg mibefradil intraperitoneally three times a day starting on postoperative day 15 (POD 15) for one week; group 2 received 8 Gy cranial RT delivered on POD 15; group 3 received RT on POD 15 followed by one week of mibefradil; group 4 received mibefradil on POD 15 for one week followed by RT on POD 22. A total of 27 glioma-bearing rats were analyzed. Two animals survived until POD 60, which is twice the expected survival for untreated C6 glioma-bearing rats. Survival was compared between groups using the Kaplan-Meier method. Median survivals in group 1, 2, 3, and 4 were 35, 31, 43, and 52 days, respectively (log-rank test, $p = 0.036$). Analysis of variance (ANOVA) and post hoc testing indicated that no tumor volume differences were noted on POD 15 and 29. However, significant volume differences were found in POD 43; mean tumor volumes of groups 1, 2, 3, and 4 were 250, 266, 167, and 34 mm^3, respectively (ANOVA, $p = 0.046$). Cox proportional hazards regression showed survival was associated with tumor volume on POD 29 ($p = 0.001$) rather than on POD 15 ($p = 0.162$). Mibefradil response is schedule-dependent, and it enhances survival and tumor reduction when combined with radiotherapy.

ET-22. EZN-2208, A NOVEL PEGYLATED SN38 DRUG CONJUGATE, MARKEDLY INHIBITS TUMOR GROWTH IN COMPARISON TO IRINOTECAN

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EZN-2208 is a water-soluble PEGylated conjugate of SN38 that results in parenteral delivery, increased solubility, higher exposure, and longer apparent half-life of SN38, as well as more profound deoxyribonucleic acid (DNA) damage and inhibition of angiogenesis, than irinotecan. As such, EZN-2208 prolongs exposure of tumors to SN38 via preferential retention of EZN-2208 in the tumor and prolonged circulation of SN38 in the blood. This study evaluated the efficacy of EZN-2208 compared to irinotecan in both an intracranial and subcutaneous brain tumor model. Intracranial and subcutaneous brain tumor xenografts (D-270 MG) were grown in athymic BALB/c mice. Three days after intracranial implantation or after tumor size reached 200-500 mm^3, groups of 10 mice were randomly treated with either drug vehicle/control, EZN-2208, or irinotecan. Tumor responses for intracranial xenografts were assessed by difference in median survival and by tumor growth delay and regression in subcutaneous xenografts. Intracranially, EZN-2208 (10 mg/kg/day x 5, intravenous) produced a 1066+ % increase in survival versus vehicle control, whereas treatment with irinotecan (40 mg/kg/day x 5, intraperitoneal) resulted in an increase of 73%. Subcutaneously, EZN-2208 produced a growth delay of 180+ days ($p < 0.001$) versus control and 160+ days versus irinotecan alone, with all EZN-2208 treated tumors regressing to undetectable size. At doses less than MTD (1 mg/kg, 3 mg/kg, and 5 mg/kg per day x 5), EZN-2208 produced growth delays of 8.38, 15.98, and 90+ days ($p < 0.004$), respectively, compared to vehicle control, whereas irinotecan alone (40 mg/kg/day x 10, intraperitoneal) produced a growth delay of 36.26 days. Our results demonstrate the therapeutic activity of EZN-2208 alone against brain tumor xenografts. Furthermore, our study demonstrates the benefit of EZN-2208 over irinotecan when given at maximum tolerated and clinically relevant doses. These results support further exploration of EZN-2208 as a single agent and in combination with other standard-of-care agents for the treatment of brain tumors.

ET-23. THE EFFECT OF INCREASING CONCENTRATIONS OF INTRACRANIAL ALBUMIN ON FLUID FLOW RATES WITHIN ADJACENT WHITE MATTER TRACTS IN RATS

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BACKGROUND: In most systemic cancers, tumor cells are passively carried by extracellular fluid flow to regional lymph nodes. However, the mechanism of glioma cell dissemination remains controversial, as the brain does not contain a lymphatic system. Because malignant brain tumors are characterized by a disrupted blood-brain-barrier that leaks albumin from blood vessels into brain parenchyma, we hypothesize that this albumin influx will pull water in osmotically and increase flow rates and glioma cell dissemination down adjacent white matter tracts. This may have implications for future radiation planning and steroid/vascular endothelial growth factor (VEGF) inhibitor dosing. METHODS: Our study examines flow rates in white matter tracts after intracranial administration of albumin. We mixed 0.12-6 mg bovine albumin with 20 μL of 2% Evans Blue and injected the mixture stereotactically into the right frontal lobes of 15 anesthetized Sprague-Dawley female rats at the gray-white matter junction over 80 minutes. Seven hours later, the rats were killed and their brains sectioned into 0.5 mm coronal slices and imaged to determine interstitial flow rates. RESULTS: All animals tolerated the infusion without difficulty. The entire brain was white except at the site of injection and in ipsilateral white matter tracts, especially the external capsule. Average flow rates (mm/hour) for rats receiving 0.12 mg of albumin were 0.43 (coronal) and 0.13 (axial) and for those receiving 6 mg albumin were 0.99 (coronal) and 0.23 (axial). Positive correlation was observed between albumin dose administered and interstitial fluid flow rates ($R = 0.57$ coronal and 0.36 axial). CONCLUSIONS: This study demonstrates higher interstitial fluid flow rates in adjacent white matter tracts in response to albumin-induced-edema. These observations provide insight into expected pathways for glioma dissemination that could be used to tailor radiation plans for individual patients. Furthermore, these findings provide a rationale for interventions to reduce glioma dissemination by limiting the extravasation of albumin through the blood-brain-barrier while local radiation is being administered.

ET-24. CHEMOTHERAPY CAN SYNERGIZE WITH ADOPTIVE IMMUNOTHERAPY TO INHIBIT MEDULLOBLASTOMA GROWTH

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New immune-based approaches for brain tumor therapy are rapidly being evaluated for first-line treatment. Despite encouraging pre-clinical data, however, results in patients have been suboptimal. Tumor-induced immune suppression and intrinsic resistance to immune attack are likely culprits. Chemotherapy and biologic agents may be able to disrupt these mechanisms and restore tumor sensitivity to immune attack. In this study, we are examining whether a combination of gemcitabine and rapamycin can sensitize medulloblastoma cells to immunotherapy in vitro and in vivo. We are using the commercial medulloblastoma cell line Daoy as the target for our experiments. First, we tested a variety of concentration combinations of

gemcitibine with rapamycin to determine if these agents have cytotoxic activity against Daoy cells. Next, we generated anti-tumor T-cells using naive T-cells stimulated in the presence of Daoy lysate-pulsed dendritic cells. We tested the ability of these cells to mediate cytotoxic killing in vitro using a flow cytometry-based assay. Finally, we tested the ability of chemotherapy alone versus chemotherapy in combination with immunotherapy to mediate tumor growth inhibition of subcutaneous medulloblastoma xenografts. Rapamycin had little activity alone against Daoy cells in vitro, and an IC50 was not reached. Gemcitibine had a 3 day IC50 alone of 10 nM but in combination with 100 nM rapamycin, it decreased to 1 nM, suggesting synergism of these two agents. Stimulated T-cells were able to mediate in-vitro cytotoxicity, although background cytotoxicity of unstimulated "naïve" T-cells was also significant. Finally, established subcutaneous Daoy cell xenografts in severe combined immunodeficient (SCID) mice were treated with chemotherapy alone or chemotherapy plus adoptive immunotherapy (simulated and unstimulated). Gemcitibine and rapamycin alone significantly slowed tumor growth, but the addition of immunotherapy further augmented inhibition, suggesting that combining immunotherapy and chemobiologic therapy may offer an effective treatment option for patients.

ET-25. VSTAT120 TEMPERS THE MICROGLIA/MONOCYTE INNATE INFLAMMATORY RESPONSE, AUGMENTING ONCOLYTIC VIRAL THERAPY

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BACKGROUND: Glioblastoma multiforme (GBM) is a grade IV malignant astrocytoma with a median patient survival of 15 months. Oncolytic virus (OV) therapy uses engineered viruses to selectively destroy cancer cells. Rapid antiangiogenesis mediated by oncolytic virus (RAMBO) is an OV that expresses the anti-angiogenic transgene Vstat120. Mice bearing intracranial tumors treated with RAMBO survived significantly longer than mice treated with a control virus. In addition to its anti-angiogenic effects, RAMBO also replicated better than a control virus in vivo. These results suggested that the expression of Vstat120 may also temper the innate immune response to viral infection. This project investigates the effect of RAMBO on host innate cellular defense responses mediated by monocytes/microglia. METHODS: Co-culture experiments were established with murine microglia and glioma cells infected with RAMBO virus or a control virus. The expression levels of various cytokines were analyzed via quantitative PCR. In other experiments, mice bearing intracranial tumors were treated with RAMBO or a control virus. The brains of these treated mice were harvested, and flow cytometric analysis was conducted to examine microglia/macrophage infiltration and activation in the brain. RESULTS: Results of co-culture experiments showed a significant downregulation of type-1/2 interferons and inflammatory cytokines and chemokines in microglia co-cultured with RAMBO-infected glioma cells compared to a control virus. Flow cytometric analysis of microglia/macrophages revealed a significant reduction in the number of these cells in the brains of mice treated with RAMBO compared to control virus. There was also a significant reduction in the activation of microglia in brains of mice treated with RAMBO. CONCLUSIONS: The expression of Vstat120 within the RAMBO virus mediates an anti-inflammatory effect that tempers the innate immune response upon infection and allows the virus to propagate and destroy tumor cells more effectively than traditional OVs. This study further validates the use of the RAMBO virus as a therapeutic for treating GBMs.

ET-26. HFE CAN BE A POTENTIAL THERAPEUTIC TARGET FOR GLIOBLASTOMAS TREATMENT

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HFE protein plays a key role in the regulation of body iron. HFE gene variants H63D and C282Y are associated with increased cellular iron accumulation and involved in multiple diseases. Although there are many publications that suggest a statistical association between HFE polymorphisms and numerous cancers, the role of HFE polymorphisms in cancer has not been studied well. In this study, we investigated the effect of HFE on brain tumor cell growth and tumor development. First, we determined the HFE expression in brain cancer cells. HFE is strongly expressed in brain tumor tissues such as meningiomas, glioblastomas, and astrocytomas and a number of commercially available astrocytoma cell lines, as determined by immunohistochemistry. We observed in our cell culture models that the C282Y HFE gene variant imparted complete resistance to chemotherapeutic agents and radiation, and cells with the H63D gene variant where more resistant than wild type. This resistance could be reversed by transfecting the cells with siRNA for HFE. Because of the importance of iron in cell proliferation, we determined the association between HFE and cancer cell proliferation. Transfecting HFE siRNA into the cancer cells resulted in a significant inhibition of cell proliferation in both astrocytoma and neuroblastoma cells regardless of HFE genotype. The antiproliferative effect of HFE siRNA was dose dependent. Cells transfected with non-specific siRNA or the transfection reagent control siRNA showed no evidence of decreased cell proliferation. HFE siRNA did not inhibit the proliferation of a normal astrocyte cells. We further tested the effect of HFE siRNA on cancer cell growth in the subcutaneous nude mouse tumor model. As observed in cell culture, HFE siRNA significantly inhibited tumor growth compared to control groups. These data indicate that HFE inhibition by siRNA may prevent cancer cell proliferation and tumor development and increase cancer cell sensitivity to treatment.

ET-27. A NOVEL CLASS OF SMALL MOLECULES PREVENT GLIOMA GROWTH AND INHIBIT HYPOXIA-INDUCIBLE FACTOR BINDING TO TRANSCRIPTION CO-FACTORS P300/CBP

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Microregional hypoxia is a well-known characteristic of glioblastoma that appears as the tumor mass outgrows the existing vascular supply. Hypoxic areas of solid tumors are resistant to traditional chemo- and radiotherapies, emphasizing the need to develop new therapies for their targeting. A common property of these cells is the expression of hypoxia-inducible factor (HIF)-1 and HIF-2, key transcription factors that coordinate adaptive responses to hypoxia, providing cancer cells the means to survive and proliferate under hypoxic conditions. To identify specific small molecule inhibitors of HIFs, we screened a combinatorial library of 10,000 natural product-like chemical compounds. We found that KCN1, the lead of a class of novel arylsulfonamides identified in this screen, potently inhibits HIF transcription in various cancer cell lines (IC50 = ~600 nM) while exerting minimal effects on the levels of HIF-1alpha, other short-lived proteins, or control proteins. To identify the molecular mechanism of action of this novel inhibitor, we examined its ability to interfere with the HIF transcriptional complex. Pulldown with an HRE-containing probe, chromatin immunoprecipitation, and co-immunoprecipitation assays demonstrated that KCN1 disrupts the interaction of HIF-1alpha with transcriptional co-activators p300/CBP, likely through binding the CH1 domain of p300/CBP. Detailed experimental evidence supporting this novel mechanism will be presented. In an effort to translate KCN1 to the clinic, we evaluated its toxicity and anti-tumor efficacy in animal models. Systemic administration of KCN1 in nu/nu mice harboring human LN229 glioblastoma xenografts revealed strong anti-tumor effects in the absence of noticeable toxic effects. These data suggest that KCN1 and the arylsulfonamides are promising new agents for translation to clinical trials.

ET-29. DESIGN OF NEW ANGIOPEP-2-ANTI-EGFR AND ANGIOPEP-2-ANTI-HER2 DERIVATIVES WITH INCREASED BLOOD-BRAIN BARRIER PERMEABILITY FOR TREATMENT OF BRAIN TUMORS

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The therapeutic potential of monoclonal antibodies (mAbs) to treat a variety of tumor types is well established. However, this class of agents is generally excluded from the brain because of poor blood-brain barrier (BBB) permeability. Although they prevent entry of xenobiotics into the brain, BBB endothelial cells do express multiple transporters and receptors to permit entry of essential molecules, such as nutrients and hormones. One such receptor, the low density lipoprotein-related receptor 1 (LRP1), enables numerous ligands to access the brain via receptor-mediated transcytosis. We have previously demonstrated that therapeutic brain levels of non-brain-penetrant peptides or small molecules can be achieved by conjugation with Angiopep-2 (An2), a proprietary 19 amino acid peptide that binds LRP1. For example, An2-paclitaxel (GRN 1005, licensed to Geron, Inc.) is currently in phase II studies for brain metastases. Applying this engineered peptide conjugate (EPiC) technology to mAbs, we have now been able to expedite transport of anti-Her2 and anti-EGFR mAbs across the BBB. Several conjugates were created using various chemical methods and conditions, which were optimized for brain penetration and pharmaceutical properties.

By measuring in vivo brain uptake in mice, we observed that incorporation of An2 increases the rate of entry into brain parenchyma by 5-10-fold over unconjugated mAbs, depending on the number of An2 molecules per mAb. EPiC-mAb conjugation does not affect target binding affinity or plasma stability. In mice, the plasma half-life of EPiC-anti-Her2 is the same as that of the unconjugated molecule, whereas the brain:plasma ratio is higher. Overall, these data extend the validation of An2 conjugation beyond small molecules and peptides to include larger molecules, such as therapeutic mAbs, for development of new brain-penetrant antitumor therapeutics.

ET-30. EFFICACY OF TUMOR TREATING FIELDS AND/OR RADIOTHERAPY IN NON-SMALL CELL LUNG CANCER CELLS

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INTRODUCTION: Tumor Treating Fields (TTFields) have been shown to inhibit tumor growth both in vivo and in vitro in several types of tumors, including gliomas. Moreover, TTFields also augment the effect of chemotherapy (CX) and sensitize tumor cells to CX. Non-small cell lung cancer (NSCLC) is characterized by a CX and radiotherapy (RT) refractory phenotype. Interestingly, TTFields have shown promising effects when applied alone in NSCLC or in combination with pemetrexed. Here we compared the cytotoxic effect of TTFields with RT in a panel of NSCLC cell lines with various degrees of RT sensitivity. METHODS: Human NSCLC cell lines A549, H1299, and U-1810 were used. Cells (10,000) were seeded in a drop and the next day treated with TTFields, 2 V/cm, 150 kHz, continually for 72 h. Parallel samples were irradiated with ^{60}Co gamma-rays (0.2 keV/μm at 0.28 Gy/min) with 2 Gy or 8 Gy. At 72 h post-treatment, the effect on cell viability was assessed with XTT or MTT assays. RESULTS: When TTFields were applied continuously for 72 h, significant inhibition of cell proliferation was observed in the A549 cell line (∼50%). Inhibition of growth was also evident in the H1299 (∼30%) and U-1810 (∼10%) cell lines. In all three cell lines, results obtained for the TTFields were comparable with the effects of 8 Gy irradiation. Cells treated with TTFields showed many abnormal mitotic figures that could be related to the interference of TTFields with the mitotic spindle formation. Further analyses of molecular signaling mechanisms as well as RT combination effects are ongoing and will be presented. CONCLUSION: We show that TTFields induce significant growth inhibition in NSCLC cells, which is of the same magnitude as can be achieved with 8 Gy of conventional RT. Further analyses of TTFields and RT combination effects might reveal novel therapeutic strategies.

ET-31. MICROENVIRONMENT EFFECTS ON MICRORNA EXPRESSION TO REGULATE THE SURVIVAL OF METASTATIC BRAIN TUMORS

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Brain metastases are a frequent complication of cancer with up to 170,000 new cases diagnosed yearly in the United States. Despite recent advances in the development of diagnostics, radiosurgery, and molecularly targeted therapies, most deaths due to cancer result from the progressive growth of metastatic, drug-resistant lesions and brain metastases and serve as an important cause of morbidity and mortality. The complexity of the metastatic process indicates that it is tightly regulated at the genetic level through the coordinated activation and repression of multiple genes. The "seed and soil" hypothesis suggests that metastatic and primary tumor cells exploit the brain microenvironment for their growth and survival through unique interactions. We have developed an experimental model of the brain microenvironment using GFP$^+$immortalized murine astrocytes co-cultured with cells from various human solid tumor types. Astrocytes promoted the proliferation of tumor cells and fostered a pro-survival, chemoprotective effect on melanoma, breast, and lung tumor cells. The astrocyte effects were optimal after direct contact with tumor cells that permitted intercellular communication. The pro-survival effect conferred by astrocytes was also inhibited by the neuronal gap junction blocker carbenoxolone. To address the molecular basis of the effect of astrocytes, microRNAs (miRs) were isolated from tumor cells prior to and after co-culture. We detected statistically significant changes in the expression of individual miRs in tumor cells after astrocyte co-culture. Current studies seek to determine whether these same miRs are similarly differentially expressed in metastatic brain lesions. Individual miRs may serve as novel therapeutic candidates for the treatment of metastatic lesions. Ongoing studies seek to exploit chemically engineered oligonucleotides antagonistic to specific individual miRs as novel therapeutics for metastatic brain tumors.

ET-32. SENSITIVITY OF GLIOBLASTOMA NEUROSPHERES AND ORTHOTOPIC XENOGRAFTS TO CABOZANTINIB (XL184)

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Poor prognosis and high recurrence rate after therapy remain prevalent for patients diagnosed with glioblastoma (GBM). Cabozantinib (XL184, Exelixis) is a novel inhibitor of the MET and VEGFR2 pathways. Consequently, they impair tumor growth, angiogenesis, and metastasis. Cabozantinib is currently in clinical trials for several malignancies, including GBM. Neurosphere cultures enriched in cancer stem cells were obtained from freshly resected GBM tumors and profiled by Illumina BeadChip array (Human HT-12 v4). Neurosphere cultures were treated with cabozantinib in vitro under hypoxic and normoxic conditions as either a single agent or in combination with temozolomide. Two GBM neurosphere lines expressing variable levels of the Met receptor were implanted intracranially into female nude mice. Tumor growth was monitored weekly by noninvasive in vivo bioluminescence imaging (BLI). Cabozantinib was administered intragastrically once daily at doses of 30 and 60 mg/kg for 3 weeks. Control mice received vehicle alone. Response to treatment was monitored by BLI and survival time. Animals were killed at the onset of tumor burden, and brain tissue was alternately frozen for molecular analysis or formalin-fixed and paraffin-embedded for immunohistochemistry. Sensitivity to cabozantinib in vitro was not dependent on oxygen levels, and sensitization to temozolomide was variable. Cabozantinib monotherapy significantly impaired orthotopic GBM tumor growth, as assessed by in vivo BLI and survival. Our results indicate that high levels of Met receptor are not required for a significant antitumor activity. No toxicity was observed in the mice during treatments. We are currently investigating cabozantinib's effect on downstream signaling, angiogenesis, and potential for use in combination therapies for the treatment of GBM. Overall, cabozantinib showed promising antitumor activity as a monotherapy at a tolerable dose, indicating that this drug has potential as an adjunctive therapy for GBM.

ET-33. COLD ATMOSPHERIC PLASMA IN THE TREATMENT OF MALIGNANT GLIOMA

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INTRODUCTION: Cold atmospheric plasma (CAP), an ionized gas generated at close to room temperature, has recently been shown to selectively target cancer cells with minimal effects on normal cells in vitro and in vivo. Its mechanism of action is generation of reactive oxygen species with possible induction of the apoptosis pathway. We have developed a device to generate CAP and demonstrated positive results with various malignancies. A primary interest of the laboratory is in assessing CAP in the treatment of glioblastoma, a very aggressive and invasive primary brain malignancy that continues to have poor survival outcomes despite multimodal therapies. METHODS: We investigated the role of CAP in the treatment of glioma in vitro. Two glioma cell lines (U87 and U373) were grown to approximately 40% confluency and exposed to CAP for various time periods from 15 to 180 seconds. The effect of CAP on cell growth was assessed with microscopy and MTT assays. Treatment effects on the cell cycle were assessed by flow cytometry. RESULTS: Treatment with CAP resulted in a dose-dependent decrease in cell proliferation both in the U87 and U373 cell lines. Treatment with CAP for 120 seconds caused a greater than 50% reduction in cell viability after 24 hours. In addition, treatment with CAP for 180 seconds caused nearly 100% reduction in cell viability after 24 hours. Treatment with CAP for 90 and 120 seconds caused S-phase arrest and a 2-fold increase in the G2/M peak after 24 hours. CONCLUSIONS: Treatment of glioblastoma cells with CAP displays promising preliminary results in decreasing cell viability. Future experiments will look into assessing the effects of CAP on normal tissue, including HUVEC and NHA cells, as well as on primary glioblastoma cells. In addition, we will examine the mechanism of action of cell death.

ET-34. HIGHLY POTENT CYTOTOXIN TARGETING IL-13Rα2 IN CANINE AND HUMAN GLIOBLASTOMA

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Most preclinical in vivo studies on high-grade astrocytomas like glioblastoma (GBM) are primarily conducted in rodents or other small mammals that rarely develop these tumors spontaneously. More recently, canine primary brain tumors have started to be recognized as a faithful

representation of the human disease because they arise spontaneously and exhibit similar clinical and pathobiological characteristics. We have recently found that canine gliomas, much like their human counterparts, overexpress receptors for interleukin 13, including IL-13Ralpha2. The first generation of human (h) IL-13-based cytotoxin targeting IL-13Ralpha2 demonstrated efficacy in phase III clinical trials in patients with recurrent GBM. Therefore, we have generated a canine IL-13-based cytotoxin to target the receptor. We have cloned Canine IL-13 (CanIL-13) based on sequences obtained from Genebank. The cytokine was further cloned in-frame to the N-terminal end of a modified *Pseudomonas* exotoxin A (P38QQR) to generate a single-chain cytotoxin. Recombinant cytotoxins were expressed in *E. coli* and purified using fast protein liquid chromatography. Primary canine GBM cells were screened by flow cytometry using a canine/human bi-specific monoclonal antibody 1B10E9 to verify presence of the IL13Ralpha2. CanIL13-PE38QQR was tested on human and canine GBM cell lines to assess potency. CanIL-13 alone was able to induce proliferation of TF-1 cells, a human pre-myeloid B cell line expressing the other physiological receptor for IL-13, IL-13Ralpha1/IL-4Ralpha. CanIL13-PE38QQR effectively killed human and canine glioma cells overexpressing IL-13Rα2 with high specificity; however, the CanIL-13-based cytotoxin was more potent in killing canine cells than hIL13-PE38QQR. CanIL-13 was able to effectively block the killing by both hIL13-PE38QQR and CanIL13-PE38QQR in human and canine GBM cells. Thus, canine GBM cells in culture overexpress IL-13Ralpha2 that can be targeted specifically by recombinant cytotoxins. CanIL13-PE38QQR represents a new translational tool in evaluating cytotoxins for the treatment of gliomas.

ET-35. TARGETING CYTOMEGALOVIRUS ANTIGENS FOR GLIOBLASTOMA IMMUNOTHERAPY

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Despite aggressive and incapacitating conventional therapy, glioblastoma multiforme (GBM) remains uniformly lethal. Immunotherapy in which the immune system (particularly cellular effectors such as T cells) is harnessed to specifically attack malignant cells offers a treatment option with less toxicity. The presence of cytomegalovirus (CMV) antigens in GBM, and not in normal brain tissue, presents a unique opportunity to target these viral proteins for tumor immunotherapy. Although many groups, including ours, have demonstrated the presence of CMV proteins in GBM, their relevance as immunological targets in GBM has yet to be established. The hypothesis of this study is that CMV proteins in GBM are relevant tumor antigens on the basis of their immunogenicity and restricted expression within GBM, and immunological targeting of CMV will result in an anti-GBM immune response. The objective of this study is to demonstrate that CMV pp65-specific T cells target and eliminate GBM tumor cells in an antigen-specific manner. To achieve this objective, we first demonstrate the ability to generate CMV pp65-specific immune responses in vitro using peripheral blood mononuclear cells (PBMCs) from GBM patients, indicated by the increase in CMV-specific tetramer-positive cells and interferon-gamma production. Primary GBM specimens were shown to express early and late CMV gene products (IE1, pp65, and gB) by Western blot analysis. Importantly, CMV pp65-specific T cells generated by in vitro stimulation of patient T cells with dendritic cells transfected with CMV pp65 mRNA specifically lyse autologous primary GBM tumor cells. Moreover, tumor-specific T cells, generated by in vitro stimulation with dendritic cells transfected with total tumor RNA derived from GBM specimens, stimulate CMV pp65-specific T cells, as evidenced by an increase in CMV pp65-specific tetramer positive cells. These data collectively demonstrate that CMV proteins can be targeted for GBM immunotherapy and CMV antigen-specific responses are effective at recognizing and lysing autologous GBM tumor cells.

ET-36. EXTENDED SURVIVAL OF GLIOMA-BEARING MICE USING PHARMACOLOGICAL INHIBITION OF MICROGLIA

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High-grade astrocytomas are characterized by diffuse infiltration of tumor cells in the normal parenchyma and resistance to radio- and chemotherapy. There is mounting evidence that microglia, specialized brain-resident macrophages, play a significant role in the development and progression of these gliomas. We have established a microglia-glioma cell co-culture system consisting of primary microglia from C57Bl/6J mice and syngeneic GL261 glioma cells. We have demonstrated that microglia strongly stimulate the invasive behavior of GL261 cells in this system. The microglia also significantly stimulate GL261 cell proliferation and resistance to ionizing radiation (IR) in these conditions. In a search for small molecule inhibitors that block the stimulatory effects of microglia on glioma cells, we found that semapimod strongly inhibits microglia-stimulated GL261 invasion and radioresistance. Semapimod (CNI-1493) is an investigational drug that is known to selectively interfere with macrophage function. We also examined the effect of intracranially-administered semapimod on orthotopic GL261 tumors in the presence or absence of whole brain irradiation. We found that in the absence of IR, treatment with semapimod only marginally increases animal survival, although it strongly inhibits glioma invasiveness. These observations strongly suggest that, at least in these settings, inhibiting glioma invasion on its own has marginal therapeutic benefit. However, although IR treatment (10 Gy total fractionated in 5 doses over 10 days) increases animal survival by 10 days, IR in combination with semapimod treatment extends survival by at least 40 days. In conclusion, our observations indicate that pharmacological targeting of microglia may be beneficial as adjuvant therapy for high-grade glioma in conjunction with IR.

ET-37. VAL-083, A NOVEL N7 ALKYLATING AGENT, SURPASSES TEMOZOLOMIDE ACTIVITY AND INHIBITS CANCER STEM CELLS, PROVIDING A NEW POTENTIAL TREATMENT OPTION FOR GLIOBLASTOMA MULTIFORME

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Glioblastoma (GBM) remains one of the most difficult tumors to treat in part because many new agents fail to cross the blood brain barrier (BBB), and to the disease also possesses intrinsic drug resistance. Temozolomide (TMZ) is a front-line therapy for the treatment of GBM; however, it is often ineffective because of drug inactivation by O6-methylguanine-DNA methyltransferase (MGMT). Cancer stem cells (CSC) are a subpopulation of tumor cells that resist therapy and give rise to relapse. Here we describe VAL-083, a novel alkylating agent that creates N7 methylation on DNA and was initially intriguing because it crosses the BBB. We addressed how it compared to TMZ, whether it could be used to overcome MGMT-driven drug resistance, and if it has activity against CSCs. Addressing these questions provided further preclinical support for VAL-083, which is currently undergoing human clinical trials in the United States against refractory GBM. VAL-083 inhibited U251 and SF188 cell growth in monolayer and as neurospheres better then TMZ and caused apoptosis after 72 h. In a 10-day colony formation assay, VAL-083 (5 uM) suppressed SF188 growth by ~95%. T98G cells are classically TMZ-resistant and express MGMT, but VAL-083 inhibited their growth in monolayer after 72 h in a dose-dependent manner (IC50 <5 uM). VAL-083 also inhibited the growth of CSCs (BT74, GBM4, and GBM8) by 80-100% in neurosphere self-renewal assays. Conversely, there was minimal effect on normal human neural stem cells. In summary, VAL-083 has better in vitro efficacy than TMZ against brain tumor cells, can overcome resistance associated with MGMT, and targets brain tumor CSCs, demonstrating that it has the potential to surpass the standard-of-care.

ET-38. RATIONAL STRUCTURE-BASED DESIGN AND EVALUATION OF NOVEL STAT3 INHIBITORS THAT TARGET MALIGNANT BRAIN TUMORS

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Aberrant unregulated activation of signal transducer and activator of transcription 3 (STAT3) has been observed in many human cancers with roles in tumor initiation, progression, drug resistance, angiogenesis, and immunosuppression. Inhibiting STAT3 is a promising target-specific chemotherapeutic strategy. We used a rational structure-based approach that exploits the central pyrazole core of celecoxib to develop a series of monovalent and bivalent compounds that directly target STAT3's active site. Pyrazole-based inhibitors (MayoNeuroSurgery1 series; MNS1) potently block IL-6-induced STAT3 phosphorylation (at tyrosine 705) in glioblastoma cells with half-maximal inhibitory concentrations (IC_{50}) values ranging between 207 nM and 50 μM and downregulate activation of downstream targets of STAT3 transcription. They inhibit tumor cell proliferation and are potent inhibitors of cell viability in glioblastoma (BT-114), medulloblastoma (DAOY), and human colorectal carcinoma (HCT-116) cell lines in a dose-dependent fashion with IC_{50} values ranging between 12.8 μM and 62.4 μM.

Furthermore, inhibitory effects on proliferation were increased with the addition of temozolomide ($\geq$ 100 μM, $p = 0.001$) in a glioblastoma cell line. Despite similar effects on STAT3 phosphorylation and cell viability, we found that a monovalent ligand blocked nuclear translocation of STAT3, whereas a bivalent ligand induced nuclear translocation, offering unique insight into mechanisms of STAT3 signaling and inhibition. Based on these findings, our pyrazole-based inhibitors are suitable lead compounds for targeting cancer cells using STAT3 signaling for tumorigenesis. The therapeutic potential of these compounds is enhanced because the central pyrazole structure is based on a FDA approved drug and is likely to retain similar pharmacokinetic and toxicity properties. This is one of the first reports of drug discovery in which a known inhibitor of one pathway has been rationally modified to target a novel pathway.

ET-39. SUBGROUP TARGETED THERAPY FOR C-MYC-OVEREXPRESSING MEDULLOBLASTOMA WITH AURORA KINASE B/C INHIBITOR AZD1152-HQPA

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INTRODUCTION: In animal models of B-cell lymphoma, cells overexpressing c-Myc are sensitized to the growth inhibiting effects of Aurora B/C inhibitor AZD1152-HQPA. We hypothesized that medulloblastoma cells expressing high levels of c-Myc would be sensitive to Aurora B/C inhibition. METHODS: Cell counting was used to assess cell viability in two different medulloblastoma cell lines that stably overexpress c-Myc (UW426myc and UW228myc) in comparison to their isogenic controls. Western blot for PARP cleavage as a marker of apoptosis was performed. Cell cycle status was determined by FACS analysis. Flank xenografts of UW426myc cells were generated in nude mice and treated with AZD1152-HQPA administered subcutaneously at a dose of 25 mg/kg/day over 4 days beginning on day 24 after cell inoculation in mice randomized to drug ($n = 9$) or vehicle ($n = 9$). RESULTS: AZD1152-HQPA at 100 nM blocked proliferation in c-Myc and control cell lines but induced apoptosis preferentially in the c-Myc overexpressing cells after a period of 48 hours. A marked reduction in cell viability of 71% and 93% was observed in UW426myc and UW228myc cells, respectively, over a period of 96 hours when compared to isogenic controls. Although c-Myc-overexpressing cells demonstrated faster endoreplication than their isogenic control counterparts, abatement of DNA replication with aphidicolin did not protect these cells from apoptosis. This implicates a non-endoreplication-dependent mechanism in the observed response to Aurora B/C inhibition. UW426myc flank tumor volume was 43% lower in the drug- versus vehicle-treated animals one day after completion of treatment with AZD1152-HQPA. Multinucleated cells, pyknotic nuclei, and disorganization of cellular arrangement were observed on hematoxylin and eosin staining. CONCLUSION: Aurora kinase B/C inhibition induces apoptosis and impairs tumor growth of medulloblastoma cells expressing high levels of c-Myc. This suggests the potential for use of Aurora B/C inhibitors as subgroup-specific chemotherapeutic agents in patients with group 3 medulloblastoma.

ET-40. A HIGH NOTCH PATHWAY ACTIVATION PREDICTS RESPONSE TO GAMMA-SECRETASE INHIBITORS IN GLIOMA STEM CELLS

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The Cancer Genome Atlas Network (TCGA) described genomic abnormalities and gene expression-based molecular subtypes of glioblastoma (GBM) that showed a strong relationship between subtypes, genomic alterations, and different neural lineages. Future studies are required to elucidate the intricate relationship between tumor subtypes and treatment response. We have thus embarked on a comprehensive effort to detect signaling profiles that are associated with response to the therapy, and these profiles may allow prospective selection of patients with high likelihood of responding to therapy. Notch signaling pathway is an evolutionarily conserved pathway that plays an important role in multiple cellular and developmental processes. Recent studies indicate that the Notch signaling pathway regulates glioma stem cell (GSC) maintenance. We investigated the effects of Notch pathway inhibition in GSCs using gamma-secretase inhibitors (GSI). Drug cytotoxicity tests on 16 GSCs showed differential growth response to GSI, stratifying GSCs into two groups: responders (6 cell lines) and non-responders (10 cell lines). Active Notch signaling seems to be an important feature for GSC because Notch inhibition only affected GSCs defined as having increased endogenous Notch activity. Treatment with GSI reduced neurosphere formation in vitro accompanied with attenuated NICD, Hes-1, Hes-3, and Hes-5. The expression of nestin, a stem cell marker, was reduced, and an increased expression of lineage differentiation markers TuJ1 and GFAP was observed. In addition, our data also showed that PTEN is an important mediator of GSI-induced attenuation of stem cell maintenance, suggesting that there might be a link between the regulatory circuitry of Notch signaling and the PTEN/PI3K/Akt pathway. In conclusion, we have identified a responder group of GSCs with Notch pathway activation that can be targeted by gamma-secretase inhibitors.

ET-41. A NOVEL HSP90 INHIBITOR, NVP-HSP990, TARGETS CELL CYCLE REGULATORS TO PRODUCE AN ANTI-GLIOMA EFFECT IN OLIG2-EXPRESSING GLIOMA STEM CELLS

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The genetic heterogeneity and signaling alterations in glioblastoma multiforme (GBM) decrease the effectiveness of single-agent therapies. Heat shock protein 90 (HSP90) is a key molecular chaperone that modulates a group of proteins, many of which are key deregulated signals in glioma cells. The ability to target multiple proteins by inhibiting HSP90 is therefore an appealing therapeutic objective for GBM. Previous work identifying glioma stem cells (GSCs) and demonstrating the initiation of gliomas by GSCs in animals offered hope for the development of anti-glioma therapeutics. Our study evaluated a HSP90 inhibitor, NVP-HSP990 (Novartis), in a panel of GSC lines. NVP-HSP990 treatment resulted in a dose-dependent inhibition of GSC growth with IC_{50} values in the low nanomolar range. Our results distinguished between two subgroups of GSCs, revealing a subset of cells expressing Olig2 that were more sensitive to NVP-HSP990. Moreover, NVP-HSP990 markedly impaired GSC maintenance and triggered neuronal differentiation, as demonstrated by the expression of neuronal markers TuJ1 and NeuN in responder GSC lines. NVP-HSP990 also disrupted the cell cycle control mechanism by decreasing CDK2 and CDK4 and induced apoptosis-related molecules. Similar to the in vitro activity of the compound, our in vivo study of an intracranial model of GSCs showed prolonged median survival times in treated cohorts. Therefore, our findings suggest that GBM with high Olig2 expression might be more susceptible to NVP-HSP990 treatment, and HSP90 signaling in GBM warrants further investigations.

ET-42. USING CHRONOTHERAPY TO OPTIMIZE TREATMENT OF GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor. This aggressive glioma responds poorly to current therapy. Treatment with chemotherapy at a specific time of day, based on endogenous physiological rhythms, has helped to improve the efficacy and tolerability of chemotherapy for other cancer types. To date, this treatment method, known as chronotherapy, has not been evaluated for the treatment of brain tumors. Current therapy for GBM includes the DNA alkylator temozolomide (TMZ). Astrocytes are known to exhibit daily rhythms in gene expression and function in vitro and in vivo. In addition, recent studies have shown that components of the intracellular clock (core clock genes) interact with and enhance function of the DNA damage-response signaling pathway. Because variation in DNA repair capacity is known to modulate response to TMZ, we hypothesized that the response of astrocytomas to TMZ would vary with time of day. To address this hypothesis, we first examined circadian rhythm in U87 cells engineered to express firefly luciferase driven by either the mouse Bmal 1 or Per2 promoter. We measured the amplitude and period of time of day-dependent variation in bioluminescence and found that U87 cells exhibit intrinsic rhythm in clock gene expression. To test our hypothesis in vivo, we established intracranial xenografts of luciferase-expressing U87 cells and evaluated the effect of daily treatment in the morning versus daily treatment at night. The effect that time of administration had on TMZ effects was measured by bioluminescence imaging and survival in these two cohorts of mice. This work was supported by NIMH grant 63104.

ET-43. TARGETING VASCULOGENESIS: A NEW PARADIGM FOR THE TREATMENT OF BRAIN TUMORS

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With current treatment of glioblastomas (GBM), 85% of the tumors recur within the field of high-dose radiation. This tumor recurrence requires

formation of new blood vessels. I have developed a new therapeutic paradigm based on the dual origin of tumor blood vessels: angiogenesis, the sprouting of endothelial cells from nearby blood vessels, and vasculogenesis, the formation of blood vessels from circulating cells. Because tumor irradiation abrogates local angiogenesis, the tumor must rely on the vasculogenesis pathway for regrowth after irradiation. This vasculogenesis pathway depends on the action of the hypoxia-inducible factor (HIF)-1 target gene stromal derived factor 1 (SDF-1), the ligand for the CXCR4 and CXCR7 receptors. I have shown with multiple GBM systems in mice and rats that this pathway can be blocked using small molecule inhibitors of the ligand or its two receptors. Doing so prevents or markedly delays the recurrence of the tumors. One of the models I have used involves administration of a single dose of the carcinogen ENU to pregnant rats, which reliably causes the pups to develop brain tumors, similar to those seen in patients, from approximately 80 days of birth. I will present data on the effect of combining the SDF-1 inhibitor NOX-A12, an L-enantiomeric RNA oligonucleotide (NOXXON Pharma), with brain irradiation. Addition of this drug after irradiation at doses and times that are equivalent to well-tolerated human doses and times and inhibit the action of SDF-1 dramatically improves tumor shrinkage compared to irradiation alone and prolongs the lifespan of the rats (median lifespans of 20 Gy alone and 20 Gy + NOX-A12 are 196 and 349 days, respectively [$p < 0.003$ versus radiation or controls alone]). I therefore believe that these encouraging data justify a human trial in first line glioblastoma patients.

ET-44. CONVECTION ENHANCED DELIVERY OF TOPOISOMERASE INHIBITORS IN A MURINE GLIOMA MODEL

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BACKGROUND: Intracerebral convection enhanced delivery (CED) of chemotherapeutics for treatment of glioblastoma (GBM) can overcome many of the limitations of systemic therapy, such as poor diffusion across the blood brain barrier and systemic toxicities. Appropriate chemotherapeutic targets must be identified to utilize CED. Topoisomerases are highly expressed in GBM, making them attractive candidates for targeted chemotherapy. The expression levels of the two types of topoisomerase, TOP1 and TOP2, vary significantly across different GBM subtypes, with proneural GBM showing the highest levels of TOP2, suggesting that proneural GBM may be particularly sensitive to inhibitors of TOP2. OBJECTIVE: To compare the therapeutic efficacy of CED of topotecan (TOP1 inhibitor) and etoposide (TOP2 inhibitor) in a murine model of proneural GBM. METHODS: A proneural GBM model was generated by injecting floxed Pten and p53 mice harboring a stop-floxed luciferase reporter with a PDGF-IRES-Cre retrovirus. Primary cell lines were cultured for cell cytotoxicity testing. Dose escalation toxicity studies of CED of etoposide or topotecan determined the maximal tolerated dose (MTD) in mice. In vivo effects of etoposide and topotecan were assessed after a 7 day CED treatment in tumor-cell injected mice using serial bioluminescent imaging to monitor tumor growth. RESULTS: Both etoposide and topotecan inhibited in vitro cell proliferation in a dose-dependent manner after 72 hours of exposure. Etoposide demonstrated greater antiproliferative effects than topotecan. In an ongoing study, 7-day CED of etoposide and topotecan at maximal doses both significantly reduced tumor growth rates, with etoposide exhibiting greater antitumor activity than topotecan. CONCLUSIONS: Topoisomerase inhibitors show significant antitumoral effects in a proneural GBM model. The apparent increased chemosensitivity of proneural glioma cells to etoposide may result from the high expression of TOP2 in these tumors. This study provides evidence supporting the clinical trial for CED of topoisomerase inhibitors in GBM patients.

ET-45. SYSTEMIC DELIVERY OF SAPOSIN C-DIOLEOYLPHOSPHATIDYLSERINE HAS ANTIANGIOGENIC AND ANTITUMOR EFFECTS AGAINST GLIOMA

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Saposin C-dioleoylphosphatidylserine (SapC-DOPS) nanovesicles are a nanotherapeutic that effectively targets and destroys cancer cells. Here we explore the systemic use of SapC-DOPS in several models of brain cancer, including glioblastoma multiforme (GBM), and the molecular mechanism behind its tumor-selective targeting specificity. Using two validated spontaneous brain tumor models we demonstrate the ability of SapC-DOPS to selectively and effectively cross the blood-brain tumor barrier to target brain tumors in vivo and reveal the targeting to be contingent on the exposure of the anionic phospholipid phosphatidylserine (PtdSer), a phospholipid normally sequestered on the inner leaflet of the cell membrane but frequently exposed on the cell surface of many cancer cells and tumor-associated vasculature. Increased surface expression of PtdSer was found to correlate with SapC-DOPS-induced killing efficacy ($P < 0.01$), and tumor targeting in vivo was inhibited by blocking PtdSer exposed on cells. Antiangiogenic effects were observed as treatment with SapC-DOPS significantly reduced the ability of endothelial cells to migrate in vitro ($P < 0.001$), eliminated the ability of endothelial cells to form tubes in Matrigel ($P < 0.01$), and inhibited vessel sprouting in an ex vivo rat aortic ring assay ($P < 0.001$). In vivo treatment of SapC-DOPS resulted in a significant reduction of CD31 positive microvessels throughout the tumor ($P < 0.001$). To elucidate the antitumor efficacy of SapC-DOPS, we compared survival of mice with intracranial tumors in two different tumor models, low PtdSer-exposing (U87ΔEGFR-Luc) and high PtdSer-exposing (X12) GBM cells. Although SapC-DOPS treatment resulted in a significant increase in survival in both models ($P < 0.0001$), it is interesting to note that mice implanted with PtdSer-low U87ΔEGFR-Luc tumors showed a 25% long-term survival rate compared to a 75% long-term survival rate in mice implanted with PtdSer-high X12 tumors. This study supports the further development of SapC-DOPS as a novel antitumor and antiangiogenic agent for brain tumors.

ET-46. HISTONE DEACETYLASE INHIBITOR SAHA DIFFERENTIALLY SENSITIZES GLIOBLASTOMA XENOGRAFTS TO TEMOZOLOMIDE AND POTENTIATES EVOLUTION OF O6-METHYLGUANINE-DNA METHYLTRANSFERASE-MEDIATED ACQUIRED RESISTANCE

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The benefit of temozolomide (TMZ) in glioblastoma (GBM) is limited by resistance. Because of the ongoing interest in testing inhibitors of histone deacetylases for therapy in GBM, we evaluated the benefit of a combined SAHA and TMZ regimen and whether SAHA influences the mechanism of resistance emergence in 3 GBM xenografts (GBM12, GBM22, and GBM14). Flank xenografts were treated with placebo, SAHA, or TMZ alone or in combination. In all 3 tumor lines, there was no difference in tumor growth between mice treated with placebo or SAHA, whereas TMZ and SAHA + TMZ significantly inhibited tumor growth compared to placebo. SAHA + TMZ significantly sensitized GBM14 to TMZ, whereas a slight sensitization was noted in GBM12 and no sensitization in GBM22. In GBM14, all sublines from TMZ therapy had elevated O6-methylguanine-DNA methyltransferase (MGMT) regardless of whether SAHA was used or not. In contrast, no MGMT elevation was observed in any of the resistant GBM22 sublines. In GBM12, despite no difference in treatment efficacy between TMZ and TMZ + SAHA ($p = 0.12$), SAHA co-treatment promoted MGMT upregulation in 5 of the 8 resistant TMZ + SAHA sublines, whereas none of the tumors treated with TMZ or SAHA alone expressed MGMT at recurrence. TMZ sensitivity was restored with O6-Benzylguanine co-treatment in the MGMT-expressing, TMZ-resistant GBM12 subline #5500 (relative neurosphere/well was 0.14 ± 0.02 as compared to 0.93 ± 0.01 for TMZ alone, $p < 0.01$), whereas no such effect was observed in the non-MGMT expressing sublines #0604 and #5485 (relative neurosphere/well was 0.88 ± 0.05 versus 0.84 ± 0.03 [$p > 0.05$] and 0.85 ± 0.01 versus 0.87 ± 0.03 [$p > 0.05$], respectively). Lastly, elevated acetylated H3K9 and increased recruitment of SP1, C-JUN, NF-kB, and p300 to the MGMT promoter were specifically observed in MGMT-expressing, TMZ-resistant GBM12 sublines. Thus, HDAC inhibitor therapy may sensitize cells to TMZ and shift the evolution of resistance mechanisms favoring MGMT upregulation in GBM.

ET-47. A MULTI-TARGETED KINASE INHIBITOR (HCI-2084) POTENTLY INHIBITS THE AURORA, AXL, AND STAT3 PATHWAYS AND CAUSES CELL CYCLE ARREST AND APOPTOSIS IN GBM STEM-LIKE CELLS

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Activation of the Aurora and Axl kinase signaling pathways is associated with poor prognosis and treatment resistance in GBM. In addition, STAT3 has been identified by our group as a key transcriptional regulator of the mesenchymal subtype in GBM and a potential therapeutic target in GBM stem cells (GSCs). HCI-2084 is a novel small molecule inhibitor initially designed

to target the Gas6/Axl pathway. Kinase profiling demonstrated that HCI-2084 is also a potent inhibitor of the Aurora and JAK family of kinases. On the basis of this promising therapeutic profile, we set out to test the activity of HCI-2084 in GBM models utilizing both GSCs isolated from individual human tumors (GSC-20 and GSC-23) and traditional glioma cell lines (U87, U251, and T98G). Using Mesoscale assays, we found a potent and concentration-dependent inhibition of phospho-Aurora A (EC50: 3-82 nM), phospho-histone H3 (EC50: 19-70 nM), phospho-STAT-3 (EC50: 181-544 nM), and phospho-Akt (EC50: 8-64 nM) across the GSCs and GBM lines. These signaling effects were associated with a dose-dependent decrease in cell viability by HCI-2084 treatment. GSC-23 was most sensitive, with an IC50 of 56 nM; U87, T98G, and GSC-20 were intermediate in sensitivity, with IC50 values of 323, 215, and 312 nM, respectively; and U251 cells were most resistant, with an IC50 of approximately 2 μM. Aurora kinases are known to be strong regulators of mitotic progression, and we previously demonstrated that STAT3 inhibition in GSCs is associated with cell cycle arrest. Consistent with these observations, we found that treatment with HCI-2084 resulted in a dose-dependent G2/M arrest. HCI-2084 also resulted in a dose-dependent increase in caspase 3/7, 8, and 9 activation, indicating that decreased cell viability was due at least in part to apoptosis. Together, these data indicate the potential of HCI-2084 as a novel and potent therapeutic agent against GBM and GSCs.

ET-48. TEMOZOLOMIDE-PERILLYL ALCOHOL CONJUGATE: EFFECTIVE TREATMENT FOR TEMOZOLOMIDE-RESISTANT MALIGNANT GLIOMAS

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Glioblastoma multiforme (GBM) is a highly invasive vascular tumor that is usually fatal within two years. Standard of care therapy for GBM consists of surgery, radiation, and chemotherapy with temozolomide (TMZ). Tumors generally become resistant to TMZ, resulting in tumor recurrence and patient death. Perillyl alcohol (POH) is a naturally occurring monoterpene that has been used orally for the treatment of a variety of systemic cancers, including breast, pancreas, and lung carcinomas. We have recently constructed a new chemical entity by conjugating temozolomide to perillyl alcohol via a carbamate bond. This new compound has been named TMZ-POH (NEO212). The studies presented here demonstrated that TMZ-POH is cytotoxic to four TMZ- resistant glioma cell lines at doses that range from 10- to 20-fold lower than either agent alone or the mixture of both agents. Furthermore, the cytotoxic effects of TMZ-POH are independent of O6-methylguanine-DNA methyltransferase (MGMT) expression. TMZ-POH does not appear to affect normal astrocytes or normal brain endothelial cells. Several mechanisms of TMZ-POH-mediated cytotoxicity have been identified, including the induction of ER stress and DNA alkylation.

ET-49. ROLE OF HEMATOPOIETIC STEM CELLS IN ENHANCING THE ANTI-TUMOR EFFICACY OF ADOPTIVE CELLULAR THERAPY

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INTRODUCTION: Adoptive cellular therapy following non-myeloablative (NMA) and myeloablative (MA) host conditioning regimens has emerged as a remarkably effective treatment modality for refractory metastatic melanoma, leading to objective clinical responses in >40% metastatic lesions within the central nervous system. However, the application of adoptive cellular therapy for other cancers has been limited by the lack of a readily expandable population of polyclonal tumor-specific lymphocytes for use in cellular therapy protocols. METHODS: We have pioneered a novel platform of adoptive T cell transfer employing the use of total tumor RNA-pulsed dendritic cells to reliably expand CD4+ and CD8+ tumor-reactive T lymphocytes in vitro. Primary dendritic cells derived from murine bone marrow were pulsed with total RNA derived from a syngeneic high-grade astrocytoma (KR158B-luc) and used to generate tumor-reactive T lymphocytes (TTRNA-T cells) ex vivo. TTRNA-T cells were adoptively transferred into intracranial tumor-bearing mice following MA or NMA host conditioning. This was followed by hematopoietic stem cell (HSC) co-transfer and intradermal TTRNA-DC vaccination. RESULTS: In a highly invasive, temozolomide and radiation resistant murine astrocytoma model, adoptive cellular therapy using TTRNA-T cells and HSC co-transfer resulted in significant prolongation of median survival and long-term cures of established tumors in animals receiving MA conditioning and HSC transfer. These studies revealed greater T cell infiltration of intracranial tumors in mice receiving MA and HSCs as well as long-term T cell persistence within the tumor microenvironment. Co-localization and persistence of tumor-reactive TTRNA-T cells and HSCs within the tumor microenvironment were consistently observed up to 68 days post-transfer. In vitro transwell migration assays demonstrated that T cell migration to tumor cells is significantly enhanced by HSCs. CONCLUSION: Interactions between HSCs and T cells in solid cancers has not been previously characterized, but we have demonstrated that T cell-HSC interactions enhance the significant anti-tumor efficacy of adoptively transferred TTRNA-T cells.

ET-50. METABOLIC ALTERATION AS AN ADJUVANT THERAPY FOR THE TREATMENT OF MALIGNANT GLIOMA: EFFECTS ON TUMOR EDEMA

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The ketogenic diet (KD) is a therapeutic high-fat, low-carbohydrate diet that alters metabolism by increasing the level of ketone bodies in the blood. We have used a bioluminescent intracranial mouse model of malignant glioma to demonstrate that a rodent ketogenic diet given ad libitum increases blood ketones without altering animal weight or overall blood glucose levels. Animals maintained on the KD have extended survival following tumor implantation, and the KD potentiates the therapeutic effects of radiation and chemotherapy. We now report that animals fed the KD had statistically significantly larger tumors just prior to death (determined by bioluminescence) then did animals fed a standard diet (SD). We hypothesize that ketosis, which is known to have a neuroprotective effect in various diseases, may reduce peritumoral edema and/or inflammation. Cyclooxygenase 2 (COX2) is a known mediator of inflammation, and we have shown that animals maintained on an SD have a significant increase in the expression of COX2 in their tumor, whereas animals maintained on the KD showed no such increase. In addition to rodent KD, we have studied the anti-brain tumor effects of KetoCal (a nutritionally complete ketogenic diet used for the treatment of refractory pediatric epilepsy). KetoCal also extended survival, potentiated the anti-tumor effects of radiation and chemotherapy, and animals fed KetoCal alone had larger tumors when they succumbed to their disease than did animals on an SD. These animals also showed changes in gene expression that could lead to reduced edema. Taken together, our data demonstrate that metabolic alteration not only affects tumor bioenergetics but also alters the expression of genes involved in other aspects of tumor growth and response to therapy. An understanding of the global effects of metabolic alteration on tumors is likely to provide additional support for the KD's use as an adjuvant therapy for the treatment of brain tumors and other cancers.

ET-51. EFFICACY OF A BRAIN-PENETRANT PI3K/MTOR INHIBITOR IN ORTHOTOPIC MODELS OF GLIOBLASTOMA

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Glioblastoma (GBM), the most common primary brain tumor in adults, presents a high frequency of alteration in the PI3K pathway. GNE-317, a dual PI3K/mTOR inhibitor optimized to cross the blood-brain barrier (BBB), was characterized for delivery and PI3K pathway modulation in the brain and examined for efficacy in orthotopic xenograft models of GBM. Consistent with the goal to develop a molecule able to evade the action of key brain efflux transporters, studies in transfected MDCK cells overexpressing P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP) demonstrated that GNE-317 was not a substrate of either of these transporters. Following oral administration to mice, GNE-317 readily achieved concentrations in the brain closely approximating those in plasma (brain/plasma ratios close to 1) from 1 to 6 hours after dosing. GNE-317 markedly inhibited the PI3K pathway in normal mouse brain, causing 40%-70% suppression of pAkt and pS6 signals up to 6 h after dosing. Previous studies with PI3K or dual PI3K/mTor inhibitors that are substrates of brain efflux transporters demonstrated efficacy in a model with a compromised BBB (U87) but not in a model lacking contrast enhancement (GS-2). In contrast to the restricted activity of these brain-impenetrant inhibitors, GNE-317 was found to exert tumor growth inhibition in both U87 and GS-2 models. These results

indicate that specific optimization of PI3K inhibitors to cross the BBB leads to potent suppression of the PI3K pathway in healthy brain and efficacy in contrast-enhancing and non-enhancing orthotopic GBM models. The efficacy of GNE-317 in two intracranial models of GBM suggests that this compound could be effective in the treatment of GBM.

ET-52. IDENTIFYING NOVEL PATHWAYS INVOLVED IN REGULATING BONE INVASION IN MENINGIOMAS

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INTRODUCTION: Although meningiomas are considered to be benign tumors, a subset invade bone, causing hyperostosis and invasion of adjacent neural and soft tissue elements. Bone-invading meningiomas represent a significant clinical challenge because complete surgical resection is often impossible, resulting in higher recurrence rates and repeat surgery. The aim of this study was to identify differentially expressed genes and proteins involved in bone invasion with the ultimate goal to establish potential novel therapeutic strategies. METHODS: We defined two distinct bone-invading meningiomas and their control counterparts: 1) spheno-orbital meningiomas and control sphenoid wing meningiomas and 2) transbasal meningiomas and control anterior skull base meningiomas. From our database, we selected 75 patients in these two categories. RNA was extracted from paraffin-embedded tissue sections and used for an Illumina whole-genome DASL assay. Data were analyzed using multi expression viewer software. Array data were verified using quantitative real-time PCR (RT-qPCR). We also performed tissue microarray (TMA) on archived paraffin sections of these tumors for immunohistochemical analysis. Both in vitro and in vivo functional studies were carried out using meningioma cell lines (IOMMA-Lee, CH157-MN, and F5 cells), focusing specifically on the functional relevance of MMP16 in meningioma bone invasion. Small animal MRI was used to study the pattern and behavior of tumor growth. RESULTS: RNA microarray data analysis identified 222 differentially expressed genes. We selected the most relevant and highly overexpressed genes to study: PDGFRα, MMP16, and MMP19. RT-qPCR confirmed upregulation of these genes. In vitro and in vivo studies demonstrated that MMP16 increased invasion, migration, and brain invasive properties of meningioma cells lines. Ingenuity pathway analysis implicated MMP2 and MMP9, which we confirmed to be upregulated by zymography. CONCLUSIONS: We have demonstrated that MMP16 plays a significant role in modulating bone invasion of meningiomas and can potentially provide a new target to pursue for therapeutic potential.

ET-53. AN NF-κB-DEPENDENT GENE-SET IDENTIFIES THE LNCRNA MALAT1 AS A NOVEL TARGET IN THE TREATMENT OF MALIGNANT GLIOMA

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Temozolomide (TMZ) has become a standard agent in the management of malignant glioma. Although newer targeted agents have been developed, they have not shown significant improvement over TMZ. Nevertheless, targeted therapies offer great potential if used in the appropriate manner. It was recently shown that the p50 subunit of NF-kappaB is necessary for cytotoxicity by TMZ. In the current study, we set out to rationally identify targets that can enhance the therapeutic index of TMZ. Initially, using an orthotopic glioma model, we found that loss of p50 renders animals resistant to TMZ. Subsequently, an unbiased analysis of gene expression was performed following treatment with TMZ in wild-type and p50-deficient gliomas. Ten transcripts were found to be significantly (false discovery rate < 0.01) altered in wild-type gliomas only. The change in expression was confirmed by qPCR. The 10-gene set was found to separate patients into those with good and poor prognosis in 3 of 5 glioma expression databases with analysis performed using the Cox proportional hazards model. When the expression of each gene was analyzed in the Rembrandt database, only the long noncoding RNA MALAT1 was found to separate patients into good and poor prognosis groups, with poor responders having higher expression levels. This finding was validated in the TCGA database. The time course of MALAT1 expression was examined, and expression in response to TMZ was shown to be p50- and p53-co-dependent. Critically, depletion of MALAT1 significantly enhances the anti-glioma effect of TMZ. The oral bioavailability, favorable toxicity, and clinical efficacy of TMZ suggest that it will remain central to the treatment of glioblastoma for the foreseeable future. However, it is likely that a multimodal therapeutic approach is necessary for successful management of this heterogeneous tumor. Rationally designed combinatorial strategies have the best potential for success, and in this regard, MALAT1 represents an ideal target for further study.

ET-54. BLOOD-BRAIN BARRIER DISRUPTION WITH FOCUSED ULTRASOUND ALLOWS BRAIN TUMOR THERAPY WITH TARGETED IMMUNE CELLS

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Natural killer (NK) cells are cytotoxic lymphocytes involved in innate immunity. The NK cell line NK-92 can be targeted to cancer epitopes and has demonstrated efficacy in treating solid malignancies, but NK-92 cells are ineffective in the treatment of brain tumors because they do not cross the blood-brain barrier (BBB). We investigated whether focused ultrasound (FUS) could be used to deliver targeted NK-92 cells to the brain using a model of metastatic breast cancer. HER2-expressing human breast tumor cells were implanted in the brain of nude rats. NK-92-scFv(FRP5)-zeta cells transduced to express a chimeric HER2 antigen receptor and transfected with iron nanoparticles were injected intravenously prior to and following BBB-disruption using FUS (558 kHz transducer, 1 Hz pulse repetition frequency, 10 ms pulses, and 120 s duration) in the presence of a microbubble ultrasound contrast agent. We obtained 7T MR images at 12-16 hours post-treatment and found a significant reduction in signal, indicating the presence of iron-loaded HER2-specific NK-92 cells in the tumor. This was confirmed with CD45 immunohistochemistry and Prussian blue histochemistry. The average ratio of NK-92 to tumor cells was 1:100 when NK-92 cells were present in the vasculature at the time of sonication, versus 1:500 and 1:1000 when delivered after sonication and without BBB-disruption, respectively ($p < 0.01$). Cytotoxicity assays revealed that a 1:100 NK-92:tumor cell ratio is sufficient for inducing effective tumor cell death. Furthermore, immunohistochemistry was used to detect apoptosis-initiating factors released from the NK-92 cells, suggesting that the cells retain their cytotoxic function following delivery into the brain. This study demonstrates that FUS can enable intravenous targeted immune cell therapy of brain tumors by allowing HER2 specific NK-92 cells to circumvent the BBB.

ET-55. INTRACRANIAL DELIVERY OF THE BISPECIFIC TARGETED TOXIN DTATEGF IN A MOUSE XENOGRAFT MODEL OF HUMAN METASTATIC NON-SMALL CELL LUNG CANCER

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PURPOSE: To investigate the anti-cancer effect of the bispecific diphtheria toxin (DT)-based immunotoxin DTATEGF, which targets both epidermal growth factor receptor (EGFR) and urokinase-type plasminogen activator receptor (uPAR) in vitro and in vivo when delivered by convection-enhanced delivery (CED) via an osmotic minipump in a human metastatic non-small cell lung cancer (NSCLC) brain tumor mouse xenograft model. METHOD: The MTT assay was used to test the inhibitory effects of DTATEGF, monospecific DTAT, DTEGF, and control DT on the proliferation of NSCLC PC9-BrM3 cells in vitro. An intracranial xenograft model of human metastatic NSCLC was established in nude mice using the NSCLC PC9-BrM3 cell line genetically marked with a firefly luciferase reporter gene. One μg of DTATEGF or control was delivered intracranially by CED via an osmotic minipump. Bioluminescent imaging (BLI) was performed at days 7, 14, 30, 60, and 90. Kaplan-Meier survival curves were generated. The brain tissue samples were stained by hematoxylin and eosin for histopathological assessment. RESULTS: In vitro, DTATEGF could selectively kill PC9-BrM3 cells and showed an IC50 less than 0.001 nM, representing a more than 100- to 1000-fold increase in activity compared to that of monospecific DTAT and DTEGF. In vivo, mice with tumors were treated intracranially with the drug via CED, and the results showed treatment was successful in providing a survival benefit, with the median survival of mice treated with DTATEGF being significantly prolonged relative to controls (87 days versus 63 days, $P = 0.006$). CONCLUSION: The results of these experiments indicate that DTATEGF kills the NSCLC PC9-BrM3 cell line in vitro, and when it is delivered via CED intracranially, it is highly effective against metastatic NSCLC brain tumors. DTATEGF is a safe and effective drug for which further preclinical and clinical development is warranted for the management of metastatic brain tumors.

ET-56. DEVELOPMENT OF AN EFFICIENT AND SAFE ONCOLYTIC HSV-1 VECTOR FOR GLIOBLASTOMA THERAPY

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For clinical use of oncolytic HSV-1 (oHSV1), safety and therapeutic efficacy are essential. We have been employing an engineered oHSV1 (rQNestin34.5) in which the nestin promoter, highly expressed in glioblastoma stem-like cells (GSCs), drives expression of the viral ICP34.5 gene to enhance viral replication in GSCs with less toxicity to normal human cells. The c-terminal moiety of the viral ICP34.5 gene is necessary to initiate protein translation through dephosphorylation of the translation initiation factor eIF2alpha during viral infection, but other moieties of ICP34.5 lead to neurovirulence in the brain because they bind to beclin-1, leading to autophagy. To circumvent the possibility of neurovirulence by low-level expression of ICP34.5, we have now engineered a novel oHSV1 vector, in which the nestin promoter drives the cellular GADD34 (NG34) or a truncated GADD34 gene (NG34C). The rationale for this is that the C terminus of GADD34 dephosphorylates eIF2alpha, like ICP34.5, but does not produce neurovirulence because it does not possess the beclin-1 binding moiety. We find that NG34 and NG34C are as effective as rQNestin34.5 against a panel of glioma cells. However, normal human primary cells did not support NG34C replication. NG34 and NG34C are expected to display less or no neurovirulence in mouse brains compared to rQNestin34.5. In summary, this newest generation of oHSV1s utilizes moieties from cellular genes that mimic those of viral genes required for efficient replication and lysis of gliomas without expressing viral gene moieties that are toxic to normal cells.

ET-57. GLYCOGEN SYNTHASE KINASE 3β INHIBITION SENSITIZES HUMAN GLIOMA CELLS TO TEMOZOLOMIDE BY MEANS OF C-MYC SIGNALING

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INTRODUCTION: Glycogen synthase kinase 3beta (GSK3beta) is a serine/threonine protein kinase involved in a range of cellular processes. We have previously demonstrated that GSK3beta inhibition sensitizes glioblastoma (GBM) cells to temozolomide (TMZ). In the current study, we have searched for molecular mechanisms of the synergistic effect of GSK3beta inhibition with TMZ against GBM, focusing on O6-methylguanine DNA methyltransferase (MGMT) gene silencing and its probable correlation with c-Myc signaling known to be upregulated by GSK3beta inhibition. METHODS: Human GBM cell lines T98G and U251 were treated with either a GSK3beta small molecule inhibitor, AR-A014418, at escalating concentrations or GSK3beta siRNA. The combined effect of TMZ and AR-A014418 on GBM cell proliferation was determined by the isobologram method. MGMT promoter methylation in the cells was measured by methylation-specific PCR (MSP) and methylight assay. The MGMT gene expression was evaluated by qRT-PCR. MGMT promoter binding by c-Myc and DNA (cytosine-5)-methyltransferase 3A (DNMT3A) was estimated by chromatin immunoprecipitation (ChIP) assay. RESULTS: Inhibition of GSK3beta resulted in decreased GBM cell viability, synergistic effects with TMZ in T98G cells, and downregulation of MGMT in T98G and U251 cells with relevant changes in methylation of the MGMT promoter in MSP and methylight assays. Moreover, we have shown for the first time c-Myc binding to the MGMT promoter with consequent recruitment of DNMT3A in T98G cells, which explains the changes in MGMT promoter methylation. CONCLUSION: Our data suggest that GSK3beta inhibition enhances the effect of TMZ by decreasing MGMT expression via its promoter methylation mediated by c-Myc activity. Provided data will facilitate developing combinations of GSK3beta inhibitors and alkylating agents for the treatment of patients with GBM.