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2013 WFNO-SNO Abstracts

EXPERIMENTAL THERAPEUTICS AND PHARMACOLOGY

ET-001. HIGH LEVELS OF TIMP-1 CAN REDUCE THE EFFECT OF TOPOISOMERASE INHIBITORS ON GLIOBLASTOMA CELLS

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The multifunctional protein - tissue inhibitor of metalloproteinases-1 (TIMP-1) - has been associated with poor prognosis in several types of cancers including glioblastomas. Glioblastomas are the most common and malignant primary brain tumor known for being highly invasive and resistant to therapy. New treatment strategies are continuously being explored and currently vascular endothelial growth factor (VEGF) inhibitors administered in combination with Irinotecan is the most promising second line treatment. TIMP-1 has been associated with decreased response to chemotherapy in breast and colorectal cancer and especially the family of topoisomerase (TOP) inhibitors, such as Irinotecan, has been suggested to be affected by TIMP-1. In the present study, we investigated whether a high TIMP-1 expression in glioblastoma cells played a role in TOP inhibitor resistance. We established two TIMP-1 over-expressing cell lines and evaluated the sensitivity towards the TOP1 inhibitor SN-38 and the TOP2 inhibitor Epirubicin using a viability and a cytotoxicity assay. In addition, we investigated the invasive features of the cells in a brain slice culture model as well as in an orthotopic xenograft model. The results showed that TIMP-1 over-expressing U87MG cell line sub-clones were significantly more resistant than the controls when exposed to SN-38 and Epirubicin. The same tendency was seen for the TIMP-1 over-expressing A172 sub-clones. No significant differences in invasion patterns were observed for TIMP-1 over-expressing sub-clones when compared to controls. In conclusion, the present study suggests that TIMP-1 over-expression reduces the effect of TOP inhibitors in the glioblastoma cell line U87MG. There was no significant effect of TIMP-1 over-expression on tumor cell invasion. The association found between TIMP-1 cellular levels and the effect of TOP inhibitors needs to be validated in clinical patient material.

ET-002. EFFECT OF IFN-ß AND LEVETIRACETAM ON RESISTANT GLIOMA CELLS TO TEMOZOLOMIDE

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Alkylating agents, such as temozolomide, are among the most effective cytotoxic agents used for malignant gliomas, but response rate remains not so high. The DNA repair protein O^6 -methylguanine-DNA methyltransferase (MGMT) plays an important role in cellular resistance to alkylating agents. IFN-ß and ntiepileptic drugs (AEDs), levetiracetam (LEV) have been reported as a drug sensitizer, enhancing toxicity against a variety of neoplasias, and is widely used in combination with other antitumor agents such as nitrosoureas. Here, we show that combination with IFN-ß and LEV sensitizes glioma cells that harbor the unmethylated MGMT promoter and are resistant to temozolomide. In vitro, when used at concentrations of IFN-ß and LEV within the human therapeutic range for glioma treatment and seizure prophylaxis, IFN-ß and LEV decreases MGMT protein and mRNA expression levels, and overcome drug resistance to temozolomide. Our results suggest that the combination with IFN-ß and LEV in patients with malignant gliomas may have an unrecognized impact in clinical practice and research trial design.

ET-003. THERAPEUTICALLY TARGETING ATRX DEFICIENCY IN LOWER-GRADE ASTROCYTOMA

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BACKGROUND: Lower-grade astrocytomas (LGAs) show protracted clinical courses, however, they eventually undergo malignant transformation into incurable secondary glioblastoma multiforme (GBM). IDH1 and IDH2 mutations have been detected in 70-90% of LGAs and secondary GBMs, but not primary GBMs. In addition, we have recently reported a high incidence (70%) of ATRX mutations in IDH1 mutant LGAs. ATRX mutations are known to induce G2/M checkpoint deficits and the accumulation of DNA double-stranded breaks (DSBs) along with more generalized genomic instability. Accordingly, we hypothesize ATRX-mutant cells may be preferentially sensitive to agents inducing DNA damage. Moreover, we expect that by facilitating DSBs, by direct induction and/or additional inhibition of the G2/M checkpoint (using PARP1 and Chk1 inhibitors), will promote cell death and/or senescence in ATRX-deficient cells. METHODS: For these studies, we employ established ALT-positive (IIICF/c, JFCF-6/T.1D and SaOS-2) and ALT-negative (U251) cell lines, along with isogenic astrocytes harboring the IDH1 R132H mutation in addition to either an ATRX-targeting or scrambled shRNA. In order to induce DNA DSBs we have used etoposide and assessed the number of dead cells staining with Annexin-FITC and DAPI followed by flow cytometry. RESULTS AND CONCLUSIONS: After treatment with etoposide, ALT cells lines showed significantly higher numbers of dead cells than ALT negative counterparts. Isogenic ATRX knockdown astrocyte cell lines also exhibit an enhanced cell death after treatment with etoposide; either alone or in combination with IDH mutation, the latter showing the highest degree of DSB sensitization. In order to test the effects of additional sensitization to DSBs we are performing inhibition assays for PARP1 (olaparib) and CHK1 (CHIR-124) either alone or in combination with etoposide treatment. We are also treating the cells with γ-radiation to further assess the effects of DSB induction by irradiation in the context of ATRX deficiency.

ET-004. THE ANTI-TUMOR EFFECT OF THE FIBRIN GLUE MIXED WITH TEMOZOLOMIDE AGAINST MALIGNANT GLIOMA, AN IN VIVO MODEL

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BACKGROUND: Temozolomide (TMZ) is the most common chemotherapeutic drug for glioblastoma (GBM) and malignant brain tumor. Previous studies showed that the treatment with TMZ induced autophagy, apoptosis and senescence in cancer cells. In this study, we focused on fibrin glue as drug delivery system (DDS) for administering a high concentration of TMZ in glioma cells. MATERIALS AND METHODS: We used 3 malignant glioma cell lines (U87MG, two established cell lines). These cell suspensions were injected into the back of nude mice to make the subcutaneous tumor. After a few days, the various condition fibrin glue sheets were placed in contact with subcutaneous tumor (4groups, n = 6/group). We measured the tumor size in the certain period and performed immunohistochemical staining to evaluate the effect of fibrin glue mixed TMZ (F.G-TMZ) against the subcutaneous tumor. Further, the purpose of investigating the effect on the normal brain, we placed F.G-TMZ on the surface of mice brain. After 48hours and 2weeks, brains were removed and the slices were immunohistochemically stained (H.E, GFAP, COX2, etc.) to evaluate the effect of fibrin glue mixed TMZ against normal brain. RESULTS: The observation periods of sucutaneous tumor were 26-28days. All mice didn't die and no significant adverse effects were observed during the period. In all cell lines, F.G-TMZ significantly suppressed the growth of subcutaneous tumor size than the other conditions. Immunohistochemical study showed that F.G-TMZ induced autophagy, apoptosis, senescence to the subcutaneous tumor. Further, severe inflammation, edema and demyelination caused by F.G-TMZ didn't occur in the normal mice brain at acute phase and chronic phase. CONCLUSIONS: F.G-TMZ would be a new tool of drug delivery system against malignant brain tumor.

ET-005. NOVEL EGFRvIII-SELECTIVE TRAIL FUSION PROTEIN FOR TREATMENT OF GLIOMA

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Glioblastoma multiforme is a devastating disease with very limited therapeutic options. Targeting of therapeutics specifically to tumor cells has been of long term interest. In this study we focused on the engineering of a recombinant therapeutic fusion protein specifically targeting a subset of aggressive brain tumors expressing mutated variant of the epidermal growth factor receptor, the EGFRvIII, and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Neural stem cells (NSCs) which possess inherent tropism to glioma were tested as delivery vehicles of therapeutic protein to glioma cells. The single-chain antibody against the EGFRvIII, scFvMR1, was genetically fused with soluble TRAIL through a flexible $(Gly_4Ser)_3$ linker (scFvMR1-sTRAIL). The recombinant scFvMR1-sTRAIL fusion was than expressed in CHO cells. The scFvMR1-sTRAIL fusion protein demonstrated specific interaction with EGFRvIII and TRAIL receptors in competitive assay using excess of soluble scFvMR1 and TRAIL neutralizing antibody. Additionally, the specificity of the therapeutic affect was shown by apoptosis assay in U87 glioma cells expressing wild type EGFR versus cells expressing EGFRvIII. The scFvMR1-sTRAIL suppressed Akt activity in U87-EGFRvIII glioma cells. To test the hypothesis if NSCs could serve as a delivery platform of therapeutic protein to glioma cells, lentivirus encoding for the scFvMR1-sTRAIL was generated and transduced in NSCs. Expression of the scFvMR1-TRAIL was confirmed by flow cytometry and in TRAIL ELISA. NSCs expressing the scFvMR1-sTRAIL but not control cells were toxic to EGFRvIII-expressing cells and to a lesser extent to cells expressing wild type EGFR, thus further confirming the specificity of interactions. Collectively, our study demonstrates that the scFvMR1-sTRAIL possesses a dual therapeutic modality. It specifically targets the EGFRvIII receptor, modulates downstream EGFRvIII signaling and initiates apoptosis in glioma cells through the interaction with TRAIL receptors. Studies investigating therapeutic effect of NSCs expressing scFvMr1-sTRAIL in animal models of intracranial glioma are warranted. This work was supported by Grant #205013 from the American Cancer Society, Illinois Division, Inc.

ET-006. CURAXIN (CBL0137) SIGNIFICANTLY INCREASES SURVIVAL IN ORTHOTOPIC MODELS OF GLIOBLASTOMA MULTIFORME ALONE AND IN COMBINATION WITH TEMOZOLOMIDE

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Curaxin (CBL0137) simultaneously activates the tumor suppressor p53 and inhibits cancer survival pathways, such as NF-κB and HSF-1. The effects of CBL0137 are mediated by inhibition of the transcription and replication factor FACT (FAcilitates Chromatin Transcription). FACT is expressed in undifferentiated progenitors and stem cells of adult tissues, but is almost undetectable in differentiated cells. It is expressed in many tumor types and is correlated with high grade and worse overall survival, making it an attractive target for therapy. We investigated the effects of CBL0137 on glioblastoma multiforme (GBM) using U87MG and A1207 orthotopic models of the disease. CBL0137 was administered either orally daily (25mg/kg) or intravenously (iv) every fourth day (35 and 70 mg/kg) or once per week (90mg/kg, A1207 only) beginning one day after implantation. In addition, CBL0137 administered iv was evaluated in combination with standard of care drug temozolomide (TMZ). In both models, intravenous CBL0137 was superior to oral administration. In the U87MG model, both CBL0137 (iv) and TMZ were efficacious and the combination of the two agents appeared more potent than CBL0137 at either monotherapy dose ($p < 0.001$ and $p < 0.05$, respectively). In the A1207 model, both CBL0137 iv regimens significantly increased median survival ($p < 0.001$), but temozolomide had no effect on survival as monotherapy and showed no synergy with CBL0137. In summary, these data demonstrate that CBL0137 administered intravenously is more effective than orally administered drug in both orthotopic glioblastoma models. In addition, CBL0137 given intravenously is synergistic with the standard of treatment drug temozolomide in the U87 model and efficacious against the A1207 model where the standard of care drug was ineffective. These data suggest that CBL0137 may provide clinical benefit in the treatment of glioblastoma.

ET-007. PD-0332991, A CDK4/6 INHIBITOR, SIGNIFICANTLY PROLONGS SURVIVAL IN A GENETICALLY ENGINEERED MOUSE MODEL OF BRAINSTEM GLIOMA

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Diffuse Intrinsic Pontine Glioma (DIPG) is an incurable tumor that arises in the brainstem of children. To date there is not a single approved drug to effectively treat these tumors and thus novel therapies are desperately needed. Recent studies suggest that a significant fraction of these tumors contain alterations in cell cycle regulatory genes including amplification of the D-type cyclins and CDK4/6, and less commonly, loss of Ink4a-ARF leading to aberrant cell proliferation. In this study, we evaluated the therapeutic approach of targeting the cyclin-CDK-Retinoblastoma (Rb) pathway in a genetically engineered PDGF-B-driven brainstem glioma (BSG) mouse model. We found that PD-0332991 (PD), a CDK4-6 inhibitor, induces cell-cycle arrest in our PDGF-B; Ink4a-ARF deficient model both *in vitro* and *in vivo*. By contrast, PDGF-B; p53 deficient model was mostly resistant to treatment with PD. We noted that a 7-day treatment course with PD significantly prolonged survival by 12% in the PDGF-B; Ink4a-ARF deficient BSG model. Furthermore, a single dose of 10 Gy radiation therapy (RT) followed by 7 days of treatment with PD increased the survival benefit to 19% in comparison to RT alone. These findings provide the rationale for evaluating PD in children with Ink4-ARF deficient gliomas.

ET-008. Bcl-2, pChk2, MGMT PROMOTER METHYLATION STATUS AND mRNA PATTERNS PREDICTCHEMO- AND RADIOSENSITIZATION EFFECTS OF HDAC INHIBITORS IN GBM CELL CULTURES

Lotte Berghauser Pont[1], Jenneke Kloezeman[1], Martin van den Bent[1], Roland Kanaar[1], Andreas Kremer[1], Sigrid Swagemakers[1], Pim French[1], Clemens Dirven[1], Martine Lamfers[1], and Sieger Leenstra[1]; [1]Erasmus MC, Rotterdam, The Netherlands; [2]Elisabeth Hospital, Tilburg, The Netherlands

HDAC inhibitors are potential chemo- and radiosensitizers for GBM treatment of which Vorinostat and Panobinostat are currently under Phase I/II and III evaluation. In this study, different relevant parameters considering the clinical applicability and response to these treatments were assessed. First, radiosensitizing and temozolomide-sensitizing effects were studied in vitro in a panel of 34 primary serum-free GBM cultures. Clinical relevant parameters under investigations were the optimal scheduling of RTx/HDACi treatment, effects of long-term treatment on glioma growth, combination treatment, and fractionized RTx. Additionally, biomarkers and molecular differences related to treatment response (ATP-based viability at day 8) were assessed, involving DNA damage repair, Bcl-2 pathways, apoptosis and autophagy. Vorinostat sensitized 60% of cultures to RTx and 70% to TMZ, for Panobinostat numbers were 47% and 80% respectively. Incubation with HDACi of one/two weeks/48h/24h pre-RTx resulted in significantly greater radiosensitization than given concomitantly with RTx or 24h post-RTx ($p < 0.05$). Long term treatment stagnated growth of the tumor cells. Fractionizing did not negatively influence the combination effects. Differences in a responder and non-responder to combined RTx/HDACi were found in H3-acetylation, γH2AX and 53BP1 foci, p21 and Bcl-2 levels, caspase-3/7 and autophagy responses. The responders had a normal pChek2 response to RTx alone, whereas the non-responders did not phosphorylate Chek2. The role of Bcl-2 and Chek2 were established by functional assays. Profiling based on mRNA expression as well as MGMT promoter methylation stratifies groups of GBM primary cultures that either respond or don't respond to treatment. HDAC inhibitors are effective radio- and chemosensitizers for GBM. This study reveals clinically relevant issues that could aid clinical studies to optimize this treatment with regard to timing, long term treatment and fractionizing of RTx dose, but also to selectively treat patients with either RTx/HDACi, HDACi alone or TMZ/HDACi, that are likely to respond to treatment.

ET-009. IDENTIFICATION OF POTENT ENHANCERS OF THE ONCOLYTIC ADENOVIRUS Delta24-RGD USING BOTH RATIONALE BASED COMBINATIONS AND THE NIH CLINICAL COMPOUND SCREEN

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Novel treatment strategies for the aggressive and lethal brain tumor glioblastoma (GBM) include oncolytic virotherapy. Currently, the oncolytic adenovirus delta24-RGD is under phase I clinical investigation in GBM. In vitro data on patient-derived glioma cultures show differential responses to the oncolytic effects of Delta24-RGD. Tumor characteristics such as integrin expression and alterations in various regulatory pathways may underlie these differential responses. Therefore, clinical compounds that sensitize cells to viral oncolysis may lead to effective treatment of all patients. We aimed to identify potent virosensitizers for Delta24-RGD in primary GBM cultures using both screening of an NIH compound library containing over 400 clinical compounds, and rationalized combination compounds based on mechanism of action (proteasome inhibition). Five different concentration ranges (1 μM–100 μM) of the library were combined with MOI 25 delta24-RGD on the (semi-) resistant GS79 and GS102 patient-derived serum-free GBM cultures. Viability was assessed five days after treatment by an ATP-based assay. Validation was performed by Chou-Talalay synergy quantification assays. The compound screen on these two cultures revealed eleven compounds as potential virosensitizers, whereas based on rationale, one compound was effective. Validation by the Chou-Talalay method confirmed 8 of the 12 to be synergistic or additive in combination with the virus. These included three experimental/phase I compounds, (two anti-neoplastic and an anti-inflammatory agent), a proton pump inhibitor, a platelet aggregation inhibitor, an antidepressant, an antipsychotic and a calcium-channel blocker. Further experiments to reveal the mechanism of combined action were performed, studying the viral infectivity, viral production, and viral oncolysis (autophagy and apoptosis) of the most effective or feasible compounds. In addition, in silico common pathway analysis of the different drugs was performed to gain insight into the combined mechanisms. The approach of compound screening was effective in identifying potent drugs that exert in enhancing oncolytic effects of Delta24RGD in primary GBM.

ET-010. VERY LOW-DOSE TEMOZOLOMIDE AND CISPLATIN TREATMENTS CAUSE TIME AND DOSE DEPENDENT DENDRITIC SPINE DAMAGE IN CULTURED HIPPOCAMPAL NEURONS

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OBJECTIVE: To determine if either the differentiated hippocampal neurons (HN) or the proliferating neural stem/progenitor cells (NSC) are more sensitive to cisplatin (CDDP) and temozolomide (TMZ) induced-injury. BACKGROUND: The cognitive effects of chemotherapy are rapidly emerging as a major neurological issue. DNA-targeted drugs such CDDP and TMZ achieve high concentrations in the brain, justifying their use in the treatment of primary and metastatic central nervous system tumors. We have recently shown in-vitro, that TMZ and CDDP are toxic to NSCs that are considered crucial for cognitive function in adult brain. DESIGN/METHODS: We treated rat HN and NSC cultures with very-low doses of CDDP and TMZ. Results were assessed in vitro by HN synaptic imaging using the psd95 marker and Sholl analysis of dendritic branching, and by measuring cell proliferation and apoptosis in NSCs. RESULTS: We show that in-vitro, CDDP treatments with very-low doses (0.05 and 0.1 mM) induced HN dendritic spine damage (loss of psd95 staining) as early as 30 minutes after treatment. One hour after treatment, same very-low doses induce loss of dendritic branching. However, in this concentration, CDDP doesn't cause NSC loss of viability and doesn't induce apoptosis. We also test the effect on HN and NSC of higher doses CDDP such as 1 mM that were still too low to cause any effect on cancer cell cells survival. Treatment with 1 mM CDDP causes immediate (15 minute) decrease in psd95 staying, as well as loss of dendritic branching. NSCs apoptosis also becomes apparent after 24 hours. Similar results were obtained after TMZ treatment. CONCLUSIONS: The CDDP and TMZ concentrations that induce significant dendritic damage are lower than those that cause NSC apoptosis. The effect of dendritic spines and branching are also earlier and more lasting. Study Supported by: NIH/NINDS K08 award 1K08NS072234 and the Stern Family Gift.

ET-011. THE THERAPEUTIC EVALUATION OF INTEGRIN αvβ3-SPECIFIC DISINTEGRIN ON BRAIN TUMOR USING MRI

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Integrins, a family of transmembrane heterodimers formed by associations of α and β subunits, play an essential role in the progression of various cancers. Among them, αvβ3 has attracted enormous interest for its specific involvement in angiogenesis contributing to tumor progression, including brain tumors. Therapeutic strategies based upon the antagonism of αvβ3 have been recognized as an important development to the future of brain cancer treatment. The present study aims to evaluate a novel disintegrin specific to αvβ3 with regard to its effects on tumor growth using magnetic resonance imaging. The disintegrin, named ARLDDL, was a mutant protein made from the snake venom of *Calloselasma rhodostoma* which is highly selective of αvβ3. A mouse U87 glioma model was used to evaluate its anti-tumor activity. The disintegrin ARLDDL was administered intraperitoneally following two regimens; one was one week long treatment before the angiogenic switch (day 9-13) and the other was continuous treatment given before and after the angiogenic switch (since day 9). T2 weighted imaging was repeatedly acquired to monitor the tumor growth. Animal survival was also recorded. The results showed that the disintegrin ARLDDL prolonged the survival of the U87 mice by more than two weeks. The saline treated animals died approximately around the time of day 30-35. The growth curves indicated a significantly slower growing pattern rendered by ARLDDL as compared to saline. Interestingly, disintegrin given before the angiogenic switch produced a therapeutic effect that was slightly better than the continuous treatment. This suggests that integrin disruptions before tumor angiogenesis may be sufficient to interfere with the growth of brain tumors. The results demonstrate that a potent and selective disintegrin specific to αvβ3, such as ARLDDL is beneficial to intervening the progression and growth of malignant brain tumors, and thus may be considered as a promising treatment target.

ET-012. POTENTIAL SYNERGISTIC EFFECT OF TERAMEPROCOL GIVEN BY CONVECTION-ENHANCED DELIVERY AND TEMOZOLOMIDE IN TREATING ORTHOTOPIC GLIOBLASTOMA XENOGRAFT

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Terameprocol is a global transcription inhibitor that affects cell division and induces tumor cell apoptosis. Phase I study of terameprocol in patients with recurrent high-grade glioma showed that intravenous administration of terameprocol has tolerable adverse effect and potential anti-tumor efficacy. Convection-enhanced delivery (CED) gives promising administration of drug locally and could be the part of combination chemotherapy to reduce synergistic systemic adverse effect. In this study, different regimens of terameprocol administrated by CED with or without enteral administration of temozolomide were evaluated in orthotopic glioblastoma xenograft. Luciferase-modified human U87MG cells were injected into the right striatum of athymic mice. All animals were monitored using bioluminescence imaging (BLI) to assess tumor growth and response to therapy. CED of 0.25mg terameprocol had already reduced tumor enlargement. Serial CED of terameprocol is superior to once administration with the same total dosage in survival analysis. Optimal regimen of combination therapy with temozolomide will be test to get the best synergistic treatment effect.

ET-013. GOLD NANOPARTICLES AS A DRUG DELIVERY SYSTEM FOR MALIGNANT BRAIN TUMORS

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The blood-brain barrier (BBB) is the most significant obstacle for the effective delivery of therapeutic agents to malignant brain tumors. Gold nanoparticles (Au NPs) with excellent biocompatibility and targeting capability are promising drug delivery platforms for brain cancer therapy. TAT peptide, derived from the HIV virus transactivator of transcription, holds the potential to overcome the lipophilic barrier of the BBB. We designed a TAT peptide functionalized Au NPs with small core size (5 nm) to target the brain tumors. Compared with Au NP without a TAT modification, the TAT peptide modified Au NP accumulation in brain tumors was four-fold higher. Based on this discovery, we further designed a pH-sensitive TAT peptide modified Au NP conjugated with doxorubicin which is a potent anti-cancer drug but cannot penetrate the BBB. Such a conjugate showed a four-fold enhancement of cytotoxicity in glioma cell lines compared to conjugates without the TAT peptide

modification, suggesting its efficient uptake and drug release in the cancer cells. Intravenous injection of the conjugates in an intracranial glioma mouse model followed by silver enhancement staining and confocal microscopy showed that both Au NPs and doxorubicin were preferentially accumulated in the tumor, suggesting that the conjugates were able to overcome the BBB effectively. Thus, the gold nanoparticles can be served as a promising drug delivery platform for brain tumors and may be extended for other central nervous diseases.

ET-014. NUCLEUS-TARGETED DOXORUBICIN AS A POTENTIAL THERAPY FOR GLIOBLASTOMA

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Doxorubicin (DOX) is a widely used anticancer agent. However, its use is limited due to the undesired toxic side effects. We reasoned that selective direct delivery of therapeutics like DOX to their target intracellular compartments can lead to an increased specificity and lower toxicity. Interleukin 13 (IL-13) receptor alpha 2 (IL-13RA2) is over-expressed in >75% of GBM tumors, and IL-13 binding to the receptor induces internalization through ligand-receptor mediated endocytosis. To this end, we constructed a DOX delivery vehicle to target the nuclei, or lysosomes, using a modified receptor ligand, IL-13.E13K, domain II (D2) of Pseudomonas exotoxin A (PE) and SV40Tantigen nuclear localization signal (NLS) [IL-13.E13K-D2-NLS; Genes and Cancer, 1:421-433, 2010], or human lysosomal-associated membrane-protein (LAMP-1) (IL-13.E13K-D2-LAMP-1). To visualize the localization of protein to lysosomes, we biotinylated IL-13.E13K-D2-LAMP-1 and treated GBM cells followed by confocal microscopic imaging with fluorescently labeled Streptavidin. IL-13.E13K-D2-LAMP-1 demonstrated specific lysosomal localization. We then cross-linked purified IL-13.E13K-D2-NLS with cysteine residue at its C-terminal end and IL-13.E13K-D2-LAMP-1, with a derivative of DOX via disulfide bonds (Nuclear-DOX and Lysosomal-DOX, respectively). Using an MTS cell viability assay, we determined that both Nuclear-DOX and Lysosomal-DOX were cytotoxic to IL-13RA2 overexpressing G48a and U-251 MG GBM cells while significantly less cytotoxic to T98G GBM cells exhibiting low levels of the receptor. In addition, the LC50 values of both DOX conjugated proteins were significantly decreased by 90-98% from the LC50 of free DOX for G48a (55.2 μM) and U-251 (91.3 μM), which was more pronounced when cells were treated with Nuclear-DOX. This suggests that DOX conjugated with a nucleus-targeted protein is a more effective therapeutic and could exhibit less toxicity than free DOX. In conclusion, the IL-13RA2 targeting proteins under investigation are potentially useful agents for treatment and imaging of GBM.

ET-015. INVESTIGATING THE ROLE OF POLY-(ADP-RIBOSE)-POLYMERASE AS A THERAPEUTIC TARGET IN PEDIATRIC HIGH GRADE GLIOMA

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Pediatric supratentorial high-grade astrocytomas (pHGAs) and diffuse intrinsic pontine gliomas (DIPG) are devastating pediatric malignancies for which no effective therapies exist. Poly-(ADP-Ribose)-Polymerase (PARP) protein expression is found in ~60% of DIPGs suggesting PARP may be a potential therapeutic target. PARP1/2 were characterized by Western-blotting in normal human astrocytes (NHA), pHGA cell lines (SJG2, SF-188), DIPG cell lines (DIPG-M, DIPG58), and one murine brainstem glioma cell line (mBSG). Cell viability in response to different dosages of Olaparib, Veliparib, or Niraparib was determined using the MTT Assay. PARP activity, apoptosis, and DNA damage was determined by Western blotting against PAR, cleaved PARP, and phosphorylated yH2AX, respectively. Cell cycle phases were analyzed using FACS and western blot for p21. Western blotting demonstrated that, compared with NHAs, PARP1 were highly expressed in SJG2, DIPG-M, and DIPG-58 cells. PARP2 expression was only detected in SJG2 cells. All PARP inhibitors reduced PARP activity as indicated by reduced PAR levels. Olaparib reduced SJG2, mBSG, DIPG58 and DIPGM cell viability at concentrations of 5 or 10uM uM ($P < 0.05$), Whereas Niraparib induced cytotoxicity at concentrations of 2uM and above ($P < 0.05$). Olaparib and Niraparib induced DNA damage and apoptosis in SJG2 at doses of 5, 10uM and 2, 5, 10uM, respectively. Niraparib induced G2 arrest in mBSG demonstrated by FACS and increased levels of p21 ($P < 0.05$). Our data provides in vitro evidence that PARP inhibition may be an effective therapeutic avenue for treatment of pHGA and DIPG. Furthermore while all PARP inhibitors suppress PARP activity not all PARP inhibitors reduce cell viability. Thus not all PARP inhibitors can be expected to be equally efficacious in a clinical trial setting.

ET-016. PRECLINICAL EVALUATION OF PENAO: A POTENT MITOCHONDRIAL SPECIFIC, ARSENICAL-BASED INHIBITOR FOR GLIOBLASTOMA

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PENAO (4-(N-(S-penicillaminylacetyl)amino)phenylarsonous acid) selectively targets cancer-specific mitochondrial metabolism. It inactivates the inner-mitochondrial membrane protein adenine nucleotide translocase (ANT), subsequently causing an inhibition of glucose metabolism, mitochondrial membrane instability and tumour growth arrest/death. In a panel of 13 glioblastoma cell lines, including both commercial and primary lines (from primary and recurrent tumours), PENAO demonstrated marked anti-proliferative activity with IC_{50} values ranging from 0.3-4.5 μM. This was up to 50-fold more specific than for normal MRC5 lung fibroblasts and 440-fold more potent than temozolomide (TMZ), the frontline chemotherapeutic used clinically for glioblastoma. Cytotoxic PENAO concentrations (0.3-5 μM) were shown to induce the intrinsic apoptotic pathway, in both normoxic (21% O_2) and hypoxic (1% O_2) conditions. PENAO exhibited other anti-glioma efficacy where subtoxic concentrations significantly impaired migration and invasion in glioblastoma cell lines and concomitant treatment with inhibitors of ABCC1/2 transporters and glutathione synthesis increased the sensitivity of glioblastoma cell lines to PENAO by up to 100-fold. PENAO efficacy was recapitulated in vivo in glioblastoma mice models. Administration of PENAO (1mg/kg/day) in mice bearing subcutaneous glioblastoma xenografts resulted in a significant inhibition of tumor growth with all mice responding to treatment (8 partial, 2 complete). Further, orthotopic intracranial xenograft models revealed PENAO in combination with TMZ results in a significant extension of median survival. Pharmacokinetic analysis demonstrated on-target abundance for PENAO which readily crossed the blood-brain-barrier and accumulated specifically in brain tumor tissue. There were also no signs or symptoms of treatment toxicity. Together these in vitro and in vivo models of glioblastoma show that PENAO is a highly promising new therapeutic for glioblastoma. As such, phase 1 clinical trials are currently underway for determination of safety and blood-brain barrier permeability in glioblastoma patients.

ET-017. AN ENGINEERED PEPTIDE FOR NON-INVASIVE OPTICAL IMAGING OF BRAIN TUMORS

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The majority of central nervous system tumors carry grave clinical prognoses due to the limited effectiveness of surgical resection, radiation, and chemotherapy. Children diagnosed with medulloblastoma, the most common childhood brain tumor, require aggressive chemotherapy and radiation due to the tumor's marked propensity to recur and metastasize. While medulloblastoma can be surgically resected, the extent of diseased tissue removal has been shown to correlate with overall survival rates. Improvements in intraoperative tumor visualization would allow for more refined surgical resection and minimize removal of healthy brain tissue. We demonstrate that mouse cerebellar medulloblastoma can be targeted and illuminated with a fluorescent, engineered peptide (termed EETI 2.5F) that binds with high affinity to αvβ3, αvβ5, and α5β1 integrin receptors. Following tail vein injection, fluorescence arising from dye-conjugated EETI 2.5F was localized to intracranial medulloblastoma compared to the normal surrounding brain tissue, as measured by

optical imaging in both orthotopic and genetic tumor models. The intensity of the imaging signal correlated with tumor volume. EETI 2.5F, which possesses a unique ability to bind $\alpha5\beta1$ integrin, showed superior in vivo and ex vivo brain tumor imaging contrast compared to other integrin-binding peptides including c(RGDfK), a well-studied peptidomimetic that binds only $\alpha v\beta3$ and $\alpha v\beta5$ integrin. Variants of EETI 2.5F were able to distribute throughout the tumor parenchyma. In contrast, brain tumor imaging signals were not detected in mice injected with proteins containing a scrambled integrin-binding sequence, demonstrating the importance of target specificity. Our results demonstrate the promise of developing EETI 2.5F as a molecular probe for image-guided surgical resection of brain tumors. Acknowledgements: V Foundation for Cancer Research, James S. McDonnell Foundation, Wallace H. Coulter Foundation Translational Partnership Award, Stanford Center for Children's Brain Tumors at Lucile Packard Children's Hospital, and Stanford Cancer Institute.

ET-018. A NEW MULTI-TARGETED HDAC INHIBITOR INDUCES APOPTOSIS IN IN VITRO AND IN VIVO MODEL OF GLIOBLASTOMA BY MODULATING P53 EXPRESSION

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Glioblastoma is the most aggressive and malignant of the astrocytic tumors. Although complete surgical resection of the tumor is associated with better outcomes but it still does not result in a cure. Therefore, identification of novel and less toxic and small molecules that target specific pathways has become a prime focus of basic research on glioblastoma. Given the challenges associated with development of chemotherapies, we propose to test a novel multi-targeted HDAC Inhibitor diallyl trisulfide (DATS) as an alternative in the treatment of glioblastoma. Current investigations involving DATS indicated significant anti-cancer effects in glioblastoma in vitro and in vivo via upregulation of p53 expression. In our ectopic U87MG (p53 wild type) tumors in SCID mice, we treated them daily with intraperitoneal injections of DATS for 7 days. Our results indicated that DATS (10 μg/kg to 10 mg/kg) dose-dependently reduced tumor mass and number of mitotic cells within tumors. Our histological and biochemical assays in vitro and in vivo demonstrated that DATS reduced HDAC activity, increased acetylation of H3 and H4, inhibited cell cycle progression, decreased pro-tumor markers (e.g., survivin, BIRC-5, XIAP, Bcl-2, c-Myc, mTOR, EGFR, VEGF), promoted apoptotic factors (e.g., Bax, mcalpian, active caspase-3), and induced DNA fragmentation. Further, our study demonstrated an increase in p21Waf1 expression, which correlated with increased p53 expression and MDM2 degradation following DATS treatment. It does not affect normal human astrocytes and neurons and has stronger efficacy than 25 μM TSA (standard HDAC inhibitor) in in vitro and in vivo model. Collectively, our results clearly demonstrated that multi-targeted DATS will suppress glioblastoma tumor growth via HDAC-mediated regulation of p53 expression, while preserving normal brain tissue. Completion of this investigation was made possible in part by grant from Jerry Zucker Fund for Brain Tumor Research at MUSC.

ET-019. IN VIVO SYNERGISTIC ACTIVITY OF mTOR AND BRAF V600E INHIBITORS LEADING TO SURVIVAL ADVANTAGE IN PEDIATRIC LOW-GRADE GLIOMA XENOGRAFTS IS DRIVEN BY CROSS TALK BETWEEN Akt AND BRAF V600E

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PURPOSE: Missense BRAF V600E mutations and upregulation of the PI3-kinase (PI3K)/Akt/mTOR pathway are found in a substantial proportion of pediatric gliomas. We hypothesized that mTOR inhibitors would enhance the cytotoxic activity of BRAF targeted inhibitors in pediatric low-grade gliomas (PLGGs) with BRAF V600E mutations, through direct cross-talk between BRAF V600E and the PI3K/Akt/mTOR pathways. METHODS: We used BRAF V600E-targeted inhibitor PLX4720 and mTOR inhibitor everolimus (RAD001). Western blotting was performed to examine phosphorylation of key mediators of the PI3K/Akt/mTOR pathway using glioma cell lines with BRAF V600E or wild-type (WT) BRAF. In an in vivo murine flank model a BRAF V600E-mutated PLGG (BT40), mice wererandomized to receive PLX4720 alone, everolimus alone, a combination of PLX4720 + everolimus, or vehicle. Animal survival was followed. Tumor-specific markers for activation of the PI3K/Akt/mTOR pathway, and proliferation were assessed by immunohistochemistry. RESULTS: Treatment of BRAF V600E gliomas with PLX4720 + everolimus led to synergistic cytotoxic activity. In an in vivo model of BRAF V600E-mutated PLGG, combined treatment with PLX4720 + everolimus led to a statistically significant survival advantage and proliferation versus PLX4720 or everolimus alone. Treatment with BRAF V600E inhibitor substantially increased baseline Akt phosphorylation, an effect augmented by the addition of mTOR inhibitors. Akt phosphorylation was most pronounced in the combination treatment group in vivo and in vitro. CONCLUSIONS: Our studies demonstrate in vivo survival advantage in gliomas treated with a BRAF V600E inhibitor (vemurafenib) and an mTOR inhibitor (everolimus) . Preliminary biochemical findings suggest direct cross talk between the BRAF V600E mutant enzyme and PI3K/Akt/mTOR pathways, the two most common genetic aberrations in pediatric gliomas. These findings correlate with studies in BRAF V600E-mutated melanomas, but not in colon cancer cells. These findings have direct implications for designing dual-targeted therapy in upcoming clinical trials of pediatric gliomas, and understanding the molecular underpinnings of emerging resistance to targeted therapies.

ET-020. BRAIN/BRAIN TUMOR PHARMACOKINETICS AND PHARMACODYNAMICS OF LETROZOLE

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In this study, the role of aromatase (CYP19) as a target for the treatment of CNS malignancies, and disposition and anti-tumor efficacy of letrozole, an aromatase inhibitor, were investigated. Cytotoxicity and aromatase activity of letrozole against human glioma cell lines were measured using MTT assay and Enzyme Immunoassay, respectively. Brain and brain tumor pharmacokinetics (PK) of letrozole was assessed in Sprague-Dawley rats with and without orthotopic implantation of C6 glioma received letrozole (4 - 12 mg/kg; i.v.). Dual probe intracerebral microdialysis was performed to determine the unbound extracellular fluid (ECF) letrozole concentrations. Serial ECF and blood samples were collected over 8 hrs. μPET/CT imaging was performed using ^{18}F FDG to evaluate changes in active tumor volumes pre- and post-treatment of letrozole and brain tissues were collected for histological evaluations. All glioma cell lines used here expressed CYP19 and letrozole exerted marked cytotoxicity against these cells (0.1 – 3.5 mM). Normal brain ECF and plasma $C_{max}/AUC_{0\text{-}8hr}$ increased linearly up to 8mg/kg letrozole dose but these parameters increased non-linearly at higher doses. The relative brain distribution coefficients (AUC in ECF/AUC of unbound letrozole in plasma; $AUC_{ecf}/AUC_{p,ub}$) ranged from 0.31 – 0.98. Furthermore, the tumoral ECF levels of letrozole were 1.5 – 2 fold higher relative to tumor-free region of the brain, resulting in tumoral ECF C_{max} values that were 10 and 35-fold higher than the observed IC_{50} value of 0.1 μM against C6 gliomas cells in culture. μPET/CT imaging showed a marked reduction of active tumor volume after 8-10 days of letrozole treatment. Thus, employing multi-faceted and cutting edge in vitro and in vivo methods, we conclude that aromatase is expressed in glioma cells, and can be targeted effectively using letrozole.

ET-021. HIGH-THROUGHOUT SCREENING TO IDENTIFY PHARMACOLOGICS CYTOTOXIC TO GLIOBLASTOMA MULTIFORME CELLSTHAT EXPRESS WILDTYPE OR THE VIII MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor and remains uniformly fatal. Despite advances in diagnostic imaging, surgical procedures and multimodal treatment regimens, median survival for GBM patients remains poor at ~12-15 months. Moreover, conventional therapies are associated with considerable mortality and result in incapacitating damage to surrounding normal brain and system tissues. To address this urgent, unmet need, a high-throughput screen (HTS) was performed to identify pharmacologics that were highly cytotoxic to brain tumor cells. The most frequent genetic alteration associated with GBM is amplification of the epidermal growth factor receptor (EGFR) gene, which results in overexpression of the EGFR, a transmembrane tyrosine kinase receptor. A significant number of GBMs with wildtype (WT) EGFR amplification also contain the mutant EGFR gene, EGFR-vIII, characterized by

the deletion of exons 2-7, resulting in a variant with ligand-independent constitutive activity. Thus, GBM represents several histologically similar yet molecularly heterogeneous tumor cells that exist as a mosaic and display distinct therapeutic responses. It is conceivable that clinically effective therapy for GBM may require multiple EGFR inhibitors or a single agent that inhibits multiple forms of EGFR. Therefore, the HTS was performed using GBM cells that differed only in the expression of either EGFR-WT or the vIII mutant. After screening thousands of compounds, three drugs were identified that were highly cytotoxic to both the EGFR-WT and vIII mutant GBM cells. These agents are potent in the nanomolar concentration range - 100-times more potent than the FDA-approved EGFR inhibitor erlotinib. The ex vivo effect of the pharmacologics on GBM patient-derived neurospheres and the in vivo effect in a mouse model will be presented. These critical insights have uncovered the critical role the EGFR signaling network and set the stage to assess the therapeutic efficacy of pharmacologics in clinical models of GBM.

ET-022. THE BRAIN MICROENVIRONMENT NEGATIVELY REGULATES MIRNA-768-3PIN LUNG CANCER TO INDUCE *KRAS* AND PROMOTE METASTASIS

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Brain metastases are a common complication of systemic cancer and an important cause of morbidity and mortality. Lung cancer brain metastases remain a daunting adversary that negatively impact the quality-of-life and overall survival of patients diagnosed with systemic lung cancers. Moreover, the incidence and prevalence of metastatic lesions continues to rise from the increase in the aging population, the advent of targeted therapies that have increased the survival of patients with systemic cancers, increased availability of superior imaging techniques for early detection of lesions and vigilant surveillance protocols to monitor disease recurrence. Importantly, the brain microenvironment promotes tumor metastasis through mechanisms that remain elusive. We demonstrate that co-culture of lung cancer cells with astrocytes - the most abundant cell type within the metastatic brain niche - leads to down-regulation of miRNA-768-3p which then drives *KRAS* expression and key signaling pathways, enhances cell viability and promotes chemo-resistance. Vector-based forced expression of sequence complementary to miRNA-768-3p or a chemically-engineered miRNA-768-3p inhibitor recapitulated the astrocyte effects and increased cell viability. Luciferase-reporter readout experiments validated that miRNA-768-3p targeted the *KRAS* 3'-UTR and a miRNA-768-3p inhibitor strikingly increased the KRAS protein as well as downstream effectors ERK1/2 and BRAF. MiRNA-768-3p was reduced in patient brain metastases compared to normal brain and was also lower in match-paired same-patient brain metastases compared to primary tumors. The brain microenvironment negatively regulates miRNA-768-3p to enhance *KRAS* expression and promote metastasis. We propose that therapeutic replacement of the metastasis suppressor miRNA-768-3p holds clinical promise.

ET-023. THE CXCR7 INHIBITOR CCX650 SIGNIFICANTLY PROLONGS SURVIVAL IN THE C6 RAT MODEL OF GLIOBLASTOMA

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The chemokine CXCL12 (SDF-1) exerts its biological effects via two distinct receptors: CXCR4 and CXCR7. We and others have shown high expression of the chemokine receptor CXCR7 on the majority of tumor cells and tumor-associated vasculature in tumor samples from GBM patients. Potent and selective small molecule inhibitors of the chemokine receptor CXCR7 have been identified using $^{[125I]}$CXCL12 binding and trans-endothelial cell migration assays. These inhibitors 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. CCX650 is one such molecule that is orally bioavailable, displays high potency (IC_{50}: 35 nM against human CXCR7) and lacks activity on any other chemokine receptor. Preliminary preclinical toxicology studies indicate a benign safety profile with no apparent mechanism-based toxicity. The therapeutic effects of CCX650 were assessed in the rat C6 GBM model. We determined that tumor-associated vasculature in C6 tumors express CXCR7, similarly to human GBM. In our studies, rats received 18Gy whole brain irradiation 7 days post tumor cell inoculation, in combination with either oral CCX650 or vehicle. Post-irradiation survival time was significantly extended in rats receiving CCX650 relative to those that received irradiation plus vehicle. Median survival time post-irradiation was 12 days (CCX650 group; $p < 0.01$) vs. 8 days (vehicle group). In the CCX650 group, 30% of the rats survived beyond 70 days, compared to 9% of vehicle-treated rats. These results, combined with earlier reports of anti-GBM efficacy in other rodent models (e.g., rat ENU-induced spontaneous GBM) indicate that CXCR7 inhibition by CCX650 is a promising strategy for the treatment of GBM in humans.

ET-024. A MICRODIALYSIS STUDY TO ASSESS INTRACEREBRAL CONCENTRATIONS OF IRINOTECAN AND ERIBULIN WITH OR WITHOUT iRGD IN AN ORTHOTOPIC GLIOMA RAT MODEL

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BACKGROUND: The blood brain barrier (BBB) presents a formidable challenge to the effective pharmacologic management of brain tumors. Co-administration of a BBB-disrupting agent with an anti-cancer agent is a potential strategy for increasing intratumoral drug concentrations in brain tumors. iRGD (internalizing RGD peptide) is a tumor-penetrating peptide recently demonstrated to enhance drug entry into somatic tumors by increasing vascular and tissue permeability. To our knowledge, this is the first investigation assessing the ability of iRGD to increase drug penetration into brain tumor interstitium. METHODS: To determine the ability of iRGD to improve drug penetration into brain tumors, the combination of iRGD with irinotecan or eribulin was studied in an intracerebral microdialysis rat glioma model. U87 human glioma cells were established in brains of nude rats. Irinotecan (20 mg/kg) or eribulin (0.5 mg/kg), either with or without iRGD (1 mg), was injected via the tail vein into 8 and 4 rats, respectively. Microdialysate samples and blood samples for measurement of plasma concentration of the drugs were analyzed by LC-MS/MS. RESULTS: Animals receiving co-administration of iRGD had a 2.9-fold higher irinotecan Cmax (11.3 vs 3.9 ng/ml; $p < 0.0001$) and 2.2 fold higher AUC (60.9 vs 27.7 ng/ml x hr; $p < 0.001$) in brain tumors as compared to irinotecan administration alone. In contrast, the concentrations of irinotecan in normal rat brain interstitium were unchanged by co-administration of iRGD, demonstrating that the effect of iRGD is tumor-dependent. The eribulin AUC was also 2.5-fold higher when administered with iRGD (20.2 vs 8.9 ng/ml x hr) in U87 brain tumors. CONCLUSIONS: Co-administration of iRGD significantly increases the penetration of both irinotecan and eribulin in intracerebral tumor interstitium specifically. Further studies will need to be performed to determine if this 3-fold improvement in drug delivery increases the therapeutic efficacy of these drugs against primary brain tumors and brain metastases.

ET-025. COMBINATION THERAPY FOR CANCER STEM CELLS ISOLATED FROM RECURRENT GLIOBLASTOMA UTILIZING ONCOLYTIC HERPES SIMPLEX VIRUSES AND TRANSFORMING GROWTH FACTOR-β INHIBITOR

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BACKGROUND AND OBJECTIVE: Glioblastoma (GBM) inevitably recurs despite the current standard therapies and the culprit may be a subset of tumor cells with stem-like properties, termed GBM stem cells (GSCs), which have been shown to be resistant to radiation and chemotherapy. Oncolytic herpes simplex viruses (oHSV) have been safely tested in GBM patients and are efficacious against GSCs isolated from newly diagnosed GBM. In this study, we isolated GSCs from recurrent GBM, investigated the therapeutic activity of oHSV against these GSCs, and explored a combinatorial approach with small molecule inhibitors of transforming growth factor β (TGF-β), which plays a role in maintaining GSC stemness. MATERIALS AND METHODS: We established multiple tumorigenic neurosphere cultures from surgical specimens of GBMs that had recurred after surgery, radiotherapy, and temozolomide (TMZ) chemotherapy (recurrent GSCs). Two genetically engineered oHSVs, G47Δ (γ34.5(-), ICP6(-), LacZ(+), α47(-)) and MG18L (US3(-), ICP6(-), LacZ(+)) were used. Dose response curves for TMZ, G47Δ, MG18L, or TGF-β inhibitor SB431542 cytotoxicity in recurrent GSCs were determined in vitro. The interaction of oHSV and TGF-β inhibitor was assessed with the Chou-Talalay method. RESULTS: All recurrent GSCs tested were resistant to TMZ. Both oHSVs replicated, spread well, and were cytotoxic at low MOIs in the recurrent GSCs. Cytotoxicity differed between GSCs, with MG18L being somewhat more potent. The TGF-β inhibitor suppressed clonogenicity, and at high doses proliferation, of recurrent GSCs. The combination of oHSV and TGF-β inhibitor synergistically killed recurrent GSCs in vitro, which was associated with a small (~2-fold) but significant

increase in oHSV replication. CONCLUSION: GSCs can be isolated from recurrent GBM. Recurrent GSCs are susceptible to oHSV and combining with TGF-β inhibitor can enhance activity. This combination strategy is a promising one for the treatment of TMZ-resistant recurrent GBM.

ET-026. Eph RECEPTOR A3 IS A MOLECULAR THERAPEUTIC TARGET FOR THE TREATMENT OF GLIOBLASTOMA

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We have identified EphA2, a receptor tyrosine kinase, as a novel therapeutic target for high-grade astrocytomas. EphA2 is over-expressed in ~60% of glioblastomas (GBM), but not in normal brain, and it is important in the biology of glioma-stem like cells (GSC). In an effort to identify additional GBM targets especially on tumor initiating cells we found that Eph receptor A3 was significantly over-expressed under neurosphere-forming culture conditions suggesting its possible role in favoring the GSC phenotype. EphA3 was found to be aberrantly expressed in many cancers, including lymphoma, melanomas, lung cancers, and gastric carcinoma. We have thus examined the presence of EphA3 in GBM in more detail. We found that EphA3 is over-expressed in 5 out of 11 specimens (45%) of the GBM tumor lysates tested, but not in normal brain, as well as in 5 out of 7 anaplastic oligodendrogliomas (71%), and less in lower grade astrocytomas and meningiomas. EphA3 was also over-expressed in 6 out of 11 GBM cell lines tested (55%) but not on SVG p12 glial cells. Immunofluorescence staining of frozen sections of human GBM demonstrated EphA3 localization in scattered areas of the tumor, in the invasive ring, and in the niches of periphery of tumor vessels. The EphA3 receptor is present on the membrane of cells, as detected by flow cytomery, but it is also highly present in cells cytoplasm. Thus, the EphA3 receptor appears to be yet another attractive molecular target of GBM among the Eph receptors. Importantly, Eph receptors A2 and A3 are both activated by ephrin-A5 ligand, inducing receptor internalization, but EphA3 receptor does not undergo ensuing down-regulation. These two EphA receptors can be exploited for concomitant delivery of therapeutics/labels to tumor cells, including GSC, invading and vascular cells and/or for interference with the signaling pathways they transduce.

ET-027. COMBINED ANTI-ANGIOGENIC AND ANTI-INVASIVE TREATMENT OF ORTHOTOPICALLY ENGRAFTED HUMAN GBM XENOGRAFTS

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INTRODUCTION: The devastating course of Glioblastoma Multiforme (GBM), despite multimodal treatment comprising surgery, radiotherapy and chemotherapy, calls for new targeted strategies. GBMs are characterised both by florid angiogenesis and highly infiltrative growth. Therefore, we wanted to investigate whether combined targeting of angiogenesis and invasion with Bevacizumab and NVP-BKM120, a PI3K inhibitor, could suppress tumour growth. MATERIAL/METHODS: 29 nude rats were implanted intracranially with human GBM xenograft spheroids, and tumour engraftment was confirmed 3 weeks later using a small animal MRI. The resulting tumours were highly representative of human GBMs, mimicking both the angiogenic and invasive phenotype. Treatment was initiated once the tumour take was confirmed by MRI, and the animals were divided randomly into controls or groups receiving; Bevacizumab, NVP-BKM120, or Bevacizumab and NVP-BKM120 in combination. The rats received Bevacizumab intravenously once a week, and NVP-BKM120 was given orally by gavage five times per week. RESULTS: Median survival was prolonged in all treatment groups compared to the controls ($p = 0.0007$). Notably, the combined administration of Bevacizumab and NVP-BKM-120 was associated with a longer median survival (37.5 days) than groups receiving monotherapies with Bevacizumab (33 days) or NVP-BKM-120 (36 days), although not significant. CONCLUSION: Both Bevacizumab and NVP-BKM120 demonstrate anti-tumour efficacy, and the rats survived longer upon received treatment. However, the combination did not potentiate the effect of either of them. Optimised combination treatment and dosage schedules will be validated in future studies to investigate whether combined anti-angiogenic and -invasive treatment may have a role in GBM therapy.

ET-028. ALTERNATING ELECTRIC FIELDS (TTFIELDS) INHIBIT DNA DAMAGE REPAIR RESPONSE IN CANCER CELL LINES

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TTFields are an antimitotic treatment modality approved by FDA for the treatment of patients with recurrent glioblastoma. TTFields act by disruption of spindle microtubule arrangement and interference with cytokinesis through the orientation of polar macromolecules in the direction of the electric fields. We hypothesized that the negatively charged, DNA fragments resulting from radiation therapy (RT) undergo similar rotation, thus interfering with proper alignment of the Double Strand Breaks (DSBs) during DNA Damage Response (DDR). Cancer cells (U-118 Glioma and MCF-7 Breast Cancer) were exposed to RT followed immediately by TTFields application for up to 24 hours. The extent of DNA repair over time was evaluated based on cell survival, gamma-H2AX foci number and the Comet Assay. A biophysical model was constructed to estimate the movement of linear DNA fragments. TTFields application for 4 and 24 hours after RT led to a decrease in the number of U-118 cells ($50 + 23$, $42 + 28\%$, respectively) as compared to cells treated with RT alone. Clongenic assays demonstrated that cells which survived the initial TTFields + RT insult, had reduced viability ($p < 0.01$). Cells treated with TTFields for 2, 4 and 24h after RT exhibited increased levels of gamma-H2AX compared to irradiated cells. The comet assay revealed significantly delayed DDR as a result of TTFields application of 2, 4 and 24 hours ($p < 0.05$). Biophysical model predicted that chains of about 400 bp oscillate over a distance of two base-pairs at a TTFields frequency of 100 kHz. TTFields treatment alone did not affect the level of gamma-H2AX and DDR compared to untreated cells. We conclude that, in addition to their known anti-mitotic effect, TTFields also inhibits DNA repair. These results raise the possibility that TTFields applied immediately after RT may potentiate the clinical efficacy of RT.

ET-029. OVERCOMING CELL SIZE ESCAPE FROM TUMOR TREATING FIELDS USING A VARYING FREQUENCY TREATMENT PARADIGM IN-VITRO

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TTFields Therapy at 200 kHz received FDA approval for the treatment of patients with recurrent glioblastoma (GBM) based on the results of a phase III clinical trial. Radiological responses were observed in 14% of the treated patients. The lack of radiological response in the remaining patients suggests that GBM cells may escape the effect of TTFields over time. The goals of the present study were to identify possible routes by which cancer cells can escape the antimitotic effect of TTFields and to explore ways to overcome such escape mechanisms. Measurements of cell size before and after 72 hours TTFields application revealed a significant volume increase in 15 cancer cell lines (33% - 116%). As previous published data demonstrated an inverse relationship between cell size and optimal TTFields frequency, such an increase in cell size may lead to suboptimal TTFields effect. In order to test the above hypothesis, A2780 ovarian cancer cells were treated at their optimal TTFields frequency of 200 kHz for 72 hours. Cell volume increase of $70 + 29\%$ ($p < 0.05$) was evident within 24 hour treatment. FACS analysis demonstrated that cell volume increase was not due to an increase in the S or G2/M population. To overcome such cell size escape we decreased the frequency of the TTFields from 200 to 150 kHz after 24 hours treatment to coincide with the increase in cell volume. We found that varying the frequency from 200 to 150 kHz increased significantly ($p < 0.05$) the inhibitory effect of TTFields ($37 + 10\%$ survival of control) compared to continuous treatment at 200 kHz ($57 + 16\%$ survival of control). This is the first evidence of an escape mechanism from the antimitotic effect of TTFields. The results presented provide a simple way to restore treatment efficacy by varying TTFields frequency in parallel to the change in cell volume.

ET-030. CD317-TARGETED IMMUNOTOXIN INDUCES APOPTOSIS IN HUMAN GLIOMA CELLS

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Despite multimodal therapy, the clinical outcome for patients with malignant gliomas remains poor. Here, a novel antibody-based targeted therapy for glioblastoma targeting CD317, also known as HM1.24-ETA' is described. The immunotoxin HM1.24-ETA' is a fusion protein of a humanized, CD317 specific single-chain Fv (scFv) fragment combined with a truncated version of Pseudomonas exotoxin A. CD317 is a type II transmembrane protein, initially described to be expressed on terminally differentiated human B cells and overexpressed on malignant plasma cells. CD317 is also overexpressed in some solid tumor types. To date, its role in cancerogenesis is unclear. Interrogations from the glioblastoma database of the Cancer Genome Atlas network (TCGA) show that CD317 mRNA levels are up-regulated in human glioblastoma in vivo. Moreover, enhanced CD317 expression significantly reduced probability of survival in this patient group. Immunohistochemistry analysis for CD317 on a tissue micro array demonstrated that CD317 is up-regulated in human glioblastoma and that CD317 protein levels correlate directly with the tumor grade of astrocytomas. We find that CD317 is expressed heterogeneously in human glioma-initiating and long-term glioma cell lines on mRNA and protein levels in vitro. The immunotoxin HM1.24-ETA' specifically targets CD317 and induces acute cytotoxicity in CD317-positive glioblastoma cells in a concentration-dependent manner. Target cell cytotoxicity in the human glioma cells in vitro occurred via caspase 3 activity, underlining apoptosis as the mode of cell death induced by HM1.24-ETA'. Interestingly, interferon-β induces CD317 mRNA and protein levels on the cell surface of glioblastoma cells and enhances the cytotoxic effect of HM1.24-ETA' in these cells. These results indicate that CD317 is an interesting novel cell-surface protein, overexpressed on glioblastoma, that can be targeted specifically by HM1.24-ETA', a novel approach of immunotherapy for glioblastoma patients.

ET-031. THERAPEUTIC OPPORTUNITIES IN DIFFUSE INTRINSIC PONTINE GLIOMAS DETERMINED BY FUNCTIONAL CHEMICAL SCREENS AND MUTATIONAL PROFILING

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Diffuse Intrinsic Pontine Glioma (DIPG) is an incurable childhood cancer affecting approximately 400 children each year in the United States. Due to the historical paucity of tumor tissue and the lack of experimental model systems, most prior clinical trials of drug therapies have been conducted in the absence of preclinical data. With recent community and/or clinical investigator efforts, postmortem and surgical biopsies procedures have facilitated: descriptions of genomic and transcriptomic alterations, establishment of cell lines and orthotopic models, and identification of recurrent/potentially critical mutation affecting epigenetic regulation in DIPG (e.g., the H3.3K27M). Armed with these newly available preclinical tools to aid design of clinical trials, we have formed an international multi-institutional collaboration to pool resources and evaluate comprehensive molecular and functional targets of DIPG therapy. Importantly, this DIPG preclinical consortium has screened 86 potential single agent therapies across the majority of DIPG cell cultures thus established worldwide. We have performed whole exome sequencing of 35 DIPG samples, completed two sets of cell viability screens (for 13 and 11 cultures, respectively) and conducted integrated pharmacogenomic data analysis to identify potentially effective therapeutic strategies. The highest value small molecule inhibitors included: SNS-032, panobinostat, alvocidib, MLN8237, carfilzomib, dasatinib, vorinostat, irinotecan, BMS 754807, and sorafenib. Integrative analysis of 9 cell lines using drug screen data and paired RNAseq expression data additionally identified multiple candidate gene targets that have the potential to yield an effective treatment, if targeted in combination. These included: PSMB5, CDKs (e.g., CDK2), HDAC2, HDAC3, HDAC4, EHMT1, STK36, GSK38, MET, SRC and NTRK1. These results along with genomics data indicating recurrent aberrations in both HDACs and CDKs in DIPG, point to the potential value of further preclinical studies of a two-drug combination, using panobinostat and vorinostat to target HDACs and alvocidib and SNS-032 to target CDKs.

ET-032. THERAPEUTIC TARGETING OF XPO1/CRM1 IN ADULT AND PEDIATRIC HIGH-GRADE GLIOMA

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BACKGROUND: High-grade gliomas (HGG) in children and adults are poorly responsive to current chemotherapy. The nuclear exporter XPO1 (CRM1) is overexpressed and predicts poor prognosis in HGG. We explored emerging inhibitors of XPO1 for use in HGG. METHODS: Five patient-derived HGG lines (three adult, two pediatric) were treated with two XPO1 inhibitors (KPT-276 and KPT-330) in neurosphere culture conditions. These two inhibitors were also evaluated in a patient-derived orthotopic murine HGG xenograft model. Cell cycle effects were assayed by flow cytometry in cultured cells and immunohistochemistry in brain sections. Apoptosis was determined by TUNEL. MCL1 expression was measured by Western blot. RESULTS: Treatment of HGG cultures with KPT-276 and KPT-330 revealed dose-responsive growth inhibition in all five HGG lines (IC_{50} range 60-354 nM). In an orthotopic patient-derived xenograft model, both compounds demonstrated significant tumor growth suppression compared to vehicle as measured by longitudinal bioluminescence imaging ($p < 0.001$) and MRI at a discrete time point ($p = 0.003$). A significant prolongation in survival was also observed in both treatment groups versus control ($p < 0.0001$). There was no change in proportion of cells in S-phase in vitro, and there was no difference in proportion of Ki-67+ cells in vivo. However, the number of TUNEL+ cells was significantly higher with treatment in both cultured cells and tumors. Mechanistic studies revealed decreased MCL1 in cultured cells treated with XPO1 inhibitors. CONCLUSIONS: Two selective XPO1 inhibitors show excellent preclinical efficacy utilizing in vitro and in vivo models of HGG. The mechanism appears to be an increase in apoptosis, perhaps mediated through inhibition of translation of the anti-apoptotic MCL1 upon XPO1 inhibition. KPT-330 is now in Phase 1 clinical trials in solid and hematological malignancies. On the basis of these preclinical data and excellent brain penetration, we are proceeding with development of a Phase 2 trial of KPT-330 in HGG.

ET-033. ANALYSIS OF COMBINATION OF TUMOR TREATING FIELDS (TTFIELDS) WITH RADIOTHERAPY IN NON-SMALL CELL LUNG CANCER

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BACKGROUND: Tumor Treating Fields (TTFields) are low intensity, intermediate frequency alternating electric fields, which are able to inhibit tumor growth both in vitro in various tumor cell lines and in vivo in tumor-bearing animals. TTFields have been already approved by the FDA for the treatment of recurrent glioblastoma multiforme (GBM). AIM: The combination of TTFields with chemotherapy, (e.g. pemetrexed in NSCLC in vitro and in animal models), has shown sensitizing, additive effect on tumor growth inhibition. So far combining TTFields with radiotherapy (RT) has not been studied. Here we analyzed if combination of TTFields with conventional RT in non-small cell lung carcinoma (NSCLC) cells sensitized to RT-induced cell death. MATERIALS AND METHODS: NSCLC A549 cells were irradiated with a total dose of 2 or 8Gy and then TTFields were applied for 24h or 72h. Effects on cell viability were analyzed by counting with Scepter2.0 cell counter and confirmed by clonogenic survival assay. Apoptosis induction was measured by PARP cleavage. DNA double strand breaks induction and repair and downstream signaling was analyzed by phospho-DNA-PK(S2056) and phospho-ATM(S1981). Expression of p53 was also analyzed by western blot as well as phospho- and total p38MAPK. RESULTS AND CONCLUSIONS: In A549 cells treatment with IR 2Gy or TTFields alone decreased viability at 24h with about 15%, whereas the combination of IR 2Gy and TTFields caused about 25% decrease in cell viability. When TTFields were applied continuously as a single modality for 72h significant inhibition of cell proliferation was observed (~40%). Results obtained for the 72h TTFields were comparable with the effect achieved by 8Gy irradiation. On the molecular level, we observed increase in DNA-PK phopsphorylation after 24h of TTFields alone and changes in phospho-p38 level. These preliminary results suggest that mechanism of action of TTFields affects DNA damage response (DDR) and will be further discussed.

ET-034. DISCORDANT SENSITIZING EFFECTS OF ABT888 COMBINED WITH TEMOZOLOMIDE IN GBM TUMOR MODELS TESTED *IN VITRO* AND *IN VIVO*

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Poly (ADP-ribose) polymerase (PARP) plays an important role in DNA repair, and there is significant interest in developing PARP inhibitors as chemosensitizing agents for glioblastoma multiforme (GBM). This study was designed to evaluate antitumor effects of ABT888 in combination with temozolomide (TMZ) in GBM models including U251, T98 and patient derived GBM12 xenograft sublines with differential TMZ sensitivities. In cell based assays, ABT888 significantly enhanced efficacy of TMZ for all GBM models tested with pronounced sensitizing effects on resistant lines. Specifically for TMZ resistant lines U251TMZ and T98, relative proliferation index recorded after TMZ treatment were 0.95, 1.10, which decreased with TMZ/ABT888 co-treatment to 0.08, 0.32, respectively. Similarly, relative neurosphere counts of 0.95, 0.40 obtained after 100 μM TMZ treatment were significantly decreased by co-treatment to 0.54, 0.04, respectively for two different resistant xenograft lines-GBM12TMZ($MGMT^{high}$) and GBM12TMZ ($MGMT^{low}$). Consistent with a mechanism involving disruption of DNA repair, TMZ/ABT888 co-treatment resulted in robust activation of ATM/ATR signaling through Chk1, Chk2 and KAP1 phosphorylation and with higher degree of γH2AX foci formation. However, unlike sensitive cells, activation of pathways diminished progressively in TMZ resistant lines implying relatively rapid resolution of DNA damage within these cells. In contrast to consistent sensitization seen *in vitro*, combination of ABT888 and TMZ demonstrated significant delay in tumor progression in the parental xenograft line (delaying median time to endpoint by 68 days, $p = 0.0029$), but not in two TMZ resistant xenograft lines. The pharmacokinetic profile of ABT888 was similar between tumors from various GMB12 sublines, although peak accumulation of ~3 μM is below the optimal concentration associated with effective sensitization *in vitro*. Pharmacodynamic analysis of GBM12 xenograft sublines is currently in progress. Lower levels of ABT-888 combined with differences in DNA damage processing may contribute to the lack of *in vivo* sensitization of TMZ resistant models.

ET-035. CIDOFOVIR: A NOVEL ANTITUMOR AGENT FOR GLIOBLASTOMA

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PURPOSE: Cidofovir (CDV) is a FDA approved nucleoside antiviral agent used to treat severe human cytomegalovirus (HCMV) infection. Until now, no clear effects of CDV have been reported outside of the setting of viral infection, including a potential role for CDV as an antineoplastic agent for the treatment of brain tumors. EXPERIMENTAL DESIGN: We investigated CDV cytotoxicity against glioblastoma (GBM) cells, U87MG and primary SF7796, in vitro and in vivo, using an intracranial xenograft model. Standard techniques for cell culturing, immunohistochemistry, Western blotting, and real-time PCR were employed. The survival of athymic mice (n= 6-8 mice per group) bearing GBM tumors, treated with CDV alone or in combination with radiation, was analyzed by the Kaplan-Meier method and evaluated with a two-sided log-rank test. RESULTS: CDV possesses potent antineoplastic activity against HCMV infected GBM cells. This activity is associated with inhibition of HCMV gene expression and with activation of cellular apoptosis. Surprisingly, we also determined that CDV induces GBM cell death in the absence of HCMV infection. CDV is incorporated into tumor cell DNA, which promotes double strand DNA breaks and induces apoptosis. In the setting of ionizing radiation treatment (RT), the standard of care for GBM in humans, CDV augments radiation-induced DNA damage and further promotes tumor cell death. Combined CDV and RT treatment significantly extended the survival of mice bearing intracranial GBM tumors. CONCLUSION: We have identified a novel anti-glioma property of the FDA approved drug CDV, which heightens RT cytotoxic effect, the standard of care therapy for GBM.

ET-036. PRECLINICAL INVESTIGATION OF CONCURRENT VS. SEQUENTIAL RADIATION + cdk4/6 INHIBITOR THERAPY REVEALS HEIGHTENED ANTI-TUMOR EFFECT FROM INHIBITOR ADMINISTRATION FOLLOWING COMPLETION OF RADIATION

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The cdk4/6 inhibitor Palbociclib arrests tumor cells in G1 cell cycle phase and has proven to be a potent cytostatic agent in multiple preclinical studies involving a variety of retinoblastoma protein (Rb) proficient cancer models, including glioblastoma (GBM) and atypical teratoid rhaboid tumor (ATRT). In the current investigation, we examined the effectiveness of Palbociclib when administered either concurrently or sequentially with radiation therapy (RT), in consideration of using this small molecule inhibitor for treating patients with newly-diagnosed, primary malignant brain cancer. Four distinct Rb proficient intracranial xenograft models were examined: two GBM and two ATRT. The regimens compared for efficacy were: Sequential Regimen 1: RT then Palbociclib; Sequential Regimen 2: Palbociclib then RT; Concurrent Regimen: RT administered during the first 5 days of Palbociclib treatment. RT was daily for 5 consecutive days (1-2 Gy/day, dependent on the previously established RT sensitivity of each xenograft being treated), and Palbociclib treatment was daily for 2 weeks at 150 mg/kg/day. For each experiment, survival results showed concurrent treatment and sequential regimen 1 significantly outperforming either monotherapy ($p < 0.05$). With only one exception, the sequence RT first then Palbociclib outperformed concurrent treatment with respect to mean and median survival benefit, though survival differences between these groups were not statistically significant for any xenograft tested. Surprisingly, administration of Palbociclib first followed by RT consistently showed the least survival benefit among the combination therapy treatment groups. Immunocytochemistry results indicate that treatment of irradiated cells with Palbociclib sustains γH2AX and 53BP1 DNA-associated foci, suggesting inhibition of the DNA repair process, thereby providing a mechanistic rationale for the enhanced in vivo anti-tumor effect from administering Palbociclib subsequent to RT. In total, our results suggest that Palbociclib may prove efficacious when used in an adjuvant setting, following completion of conventional radiation treatment of patients with malignant brain tumors.

ET-037. TARGETING THE HISTONE H3.3-K27M MUTATION FOR THE TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMAS

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INTRODUCTION: Diffuse intrinsic pontine gliomas (DIPGs) carry a dismal prognosis despite the use of aggressive multi-modality treatment. Recent studies have identified recurrent somatic mutation of the H3F3A gene, resulting in replacement of lysine 27 by methionine in the encoded histone H3.3 protein (H3.3-K27M). We hypothesize that the expression of this mutant protein in DIPG confers growth advantage and provides a unique therapeutic opportunity for treating this cancer. METHOD: H3.3-K27M mutation status was determined by DNA sequencing from two pediatric DIPG and three pediatric GBM cell lines established from surgical biopsies. Histone H3.3 lysine 27 (H3K27) methylation status was evaluated by western blotting. Cell proliferation assays were performed to assess the response to pharmacological inhibition with GSK-J4, a selective inhibitor of H3K27 demethylase JMJD3. In vivo tumor growth and response to therapy were quantitatively measured by bioluminescence imaging and animal survival. RESULTS: The H3.3-K27M mutation was identified in the two DIPG but not pediatric GBM cell lines. H3.3-K27M mutant DIPG cells showed global reduction of H3K27 methylation compared to H3.3 wild-type glioma cells. GSK-J4 increased H3K27 methylation and induced a marked dose- and time-dependent inhibition of growth in H3.3-K27M mutant DIPG cells whereas H3.3 wild-type glioma cells showed no response to GSK-J4. Finally, GSK-J4 inhibited orthotopic human DIPG tumor growth and extended the survival of animals with brainstem tumor. Gene expression changes, as a result of GSK-J4 treatment, will be examined in cells with the K27M mutation. CONCLUSION: Our findings suggest that altered histone H3.3-K27 methylation is caused by the H3.3-K27M mutation, and is associated with tumor cell sensitivity to GSK-J4.

ET-038. THE IMMUNOHISTOLOGICAL AND RADIOLOGICAL SURVEY ON TEMOZOLOMIDE-INDUCED CYTOTOXICITY ENHANCED BY cdk INHIBITOR IN VIVO

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Recent progress in chemotherapy for malignant gliomas has been made by introduction of temozolomide (TMZ); however, drug resistance remains a

major problem. We have investigated the linkage between G2 checkpoint and DNA repair mechanisms of TMZ-treated glioma cells because previous studies have shown that TMZ resistance could be promoted by enhanced DNA repair activity in the G2–M transition. We analyzed the effect of a cdk inhibitor flavopiridol (FP) against the glioma cells since this compound has been reported to suppress DNA repair in several cancer cells arrested at G2-M phase. Western blotting revealed FP induced both the suppression of the key proteins at the G2-M transition and an increase of DNA double strand break marker in U87MG human glioma cells treated by TMZ. To confirm the chemosensitizing effect of FP in vivo, we then evaluated the effect of FP in xenografted glioma cells in nude mice treated with TMZ. We observed that necrotic change consisting of the swollen and calcified glioma cells in the animal without any treatment, whereas the fibrous change was dominantly observed in those treated with TMZ and FP. The apoptotic change and DNA double strand break were significantly increased in the xenograft treated with TMZ and FP. Reduction of proliferative cells was histologically confirmed, and the reduction of tumor bulk was radiologically confirmed along with time course in TMZ + FP group. In conclusion, present study suggests that FP enhanced the action of TMZ on glioma cells and that application of cdk inhibitor might provide a new therapeutic approach against malignant gliomas.

ET-039. AN ENGINEERED KNOTTIN PEPTIDE FOR NON-INVASIVE TARGETING OF INTRACRANIAL MEDULLOBLASTOMA

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Improved strategies are critically needed for targeted treatment and imaging of central nervous system tumors. Intregins, membrane proteins present at elevated levels in brain tumor cells and tumor vasculature, are essential for tumor growth and angiogenesis. Agents which target integrins show great promise as novel, effective treatments. Cystine-knot peptides (knottins), are engineered peptides with modifiable binding affinity. Knottins are resistant to proteases, non-immunogenic, and small (3-4 kDa), allowing them to penetrate tumors and clear quickly from non-target tissues. We demonstrate that mouse cerebellar medulloblastoma (MB) can be targeted and illuminated with a fluorescent, engineered, knottin peptide (EETI 2.5F) that binds with high affinity to αvβ3, αvβ5, and α5β1 integrin receptors. Following a tail vein injection of a dye-conjugated EETI 2.5F, fluorescence localized specifically to the intracranial tumor, as measured by optical imaging. Due to its unique ability to bind to α5β1 integrin, EETI 2.5F showed superior in vivo and ex vivo brain tumor imaging contrast compared to other engineered integrin-binding knottin peptides and to c(RGDfK), a well-studied integrin binding peptidomimetic. Even when fused to an antibody FC domain (EETI 2.5F-Fc), the larger integrin-binding protein still targeted intracranial brain tumors after intravenous injection. In contrast, brain tumor imaging signals were not detected in mice injected with EETI 2.5F proteins containing a scrambled integrin-binding sequence, demonstrating the importance of target specificity. These results highlight the potential of using EETI 2.5F and EETI 2.5-Fc as targeted molecular proteins which can be modified for brain tumor imaging or therapeutic potential. Unlike antibodies targeting integrins, knottins peptides such as EETI 2.5F can be linked to brain tumor chemotherapeutics, acting as carriers to target tumors within the CNS. Likewise, when conjugated to a fluorescent molecule, knottins can aid in intraoperative tumor resection. Knottin peptides have great potential to revolutionize the operative and medical treatment of CNS tumors.

ET-040. EXPLORING MULTIPLE ASPECTS OF STEM CELL-BASED THERAPY FOR CANCER USING NOVEL MULTI-FUNCTIONAL MOLECULES

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Due to unique tumor-specific homing and prolonged delivery profile, stem cells are promising therapeutic delivery vehicles for a variety of lethal cancers. However, this field is in its infancy and many vital questions remain to be answered before stem cell-based therapy for cancer can be realized as an effective clinical treatment. Creating new molecules that simultaneously enhance tumor cell killing and permit diagnostic tracking is vital to answering many of these questions. To this end, we have created unique first-generation fusion proteins SRL_oL_2TR and SM7L containing both diagnostic (luciferase) and therapeutic (TRAIL and MDA-7/IL-24) domains designed to allow simultaneous investigation of multiple events in stem cell-based cancer therapy in vivo. Engineering stem cells with these molecules, we demonstrated various stem cell lines display marked differences in the levels and duration of secretion of anti-cancer therapies, while in vitro assays revealed the efficacy of different stem cell-based therapies against human cancer cells. In vivo, simultaneous diagnostic and therapeutic monitoring revealed that stem cell-based delivery significantly improved pharmacokinetics and anti-tumor effectiveness of two different anti-cancer proteins compared to intravenous or intratumoral delivery. As treatment for highly malignant brain cancer xenografts, we demonstrated that stem cell-based therapy significantly attenuated progression of established intracranial tumors. Furthermore, we showed the anti-tumor efficacy of stem cell-based therapy can be augmented in vitro and in vivo by the concurrent activation of complementary cytotoxic and cytostatic therapeutic pathways utilizing SRL_oL_2TR and SM7L in a combination therapy. Collectively, these studies define a promising new approach to treating highly aggressive cancers using stem cell-based delivery of novel multifunctional molecules.

ET-041. THIRD GENERATION ST-DRUGS HAVE AN INCREASED POTENCY IN PROLIFERATIVE CLASSICAL GLIOBLASTOMA SUBTYPE

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Glioblastoma Multiforme (GBM) is the most common and aggressive primary brain tumor in adults, with a five year survival of 2% and no available cure for these patients. Recent studies suggest that cannabinoid compounds possess potent anti-tumorigenic properties while exhibiting a favorable safety profile. Starting from the aminoalkylindole, WIN55,212-2, a potent cannabinoid agonist, we developed first generation, JWH-451, and second generation, ST-25 and ST-34, ST-compounds which promote apoptosis in GBM cells lines while maintaining a promising therapeutic index. Remarkably, these compounds act independently of cannabinoid CB_1 and CB_2 receptors. Here we developed and optimized third generation compounds by conducting a structure-activity-relationship study around ST-34. Thus forty rationally-designed ST-compounds were tested against a panel of 6 patient derived primary GBM (PDGBM) cells including the proneural, neural, classical, and mesenchymal subtypes of GBM. Our results identified 4 compounds that are particularly potent at all PDGBM types. Two of these compounds, ST-59 and ST-377, showed an increased potency at PDGBM cells from patients with the classical proliferative genotype of GBM. ST drugs work through a novel mechanism of action leading to the activation of caspase3/7 and apoptosis in GBM cells and do not affect the cell viability of healthy cells. In vitro toxicity profiling indicated that these compounds have a much improved therapeutic index compared to Temazolomide. Our next step is to determine their in vivo maximal tolerated dose and pharmacokinetic studies of the 4 most promising compounds to identify a lead compound that exhibits the optimal exposure and safety profile and advanced into in vivo efficacy studies in a proliferative classical patient-derived xenograph mouse model.

ET-042. DEVELOPMENT OF FLUORESCENT GRAPHENE NANOSHEETS WITH BBB PERMEATION FOR DRUG AND GENE CO-DELIVERY IN MALIGNANT BRAIN TUMOR TREATMENT

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Malignant brain tumor is a common and severe primary brain tumor with a high recurrence rate and a poor prognosis. Because of the infiltrative nature of malignant brain tumor, tissues surrounding the tumor that remain after surgery might contribute to recurrence. Intravenously administered chemotherapeutic drugs have limited use because of their adverse systemic effects and poor blood-brain barrier penetration. Thus, development of an effective new therapeutic system to delivery drugs and gene against malignant brain tumor is in urgent need. In this study, we plan to develop new fluorescent graphene nanosheets then modify the surface with transferrin receptor antibody (anti-TfR antibody) to form a nanocarrier which can permeate the blood-brain barrier (BBB). Besides, this nanocarrier can carry epirubicin (EPI) and miRNA simultaneously to increase the accumulation in brain tumor tissue for effective chemotherapy and gene therapy in brain tumor. This novel nano-drug carrier

revealed significant anti-proliferation effect in different tumor cells including glioma, ovarian cancer and lung cancer. Animals carrying brain tumor or subcutaneous tumors were treated by the multifunctional nano drug carrier, and the therapeutic effects were extensively evaluated. Such innovative applications of emerging technologies promise to provide more effective means of tumor treatment, with lower therapeutic doses and potentially fewer side effects.

ET-043. TOCA 511 GENE TRANSFER IN COMBINATION WITH TEMOZOLOMIDE APPEARS SAFE AND DEMONSTRATES SYNERGISTIC THERAPEUTIC EFFICACY IN A MOUSE GLIOBLASTOMA MODEL

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Toca 511 (vocimagene amiretrorepvec), an amphotropic retroviral replicating vector (RRV), can successfully and safely deliver a functional, optimized yeast cytosine deaminase (CD) gene to tumors in orthotopic glioma models. Within infected cells, CD converts 5-fluorocytosine (5-FC) to the anti-cancer drug 5-FU. The combination of Toca 511 with oral extended release 5-FC (Toca FC), is currently in clinical trials for recurrent High Grade Glioma (HGG, NCT01156584 and NCT01470794). Temozolomide (TMZ), in combination with radiation therapy, is the most commonly used first-line chemotherapy treatment for patients with glioblastoma, the most common and aggressive form of primary brain cancer. A separate study (Takahashi et al., this meeting) addresses the potential radiation synergy with Toca 511/5-FC treatment. A subset of patients with certain genetic alterations does not respond well to TMZ treatment and the overall median survival for patients who respond remains poor, suggesting combinatorial approaches may be necessary to significantly improve patient outcomes. To determine whether Toca 511 and 5-FC therapy is compatible with TMZ, we examined the effect of TMZ in combination with Toca 511 and 5-FC in TMZ-sensitive and resistant glioma lines both in vitro and in vivo. We show that in vitro TMZ delays but does not prevent RRV spread, nor interfere with Toca 511 and 5-FC mediated cell killing in glioma tumor cells, and in vivo there is no significant hematologic effect from the combination of 5-FC and the clinically relevant dose of TMZ. A synergistic long-term survival advantage is observed in mice bearing an orthotopic TMZ-sensitive glioma tumor after Toca 511 administration followed by co-administration of TMZ in combination with 5-FC. These results provide support for the investigation of this novel combination treatment strategy for patients with newly diagnosed glioblastoma.

ET-044. BORTEZOMIB-INDUCED UNFOLDED PROTEIN RESPONSE INCREASES ONCOLYTIC HSV-1 REPLICATION RESULTING IN SYNERGISTIC, NECROPTOTIC CELL DEATH

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BACKGROUND: Bortezomib is an FDA-approved proteasome inhibitor, and oncolytic HSV (oHSV) a promising therapeutic approach for cancer. Their combination has not been previously tested for anti-tumor efficacy. METHODS: The synergistic interaction between oHSV and bortezomib was calculated using Chou-Talalay analysis. Viral replication was evaluated using plaque assay. Western-blot, flow cytometry and caspase 3/7 activity assays were used to evaluate induction of ER stress, unfolded protein response (UPR), JNK activation and apoptosis. Inhibitors/shRNA targeting Hsp90, JNK and RIP1kinase were utilized to investigate the mechanism of cell killing. Subcutaneous and intracranial tumor xenografts for head and neck cancers and glioma were utilized to evaluate anti-tumor efficacy of the combination. Survival was analyzed by Kaplan-Meier curves and two-sided log rank test. RESULTS: Combination treatment with bortezomib and the oHSV, 34.5ENVE, displayed strong synergistic interaction in ovarian cancer, head & neck cancer, glioma, and malignant peripheral nerve sheath tumor (MPNST) cells. Bortezomib treatment induced ER stress, evident by strong induction of Grp78, CHOP, PERK and IRE1α (western blot analysis) and the UPR (induction of hsp40, 70 and 90). Bortezomib treatment increased viral replication (p value <0.001), but inhibition of Hsp90 ablated this response, reducing viral replication and synergistic cell killing. Increased phosphorylation of JNK and c-Jun was observed in cells treated with bortezomib and 34.5ENVE. Inhibitors/shRNA against JNK/RIP1 kinase rescued synergistic cell killing. The combination of bortezomib and 34.5ENVE significantly enhanced anti-tumor efficacy in vivo. CONCLUSIONS: Synergistic efficacy of bortezomib and 34.5ENVE is mediated by multiple signaling pathways and warrants future clinical testing.

ET-045. OMX-4.80, AN ENGINEERED OXYGEN CARRYING PROTEIN, OXYGENATES HYPOXIC TUMORS AND INCREASES THE EFFICACY OF RADIOTHERAPY IN ORTHOTOPIC MODELS OF GLIOBLASTOMA

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BACKGROUND: Oxygen is essential for the tumor-killing effects of radiotherapy (RT). Overcoming tumor hypoxia is a significant unmet need in cancers like glioblastoma (GB). We engineered a novel oxygen-binding protein family called H-NOX to selectively deliver oxygen to hypoxic tissues in order to increase the efficacy of RT. Specifically, OMX-4.80 decreases hypoxia in GB models over 24 hours and may improve survival for GB patients when used in combination with RT. METHODS: In vivo imaging and immunohistochemistry were performed on an array of orthotopic GB tumors including human GB cell lines, patient-derived xenografts, and a murine glioblastoma cell line (GL261) to determine the extent of OMX-4.80 accumulation and oxygenation after IV administration. To address whether OMX-4.80 tumor oxygenation leads to increased RT efficacy, mice with intracranial luciferase-modified U251 tumors were treated with OMX-4.80 prior to either a single 10 Gy dose (10 Gy x 1) or treated prior to each of three 2 Gy doses of RT (2 Gy x 3). RESULTS: Both in vivo imaging and immunohistochemical analyses demonstrate that OMX-4.80 preferentially accumulates in intracranial tumors of both human xenograft and immunocompetent murine GB models and significantly decreases levels of exogenous (pimonidazole) and endogenous (HIF-1a) hypoxia markers. Pretreatment with OMX-4.80 increases the efficacy of either single or multiple fractions of RT (compared to RT-only groups) as evidenced by decreased tumor growth, measured by tumor bioluminescence, and statistically significant increases in survival ($p < 0.05$, by logrank). These data were confirmed in multiple other models of tumor hypoxia, demonstrating the ability of OMX-4.80 to specifically oxygenate hypoxic tumor areas in a wide range of in vivo settings. Safety data are also exceptionally encouraging in both mice and rats. CONCLUSIONS: Results indicate that OMX-4.80 is a potent and promising radiosensitizer that may enhance RT treatment of GB patients and warrants clinical development.

ET-046. PROMISCUOUS TRANSACTIVATION OF MULTIPLE RECEPTOR TYROSINE KINASES BY EGFRvIII: MECHANISM AND THERAPEUTIC SIGNIFICANCE

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De2-7EGFR (or EGFRvIII) is an extra-cellular domain mutation of the EGFR expressed in 30% of high grade gliomas. Previously we reported that the de2-7EGFR mediates its tumorigenic activity in part through the promiscuous transactivation of other receptor tyrosine kinases (RTKs), including c-Met. Using proximity ligation assay technology we now show that de2-7EGFR directly associates with c-Met in a heterodimeric complex through a scaffold containing focal adhesion kinase (FAK). Chemical and siRNA ablation of FAK prevented the de2-7EGFR-mediated transactivation of c-Met. The combination of a FAK TKI and Panitumumab (anti-EGFR) was effective in preventing the in vitro growth of U87MG.?2-7 cells. U87MG glioma cells have a c-Met/HGF autocrine loop critical for in vivo

growth and are inhibited by Rilotumumab; an antibody that inhibits HGF the c-Met ligand. Transfection with the de2-7EGFR (U87MG.Δ2-7) switches the driver of in vivo growth to this receptor. U87MG.Δ2-7 xenografts respond to Panitumumab but are resistant to Rilotumumab due to the c-Met transactivation. To further elucidate the clinical significance of c-Met transactivation, we treated U87MG.Δ2-7 cells with the EGFR TKI erlotinib in the presence of Rilotumumab. Concentrations of erlotinib that partially inhibited the de2-7EGFR, lead to increased activation of c-Met. Mechanistically, Rilotumumab increased the amount of cell surface c-Met by decreasing its internalization, prolonging its interaction with de2-7EGFR. We examined the anti-tumor activity of Panitumumab in combination with Rilotumumab or AMG 706 in U87MG.Δ2-7 xenografts. AMG 706 was selected as it inhibits several RTKs transactivated by de2-7EGFR. Rilotumumab had no single agent activity, while AMG 706 displayed modest activity. Unexpectedly, the combination of Rilotumumab and AMG 706 had significant anti-tumor activity despite neither therapeutic directly targeting the de2-7EGFR; suggesting that targeting de2-7EGFR transactivation is a viable therapeutic approach. Greater than additive anti-tumor responses were seen with Rilotumumab and AMG 706 when used in combination with Panitumumab.

ET-047. COMBINED INHIBITION OF HER1/EGFR AND RAC1 YIELDS CYTOSTASIS AND INDUCES APOPTOSIS IN A CASPASE-INDEPENDENT MANNER BY DOWN-REGULATION OF SURVIVIN

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BACKGROUND: We have previously reported that combined inhibition of the epidermal growth factor receptor by erlotinib and of RAC1 by NSC23766 yielded a synergistic antiproliferative effect on established and primary glioblastoma cells. The current study aimed at identifying the cellular and molecular mechanisms underlying this observation. METHODS: Staining for annexin/PI or carboxyfluorescein succinimidyl ester was performed prior to flow cytometric analysis in order to determine the induction of apoptosis, necrosis or cytostasis in U87 and A172 glioblastoma cell lines. Moreover, expression of Ki67 was determined by immunofluorescence and microscopic analysis. Induction of senescence was examined by staining for b-galactosidase, and the expression of cell cycle proteins was analysed by Western blot. All analyses were performed after 144 h of continuous exposure to erlotinib, NSC23766 or both at the respective inhibitory concentration 50. RESULTS: Combined treatment with erlotinib and NSC23766 resulted in a reduced number of cell divisions and a significantly decreased Ki67 expression when compared to single agent treatments. In addition, apoptosis was induced independent of activation of caspase3. On the molecular level, concomitant treatment with both agents resulted in a pronounced downregulation of cyclin D1, cyclin-dependent kinases 2, 4 and 6, as well as of survivin when compared to treatments with either agent alone. CONCLUSION: In this study, we demonstrate that the combined treatment with erlotinib and NSC23766 markedly inhibits cell cycle progression and induces apoptosis which is most likely responsible for the antiproliferative synergism of both agents. Overall, our data suggest that the combination treatment with erlotinib and a RAC1 inhibitor may represent a promising novel therapeutic approach in glioblastoma.

ET-048. OLANZAPINE INHIBITS PROLIFERATION, MIGRATION AND ANCHORAGE-INDEPENDENT GROWTH IN HUMAN GLIOBLASTOMA CELL LINES

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BACKGROUND: The poor prognosis of patients with glioblastoma fuels the search for more effective therapeutic compounds. This study is based on the strategy to identify drugs that are widely used in non-oncological diseases and possess anti-neoplastic activity as a "side effect" within their original indication. Our data show that the neuroleptic agent olanzapine has anti-tumor activity against glioblastoma cell lines in vitro and may have potential for repurposing. METHODS: The anti-proliferative effect of olanzapine was examined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays and cell count analysis. Staining for annexin V/propidium iodide or carboxyfluorescein succinimidyl ester was performed prior to flow cytometric analysis in order to determine induction of apoptosis, necrosis or cytostasis in U87MG and A172MG human glioblastoma cell lines. Moreover, soft-agar assays were performed with U87MG cells exposed to 10 μM, 20 μM or 40 μM olanzapine for 21 d. In addition, the inhibitory effect of olanzapine on the migratory capacity of U87MG and A172MG cells was analyzed by transwell® assays. RESULTS: Treatment with olanzapine resulted in a marked anti-proliferative effect on U87MG, A172MG, and U118MG cell lines with IC_{50} values ranging from 25-50 μM. Olanzapine induced apoptosis and inhibited cell division in U87MG and A172MG cell lines. In A172MG cells, migration was shown to be inhibited in a dose-dependent manner. Moreover, in U87MG cells, anchorage-independent growth was also dose-dependently inhibited. CONCLUSIONS: These data demonstrate that olanzapine, a neuroleptic agent with a reasonable clinical safety profile, has potential for repurposing against glioblastoma. Further studies are highly warranted.

ET-049. NANOVEHICLE-MEDIATED DELIVERY OF ANTI-APE1 siRNA SENSITIZES PEDIATRIC BRAIN TUMOR CELLS TO THE DNA DAMAGING EFFECTS OF RADIATION THERAPY

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Radiotherapy (RT) is an integral component of the treatment for medulloblastoma (MB) and the only effective adjuvant therapy for ependymoma (EP). Survival is frequently accompanied by one or more radiation-induced adverse developmental and psychosocial sequelae, as MB and EP most frequently occur in children less than 10 years old. These considerations emphasize the need to develop new strategies to enhance the tumoricidal action of RT while sparing adjacent normal tissue. The multifunctional DNA repair protein Ape1/Ref-1 has been implicated in conferring radiation resistance in pediatric brain tumors. Therefore, we hypothesized that knockdown of Ape1 activity through RNAi would sensitize pediatric brain tumor cells to the DNA damaging effects of RT. However, the use of siRNAs in the clinic has been hindered by the lack of safe and effective delivery vehicles. In our previous work, we developed a nanoparticle that could deliver siRNA specifically to brain tumor cells for efficient knockdown of GFP both in vitro and in vivo. Here, we aimed to deliver siApe1 to improve cell kill after RT. Nanoparticles were loaded with siApe1, or siGFP as a control, and used to treat DAOY (MB) and Res196 (EP) cells. Specific knockdown of Ape1 was confirmed at the mRNA level using PCR and protein level using Western blot. Reduction in the abasic endonuclease activity of Ape1 was measured using an Ape activity assay. Cells were then treated with H2O2 and HOCl to mimic the oxidative damage of RT and cell survival monitored through clonogenic assays. Knockdown of Ape1 mRNA decreased Ape1 protein concentrations in the cells and corresponded to reduced Ape1 activity. This, in turn, led to increased sensitivity to the oxidative damage caused by RT. Therefore, these siApe1 loaded nanoparticles may help enhance the therapeutic effect of RT in pediatric brain tumor patients and lead to better life-long outcomes.

ET-050. GENOME WIDE IDENTIFICATION OF MODULATORS OF TEMOZOLOMIDE RESPONSE IN GLIOBLASTOMA

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Temozolomide (TMZ) is a key component of standard therapy for newly diagnosed patients with glioblastoma (GBM). However, the benefit of this drug is limited by the intrinsic or acquired resistance, of which the underlying mechanisms are poorly known. The DNA repair protein methylguanine-DNA-methyltransferase (MGMT) plays a critical role in TMZ resistance. However, even patients with low MGMT often fail TMZ therapy suggesting distinct MGMT-independent resistance mechanisms. We hypothesized that identification of these unrecognized critical molecular targets modulating TMZ response in GBM will provide information that can be exploited clinically. To that end, we used an shRNA library to conduct genome wide screening for modulators of TMZ response in GBM xenograft 22 (GBM22). Using this approach, we have identified a total of 421 candidate modulators of TMZ response, which included 86 (24.3%) enzymes (excluding kinases and phosphatases), 23 (6.5%) kinases, 29 (8.2%) transcription regulators and 6 (1.7%) phosphatases. Some candidates

regulate signaling pathways known to modulate cell survival through modulation of apoptosis and DNA damage repair response (DDR). Of particular interest was retinoblastoma binding protein 4 (RBBP4), a gene which encodes a protein involved in chromatin remodeling. In a confirmatory study, silencing of RBBP4 significantly sensitized TMZ in GBM-derived stem cell neurospheres from GBM12 and GBM22 (relative NS/well for GBM12 treated with 3 microM TMZ: NT siRNA = 0.41 ± 0.08 and RBBP4 siRNA = 0.06 ± 0.1 ($p = 0.002$), whereas for GBM22 treated with 10 microM TMZ was: NT siRNA = 0.58 ± 0.09, RBBP4 siRNA = 0.22 ± 0.05 ($p < 0.01$). In conclusion, shRNA library screening is a useful high throughput approach for identification of modulators of TMZ response in GBM. The ongoing studies are aimed at further evaluating the roles of RBBP4 and other potential druggable targets in the modulation of TMZ response.

ET-051. COMBINATION TREATMENT WITH DELTA24-RGD AND TEMOZOLOMIDE IMPROVES SURVIVAL AND INFLUENCES IMMUNE CELL SUBSETS IN A MURINE MALIGNANT GLIOMA MODEL

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INTRODUCTION: The conditionally replicating oncolytic adenovirus Delta24-RGD specifically lyses tumor cells and is currently tested in clinical phase I/II trials for recurrent malignant glioma. For further clinical development, assessment of the effects of combining Delta24-RGD with conventional therapy is important. We have previously shown in an immune competent glioma mouse model that the efficacy of Delta24-RGD is mainly dependent on an induced anti-tumor immune response. Temozolomide (TMZ) has pronounced effects on the immune system, however synergistic effects of TMZ and Delta24-RGD has been described also. To assess the combined effects, we tested different treatment regimens in an immune competent glioma mouse model. METHODS: C57BL/6 mice were injected intracranially with GL261 cells. TMZ was given for 5 days before (pre-treatment regimen) or after (post-treatment) intratumoral injection of Delta24-RGD. Mice were followed for survival, sacrificed and brains were used for immunohistochemical stainings. RESULTS: The post-treatment regimen significantly improved survival compared to single treatments alone. The pre-treatment regimen does not differ significantly from the virus mono treatment. The immunohistochemical stainings showed no effect of TMZ treatment on the amount of CD4+, CD8+ and CD11b+ cells in the tumors compared to the controls, however MHCII expression and F4/80+ macrophages were severely decreased. Interestingly, the combination regimens showed an increase in these subsets of cells in the tumor, while Delta24-RGD treatment alone did not induce this. CONCLUSION: The post-treatment regimen, where TMZ is administered after viral treatment, improves survival significantly. The most pronounced effect on immune cells is visible in the MHCII+ and F4/80+ subsets, which are strongly increased in the tumor in both combination regimens compared to either treatment alone. Further studies are ongoing to assess synergistic effects of both treatments and the effects on immune cells at earlier time-points.

ET-052. MerTK INHIBITION AS A NOVEL THERAPEUTIC APPROACH TO GLIOBLASTOMA MULTIFORME

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GBM is an aggressive disease known for a highly migratory and invasive phenotype and propensity for recurrence. Clinical therapy of glioblastoma multiforme (GBM) requires innovation because the current standard of care of resection followed by radiotherapy and temozolomide treatment only increases survival time from 12 to 15 months. Foretinib, currently in Phase II clinical trials, is designed as an inhibitor of Met and VEGFR2 receptor tyrosine kinases (RTK). However, it inhibits the TAM subfamily (Mer, Axl and Tyro3) of RTKs, at even lower concentrations. Our laboratory has previously established that the TAM family is found to be upregulated in GBM and responsible for survival and migration while they exhibit little to no expression in normal brain. Short- and long-term proliferation were quantified in GBM cell lines in response to foretinib treatment. Phosphorylated and total levels of the TAM subfamily of receptors and downstream signaling molecules in response to foretinib were assessed by western blotting. Foretinib treatment of a subcutaneous xenograft mouse model under two different treatment paradigms was quantified and overall survival was enumerated. Our findings show that foretinib inhibits MerTK and its downstream targets of Akt, ERK and STAT3, resulting in apoptosis as evidenced by PARP cleavage and reduced survival. Most notably, a subcutaneous xenograft mouse model shows the robust efficacy of foretinib against GBM. This proof of concept study demonstrates that the TAM family of receptors is a relevant target in GBM and foretinib is a novel therapy well suited to combat this disease.

ET-053. ANTI-CANCER AGENT SIRAMESINE INDUCES EFFECTIVE TUMOR CELL DEATH IN GLIOBLASTOMA CELL LINES AND TUMOR STEM CELL CONTAINING SPHEROIDS

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Glioblastoma is the most frequent and malignant brain tumor with the patients having a median survival of 14.6 months. Although these tumors are treated with surgery, radiation and chemotherapy, recurrence is inevitable. A tumor stem cell population combined with the invasive properties of the tumors is believed to be critical for treatment resistance. In the present study, the aim was to investigate the effect of a novel therapeutic strategy using the lysosomotropic detergent siramesine on glioblastomas including both tumor stem cells and invasive tumor cells in the study. The results showed that siramesine effectively killed the standard glioma cell lines U87, U251, T98G and A172 detected by a proliferation and cytotoxicity assay. Loss of acridine orange staining in siramesine treated cells suggested a compromised lysosomal membrane. Co-treatment of the cell lines with siramesine and inhibitors of caspases or cathepsins suggested a differential involvement in cell death of both cathepsins and caspases. Siramesine effectively caused tumor cell death in glioblastoma stem cell containing spheroid (GSS) cultures as shown with propidium iodide uptake. Subsequent immunohistochemical staining with a panel of stem cell markers, a proliferation marker, lysosomal markers, and a caspase 3 marker showed effects of siramesine on all markers except the proliferation marker. The effect of siramesine on cells with an invasive phenotype was investigated in an in vivo-like in vitro invasion model implanting spheroids into rat brain slice cultures. The results suggested the implanted spheroids to be less sensitive towards siramesine, even in concentrations damaging the brain slice cultures. In conclusion, siramesine effectively killed the standard cell lines and the GSS spheroid cultures. The cell death mechanisms varied in the different cell lines used. Only minor effects of siramesine were found on the invasive cells suggesting that the drug is efficient but may have limitations regarding the infiltrative cells.

ET-054. THE BRAIN-PENETRANT anti-HER2 mAb, ANG4043, TARGETS HER2+ BRAIN TUMORS AND INCREASES SURVIVAL IN MICE WITH INTRACRANIAL BT-474 CELL TUMORS

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Availability of targeted therapies for HER2 positive breast cancer, such as the monoclonal antibody (mAb) trastuzumab, has dramatically improved outcomes for this type of cancer, previously associated with poor prognosis. Because mAbs do not readily cross the blood-brain barrier (BBB), the unfortunate consequence of this treatment is that metastatic tumors find the brain to be a sanctuary site, and at least half of HER2+ breast cancer deaths are caused by metastatic tumors in the brain. BBB permeability has been achieved by conjugating a proprietary peptide, Angiopep-2 (An2) to a mAb directed against HER2. An2 is recognized by low density lipoprotein receptor-related protein-1 (LRP1), a transmembrane receptor highly expressed on BBB endothelial cells whose functions include receptor-mediated transcytosis of multiple proteins into the brain. The peptide-mAb conjugate, ANG4043, displays HER2 binding affinity and in vitro anti-proliferative properties similar to those of the native mAb, and achieves therapeutic concentrations in brain following systemic exposure. When athymic nude mice were intracranially implanted with BT-474 tumor cells expressing HER2 and dosed systemically with fluorescently labeled ANG4043, intense fluorescence was observed in brain tumors, whereas little to no signal was detected for the native mAb. Survival studies were also conducted using this model. At 5 mg/kg, a significant survival advantage was observed in mice treated with ANG4043 over those treated with the native mAb or vehicle control. These results demonstrate that ANG4043 enters the brain, targets tumors, and increases survival in a murine model of HER-positive breast cancer brain metastasis. This peptide-mAb conjugate extends the validation of An2 conjugation beyond

small molecules and peptides to include larger molecules such as therapeutic mAbs for development of new brain-penetrant anti-tumor therapeutics.

ET-055. TARGETING HYPOXIA IN GLIOBLASTOMA MULTIFORME WITH A NOVEL OXYGEN CARRIER PROTEIN

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BACKGROUND: To overcome tumor hypoxia, we have developed therapeutic oxygen carriers called Heme-Nitric oxide/Oxygen binding (H-NOX) proteins that are affinity-tuned to selectively deliver oxygen to hypoxic tissues. Solid tumors are often characterized by an abnormal vascular network that leads to regions of hypoxia within the tumor. Hypoxia promotes a variety of biological processes in and around tumor cells such as vasculogenesis, angiogenesis, invasiveness, resistance to cell death, altered metabolism and genomic instability, thus altering tumor cell phenotypes and the local microenvironment. Given its central role in tumor progression and resistance to radiotherapy and chemotherapy, hypoxia is considered a high priority target for cancer therapy. The aim of this study was to examine the hypoxic volume of several murine orthotopic glioblastoma (GBM) models and validate H-NOX-mediated tumor re-oxygenation and its effect on tumor cell proliferation. METHODS: We collected brain tumors from i.v. H-NOX-injected mice bearing intracranial GBM tumors (U251, GBM6, GBM39, GBM43 and GL261). We evaluated hypoxia by immunohistochemistry of endogenous markers: the transcription factor hypoxia-inducible factor 1a (HIF-1a) as well as its downstream target Glut1, and by the exogenous clinically relevant hypoxia marker, pimonidazole. RESULTS: All hypoxia markers exhibited high signal in poorly vascularized areas of U251 and GL261, as determined by co-staining with CD31 endothelial marker. In contrast, GBM6, GBM43 and GBM39 tumors showed significant range in hypoxia marker signal intensity between samples, indicating heterogeneity in extent of hypoxia within and between GBM models. When H-NOX was administered to mice bearing intracranial U251 tumors, H-NOX significantly decreased HIF-1a stabilization and pimonidazole accumulation, demonstrating that H-NOX increases tumor oxygenation. In addition, H-NOX treated tumors exhibited fewer Ki67-positive cells, suggesting that H-NOX-mediated acute re-oxygenation inhibits tumor cell proliferation. CONCLUSIONS: These studies support H-NOX as a promising agent for overcoming tumor hypoxia and enhancing radiotherapy and chemotherapy treatment of GBM.

ET-056. DEVELOPMENT OF NOVEL THIOBARBITURIC ACID DERIVATIVE COMPOUNDS FOR TREATMENT OF GLIOBLASTOMAS

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Previously, we reported the identification of a cytotoxic chemotype compound CC-I (a derivative of thiobarbituric acid) that is effective against chemotherapy resistant glioblastomas (GBMs) and neuroblastoma in an in vitro cell culture and in vivo mouse tumor models. In this study, we determined the cytotoxicity of structurally similar compounds along with CC-I to identify analog compounds that could be more toxic than CC-I. The initial data clearly demonstrate that the thiobarbituric acid compounds with the diene motif have better toxicity than the compounds that have a diene motif which is replaced by single alkene substituents. The study also suggests that the functional group at N1- and N3- sites are important for toxicity. Therefore, we further designed several CC-I analog compounds by manipulating functional substitutions at N1-, N3- and C5-positions (either furan ring or benzene ring) of CC-I. The novel compounds were synthesized from substituted thioureas (key precursors for the synthesis of substituted thiobarbituric acids) and finally by condensing cinnamaldehyde (CMC-2 series: benzene ring compound) or trans-3-(2-furyl)-acrolein (CC-I series: furan ring compound) with appropriately substituted thiobarbituric acids in the presence of catalytic amounts of pyridine. Among the compounds with similar structures, CC-I-v1 and CC-I-v4 are the two most cytotoxic compounds to therapy sensitive (e.g., SW1088, U87-MG, SNB-19) and resistant (e.g., CCF-STTG1, T98G, U343-MG) GBMs. Depending on the GBM cell lines, CMC-2 and CC-I-v3 compounds also showed stronger toxicity than CC-I compound. Currently, we are studying the anti-tumor effect of CC-I-v1 and CC-I-v4 in an in vivo nude mouse tumor model. In summary, this structure-activity study clearly indicates that we can develop more efficacious compounds compared to our original compounds by rational modifications of the chemotype compounds. [This project is supported by the National Cancer Institute of the National Institutes of Health under Award Number R21CA167406]

ET-057. NOTCH PATHWAY ACTIVATION AND RESPONSE TO NOTCH INHIBITION THERAPY IS HETEROGENEOUS IN HUMAN GLIOBLASTOMA

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NOTCH signaling is thought to play an important role in glioblastoma growth and recently has been a focus of study in clinical trials. However, reliable methods for identification of patients with pathway activation are needed, as gamma secretase inhibitors (GSI) used for study are not NOTCH specific and may achieve effects through other pathways. To better assess NOTCH1 activation in patients we utilized a specific and sensitive antibody to directly visualize NICD1 by immunohistochemistry. Analysis of 119 GBM cases revealed nuclear NICD1 in two compartments: tumors cells and vascular endothelial cells. NICD1 activation in tumor cells showed a remarkably heterogeneous pattern within and across patients. No patient exhibited more than 30% of tumor cells with activation. 28% had high NOTCH1 activation (>15% of tumor cells positive), 21% medium activation (10-15% of tumor cells positive), and 15% trace activation (5-10% of tumor cells positive). All patients had similar levels of intensity for NICD1 staining in positive cells. Interestingly, 36% of patients showed no evidence of significant NICD1 expression in tumor cells. This suggests such populations of GBM patients may not be reasonable candidates for NOTCH therapy, a principle unexamined in prior clinical trials. Tumor vessels showed strong NICD1 signal in the endothelial cells of most patients, with higher intensity in proliferative abnormal tumor vessels relative to normal vessels. As a foundation for preclinical evaluation of pathway specificity, we identified one negative and three positive GBM cell lines for NICD1 expression and demonstrated preservation and faithful recapitulation of NICD1 activation states in matching xenograft and patient primary tumors. Treatment of these models with GSI resulted in an appropriate reduction in NICD1 expression and growth decrease only in NICD1 activated GBM lines. These studies provide strong evidence and tools for further evaluation of NOTCH in GBM and the clinical trial setting.

ET-058. HYPEROXIA RESENSITIZES CHEMORESISTANT GLIOBLASTOMA CELLS TO TEMOZOLOMIDE THROUGH PROLONGED ACTIVATION OF UNFOLDED PROTEIN RESPONSE (UPR)

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INTRODUCTION: Intra-tumoural hypoxia is associated with increased cell survival and chemoresistance in glioblastoma through the adaptive unfolded protein response (UPR). The latter maintains cellular homeostasis during endoplasmic reticulum (ER) stress response but its prolonged activation may also activate multiple apoptotic pathways. We have previously shown that the UPR chaperone protein prolyl 4-hydroxylase, beta polypeptide (P4HB) played an important role in determining TMZ-sensitivity. Moreover, normobaric hyperoxia could resensitize chemoresistant glioblastoma cells to temozolomide (TMZ). The relationships between these findings were unclear. OBJECTIVE: To investigate whether and how the chemosensitizing effect of hyperoxia is mediated through UPR-related pathways. MATERIALS AND METHODS: Isogenic TMZ-resistant human GBM cells (D54 and U87) were treated with/without TMZ at different concentrations of atmospheric oxygen. We studied changes in the expressions of two UPR activators (PERK and IRE1-α) and other UPR transcriptional factors (XBP-1, ATF3, ATF4 and CHOP) using western blotting and qPCR. We also studied their potential upstream regulator, P4HB, in cells with forced P4HB expression for differential responses. RESULTS: Combining hyperoxia with TMZ enhanced cell death that was associated with prolonged UPR activation. Normobaric hyperoxia sensitized chemoresistant cells to TMZ through CHOP-induced apoptosis by reducing P4HB expression and activating

PERK and 1RE1-α. The role of P4HB in mediating hyperoxia-induced UPR was confirmed in P4HB over-expressing cells, which showed reduced cell death and diminished expressions of UPR activators and their downstream regulators. CONCLUSION: UPR has received considerable attention recently for its role in determining malignant behavior and chemosensitivity. Hyperoxia can enhance response to TMZ in glioblastoma and its effect is likely to be mediated through UPR-related pathways. Hyperoxia should be further investigated as a potential chemotherapeutic adjunct. UPR-related regulators may also be exploited as potential markers of intra-tumoural hypoxia and TMZ-resistance, and predictors of treatment response to hyperoxia-enhanced therapy.

ET-059. CLINICAL CHARACTERISTICS AND TREATMENT OF ECTOPIC MENINGIOMAS

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We have examined the clinical characteristics and treatment of ectopic meningiomas (EMs). Samples from 17 patients with EMs were analyzed, and their clinical char-acteristics, mechanism, and treatment were studied in combination with the literature. The main clinical manifestations of EMs included increased intracranial pressure, epilepsy, local mass, and local occupying effects, but diagnosis of EMs depended on the pathology. Surgical removal can achieve the double objectives of confirmed diagnosis and treatment of tumors. The clinical characteristics of EMs vary with the sites of tumors. Operation is the treatment of first choice. Prognosis is better than that of typical meningiomas.

ET-060. TARGETED MRI CONTRAST AGENT ALONGSIDE OF DRUG: INTERLEUKIN-13-GADOLINIUM-DTPA-DOXORUBICIN-LIPOSOMES IN IMAGE-GUIDED DRUG DELIVERY FOR DETECTION AND TREATMENT OF GLIOMA

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Treatment of glioma, a malignant central nerve system (CNS) cancer, is challenging due to the blood-brain barrier (BBB) that prohibits most of anti-cancer chemo agent to penetrate. Furthermore, to detect brain cancer, MRI contrast agent such as Gadolinium-DTPA (Magnevist) is limited to the cases where the BBB is significantly compromised by the cancer because Gd-DTPA itself is not able to cross the BBB. As a result, the cancer boundary presented by Gd-DTPA-enhanced MRI reflectes only the extent where BBB is compromised instead of all the cancer-infiltrated regions. It is most advantages to develop a delivery system that carries MRI contrast agent with drug alongside, which could penetrate brain tissue, detect and treat glioma at same time. Previous work demonstrated: 1) IL-13Rα2 was over-expressed in glioma, 2) IL-13-liposomes-doxrubinsine inhibited an intracranial glioma growth and 3) IL-13-Gd/DTPA-liposomes as a MRI contrast enhanced and detected infiltrating glioma. Based on these findings we hypothesize that IL-13-liposome encapsulating Gd-DTPA and doxorubicin (Dox) is a theranostic agent for detection and treatment of gliomas. To test our hypothesis, glioma U251, U87 and glioma stem cells T3691 and athymic nude mice with intracranial glioma were selected as our in vitro/ and in vivo model. IL-13-Gd/DTPA-Dox-Lip was developed and characterized. Its feasibility and therapeutic efficacy will be further evaluated through targeted MRI guided drug delivery. In summary, the development of IL-13-Gd/Dox-Lip will lead to not only a translation into clinical neuro-oncology, but also into treatment of other tumors such in breast, lung, liver and pancreas.

ET-061. ENHANCEMENT OF CEDIRANIB ANTI-GLIOMA EFFICACY VIA COMBINATION WITH AUTOPHAGY INHIBITOR QUINACRINE

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Despite robust vascularity of malignant gliomas, anti-angiogenic therapy largely fails to induce durable responses. Autophagy, a cytoprotective cellular degradation pathway, can promote drug resistance and its late-stage inhibition can induce tumor cell death. We hypothesized that efficacy with Cediranib, a VEGF/PDGF receptor tyrosine kinase inhibitor, can be enhanced via combination with the late-stage autophagy inhibitor Quinacrine. To investigate this, we exposed 4C8 glioma cells to Cediranib, Quinacrine or the combination, under normoxic and hypoxic (0.5% O_2) conditions. Cell viability assays revealed dose-dependent cytotoxic effects of each agent under both conditions but a greater than additive combined efficacy under hypoxic conditions. Western blotting for autophagic vacuole (AV) marker LC3-II, indicated increases with Quinacrine as well as Cediranib, suggesting that Cediranib stimulates autophagic flux. Maximal LC3-II accumulation and Caspase 3 cleavage were observed with Cediranib + Quinacrine/Hypoxia. This effect was abrogated with 3-Methyladenine, an early-stage autophagy inhibitor which prevents AV formation. Employing a dual-bolus perfusion MRI approach, we assessed relative cerebral blood flow and volume (rCBF, rCBV), and vascular permeability (K^{trans}) in intracranial 4C8 mouse glioma. Cediranib or quinacrine monotherapy did not alter tumor growth, while combined Cediranib/quinacrine reduced it by over 2-fold ($p < 0.05$). Cediranib or quinacrine monotherapy did not significantly alter mean tumor rCBF or K^{trans} compared to untreated while combined Cediranib/quinacrine substantially reduced both ($p < 0.05$), indicating potent tumor devascularization. Tumor necrosis and mean vessel density (MVD), assessed immunohistologically, were unchanged by Cediranib or quinacrine monotherapy. In contrast, MVD was reduced by nearly 2-fold ($p < 0.01$), and necrosis increased by 3-fold ($p < 0.05$ one-tailed) in Cediranib/quinacrine treated versus untreated. In conclusion, hypoxia-potentiated autophagic flux stimulation by Cediranib, combined with late stage autophagic inhibition by Quinacrine, induces AV accumulation and glioma cell death. Combined Cediranib/quinacrine treatment synergistically increased anti-vascular/anti-tumor efficacy in intracranial 4C8 mouse glioma, suggesting a promising and facile treatment strategy for malignant glioma.

ET-062. IN VIVO MODELING OF ACQUIRED DRUG RESISTANCE: GBM ADAPTATION TO SUSTAINED cdk4/6 INHIBITION

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We have previously shown that cdk4/6 inhibitor PD0332991 (Palbociclib) has significant anti-proliferative activity against, and significantly extends the lives of animal subjects with orthotopic GBM xenografts having intact Rb function. Despite its activity, Rb proficient xenografts exposed to daily administration of Palbociclib eventually grow, with treated animals ultimately succumbing to tumor burden. In attempt to understand GBM adaptation to sustained cdk4/6 inhibition, mice with intracranial U87 xenografts were administered daily Palbociclib, and resected tumors from four mice, at time of required euthanasia from tumor burden, were transplanted subcutaneously into the flanks of 4 mice that continued to receive daily Palbociclib. After 58 days of additional daily treatment with Palbociclib, one of the subcutaneous tumors began growing rapidly, and this tumor was resected, then disaggregated and used to intracranially inject a new series of mice. Mice injected with Palbociclib-resistant U87, and receiving continued daily Palbociclib treatment, showed no survival benefit relative to mice left untreated following intracranial injection of Palbociclib-treated U87, supporting our development of a Palbociclib resistant derivative of U87. Palbociclib resistant U87 cells showed more rapid intracranial growth than did cells obtained from untreated U87 subcutaneous xenografts, and intracranial injection of the resistant cells resulted in significantly reduced lengths of survival relative to mice receiving intracranial injection of previously untreated U87. Data from the molecular analysis of paired Palbociclib-resistant vs. untreated tumors, from two distinct xenograft models, are being collected and analyzed now, and will be presented at the meeting. Importantly, our approach for developing therapy resistant GBM xenografts should prove generalizeable for use in identifying tumor changes that are potentially responsible for acquired resistance to treatment, some of which may provide opportunity for informed treatment of recurrent GBM with rational therapies.

ET-063. DUAL mTORC1/2 BLOCKADE WITH AZD8055 INHIBITS GROWTH OF GLIOBLASTOMA BRAIN TUMOR STEM CELLS

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The emerging field of targeted molecular therapeutics holds significant promise for treatment of glioblastoma (GBM), but has failed to achieve

more than modest efficacy in select patient subsets in recent clinical trials. Data from the Cancer Genome Atlas and other large genomic studies demonstrate that the epidermal growth factor receptor (EGFR) and PI3Kinase/mTORC1/2 pathways are frequently altered in GBM. However, pharmacological targeting of EGFR and PI3Kinase signaling in GBM has been not been promising. A lack of relevant models has rendered it difficult to assess whether targeting these pathways might be effective in molecularly defined subgroups of GBMs. In this study, human brain tumor stem cell (BTSC) lines with different combinations of endogenous EGFR wild type, EGFRvIII and PTEN mutations were used to investigate response to the EGFR inhibitor Iressa, the mTORC1 inhibitor rapamycin and the dual mTORC1/2 inhibitor AZD8055. We confirm that Iressa and rapamycin have modest effects in most BTSC lines, but AZD8055 was highly effective at inhibiting Akt/mTORC2 activity and dramatically reduced the viability of BTSCs regardless of their EGFR and PTEN mutational status. Moreover, AZD8055 was synergistic with the alkylating agent temozolomide (TMZ). These data suggest that dual inhibition of the mTORC1/2 may be of benefit in GBM including the subset of TMZ-resistant GBMs. Funding acknowledgements: Alberta Cancer Foundation and Stem Cell Network

ET-064. DISULFIRAM WHEN COMBINED WITH COPPER IS AN EFFECTIVE ADJUVANT THERAPY WITH TMZ FOR TREATMENT OF HUMAN GLIOBLASTOMA

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GBM is one of the most lethal cancers in humans, and with current therapy, survival is limited to just 15 months. Current barriers to successful treatment include the identification of a stem-like population of glioma cells, termed brain tumor-initiating cells (BTICs) that have been shown to re-populate a tumor and confer resistance to conventional therapies. To develop therapeutic strategies that target BTICs we have been investigating treatments adjunctive to current standard-of-care. Our focus has been on exploring already-marketed (clinically approved) drugs that show therapeutic potential against the stem-like glioma population. Using a high-throughput in vitro drug screen we found that Disulfiram (DSF), an off-patent drug previously used to treat alcoholism, in the presence of copper gluconate, had low nanomolar efficacy against patient-derived-BTICs, including the highly infiltrative, quiescent population. We determined that disulfiram inhibited the 26S proteasome in a copper-dependent manner. Consistent with being a potent proteosome inhibitor, DSF-Cu induced activation of both heat shock and unfolded protein responses in patient-derived BTICs as assessed by global gene expression profiling. In addition we found that all patient-derived BTICs tested, including those resistant to TMZ, were sensitive to DSF-Cu, and that low dose DSF-Cu significantly augmented TMZ activity in vitro. Moreover and importantly, we found that DSF-Cu in combination with TMZ significantly prolonged survival in intracranial patient-derived BTIC models in vivo. This observation suggests that DSF-Cu has the potential to be repurposed for the treatment of refractory GBM, including highly infiltrative glioblastoma.

ET-065. TARGETED LIPOSOMES FOR THE TREATMENT OF PERIPHERAL NERVE SHEATH TUMORS

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Neurofibromatosis type-1 (NF1) is an autosomal dominant disorder often leading to neurocutaneous tumors either benign or malignant. Currently NF1 tumors are treated by surgery, anti-angiogenic or farnesyl transferase inhibitor therapy. In our present investigation we observed the IL13Ra2, a receptor for interleukin-13 expression in several peripheral nerve sheath tumors and corresponding cell lines which can be a potential target. As NF1 associated MPNST's has elevated Ras-GTP compared to sporadic MPNST, combination of Ras inhibitors with other chemotherapeutic agents is a significant approach to combat this aggressive tumor. Our aim is to combine IL-13 conjugated liposomal doxorubicin and Ras inhibitors to target the tumors selectively and enhance its therapeutic potential. The specific binding and cytotoxic effect of the IL-13 conjugated liposomal doxorubicin (IL13LIPDXR) in the in vitro monolayer and 3D MPNST cell culture models were demonstrated. We also investigated the cytotoxic effect of farnesyl thiosalicylic acid (FTS), a Ras inhibitor and IL13LIPDXR when added concomitantly to established MPNST cells lines and demonstrated enhanced cytotoxicity. The lipid bilayer of the doxorubicin liposomes was modified with FTS and their in vitro cytotoxic effect was studied. Experiments are in progress to determine the ratio of Ras inhibitor/doxorubicin in the IL-13 conjugated liposomes for optimal efficacy and the mechanism of action on PNST cells. Sciatic nerve tumor model was developed by implanting sNF96.2 or ST88-14 cells in the sciatic nerves and the tumor growth was observed in 8 weeks as evidenced by the morphology of the sciatic nerves microscopically. IL13Rα2 receptor expression in sciatic nerve tumor tissues was evident from the immunohistochemistry (IHC) and immunoblots but not in the normal control nerves. IHC was also performed for determining the expression Ki-67 (cell proliferation), heavy chain ferritin, S100 (myelin forming Schwann cells). Therapeutic evaluation of the targeted liposomes in the tumor model is in progress.

ET-066. CILENGITIDE AFFECTS MENINGIOMA CELL SURVIVAL AND MOTILITY

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We analyzed the impact of the integrin inhibitor cilengitide on migration, proliferation and radiosensitization of meningioma cells. We first measured integrin expression in tissue microarrays of human meningiomas, derived from tumors of all WHO grades. We further studied the anti-meningioma properties of cilengitide in cell cultures, subcutaneous and intracranial nude mouse models, with and without irradiation. The heterodimer $\alpha v\beta 5$ was the predominantly expressed integrin in meningiomas, while $\alpha v\beta 3$ was mainly observed in tumor blood vessels. The cilengitide-induced decrease of proliferation and cell viability, as measured in BrdU- and microtitertetrazolim (MTT)-assays was only moderate, even if up to 100 μg/ml cilengitide were applied. However, irradiation enhanced the effects on cell survival. As expected, a much lower concentration (1 μg/ml) cilengitide was sufficient to significantly inhibit meningioma cell migration and invasion in vitro in transwell-assays, using matrigel-covered polycarbonate filters with 8 μm pores. A daily dosage of 8 or 75 mg/kg (i.p.) did not affect the volumes of tumors growing under the skin of nude mice or intracranially, as determined by calliper rule estimation or MRI-based tumor volume determination, respectively. However, a combination of 75 mg/kg cilengitide daily and irradiation (2 x 5 Gy) led to a 67% reduction of MRI estimated tumor volumes in the intracranial model ($p < 0.01$), whereas the corresponding reduction reached by irradiation alone was only 55% ($p < 0.05$). These results suggest that cilengitide has only mild cytostatic properties in meningioma cells, but strongly inhibits meningioma cell migration and invasion. Although further mouse experiments are required to support a potential synergism between the integrin inhibitor and irradtiation, these preliminary results suggest that a combination of the drug with radiotherapy may help to further reduce meningioma recurrence in cases of incomplete resection.

ET-067. HIGH-THROUGHPUT IN VITRO SCREENING OF GLIOMA STEM CELL LINES: EVALUATION OF OVER 350 TYROSINE KINASE INHIBITORS

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Despite the availability of hundreds of drugs, there is little data on the efficacy of these agents in the extremely heterogeneous populations of tumor cells observed in glioblastoma. To identify potentially efficacious inhibitors, a high-throughput compound-screening assay was used to identify drug sensitivities in a panel of glioma stem cell (GSC) lines to 350 chemotherapeutic tyrosine kinase inhibitors (GSK PKIS1) with varying cellular targets and mechanisms of kinase inhibition. Five glioma stem cell lines have been screened at the time of this abstract with 10 additional lines in the queue, with the complete panel being representative of the classic TCGA subtypes. Cell lines were screened in 384-well plates at a density of 1000 cells/well with drug concentrations of either 5 or 0.5 μM. Cell viability measurements were taken using Cell-Titer Glo 5 days after drug treatment and compared to control wells to calculate percentage inhibition. Compounds were grouped based on inhibition of cell proliferation: >80%, 80-50%, 50-30%, and <30%. Of the 350 compounds, 101 compounds inhibited >50% of the cells in at least 1 of 5 cell

lines. Most impressively, 6 compounds exhibited >80% inhibition in all 5 lines at the lower 0.5 uM concentration. These compounds belonged to two drug classes- three glycogen synthase kinase-3 (GSK-3) inhibitors and three cyclin-dependent kinase (CDK) inhibitors. Five additional compounds were found to inhibit viability above 80% at 0.5 μM in 2 of 5 cell lines. A separate set of compounds containing commonly used tyrosine kinase inhibitors was screened for comparison of drug efficacy. The relationship between GSC molecular phenotype and drug sensitivity as well as the relationship between drug sensitivity and drug structure will be presented. HTS is an effective and efficient method to identify effective inhibitors in subsets of molecularly distinct gliomas and may accelerate clinical trial development.

ET-068. A COMBINATION OF INTERFERON-BETA AND TEMOZOLOMIDE AUGMENTS ANTI-TUMOR EFFECTS THROUGH p73/YAP-MEDIATED APOPTOSIS BY PML IN GLIOBLASTOMA

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BACKGROUND AND PURPOSES: Interferon-β (IFN-β) sensitizes temozolomide (TMZ)-resistant glioma cells by the transcriptional down-regulation of the DNA repair gene methyl guanine methyl transferase (MGMT) and augments anti-tumor effects. Promyelocytic leukemia (PML) is an important regulator of p53 function for certain kinds of DNA and a gene induced by IFN-β. We previously reported that the level of PML protein in human glioma tissue decreased as the degree of malignancy increased. and that PML overexpression in GBM cells transfected with plasmid DNA induced apoptosis, which was mediated by caspase-8 activation. Based on these findings we hypothesized that the increase in endogenous PML expression induced by IFN-β contributes to its anti-tumor effects independently of MGMT in glioma cells subjected to combination therapy with IFN-β and TMZ. We focused on the role of PML and the interaction between PML and p73/YAP. METHODS: The human glioblastoma cell lines U87MG, T98G, U251MG and the U87MG xenograft mice model were used. The antitumor effects and the mechanisms underlying the efficacy were assessed by genomic and proteomic analysis. The GBM cells were transfected with siRNA to suppress the human PML gene using the HiPerFect transfection. RESULTS: In the combination therapy, an IFN-β-induced increase in endogenous PML contributed the anti-tumor effects in p53 wild- and mutant glioma cells and in the xenograft mice model. The increased PML promoted the accumulation of p73, a structural and functional homolog of p53, to fuse the coactivator Yes-associated-protein in the PML nuclear bodies. CONCLUSION: The adjuvant therapy targeted at PML may be a promising therapeutic strategy for glioblastoma.

ET-069. NOVEL EGFR/DNA BINARY TARGETING MOLECULE ZRBA1 POTENTIATES RADIATION RESPONSE IN GLIOBLASTOMA TUMOR MODEL

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Glioblastoma is the most common and aggressive malignant primary brain tumor in humans with a poor clinical outcome. Although no contemporary therapies are completely curative combined chemo-radiation (STUPP) has led to improved overall survival. Therefore development of new targeted drugs and their combination with radiation offer new treatment possibilities. The purpose of our study was to evaluate the radiosensitizing abilities of ZRBA1 combi-molecule designed to block both EGFR-TK signaling pathways while additionally inducing significant levels of DNA damage. In cancer cells the binary property of ZRBA1 can be limited by DNA repair mechanism therefore in order to increase its efficacy we combined this drug with ionizing radiation. The effect of ZRBA1 on the radiosensitivity of three human glioblastoma cell lines (U87, U343 and U373) was evaluated using MTT and clonogenic assays whereas DNA damage/repair and cell cycle were evaluated using γH2AX antibody and FACS assay. Our results demonstrate that exposure of cells to 25uM of ZRBA1 resulted in an increase in radiosensitivity of all tested cell lines with dose enhancement factors at a surviving fraction of 0.1 ranging from 1.4 to 1.7. Importantly, in contrast to Temozolomide which enhances radiation response most effectively in MGMT-negative cells, radiosensitizing proprieties of ZRBA1 does not depend on the MGMT methylation status. Additionally, as a measure of DNA double strand breaks, the number of gH2AX foci per cell was significantly greater at 24h after the combined modality compared with the individual treatments with radiation, Temozolomide or ZRBA1 alone. Overall, our results have demonstrated of increased levels of DNA double strand breaks when ZRBA1 was combined with radiation and suggests that the radiosensitizing proprieties of ZRBA1 relay on inhibition of DNA repair and cell cycle arrest. Therefore we postulate that ZRBA1 may be developed as a potent and innovative radiosensitizing agent for malignant glioma tumors.

ET-070. FORETINIB INHIBITS INVASION, A HALLMARK OF MALIGNANCY, IN GLIOBLASTOMA MULTIFORME

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Malignant glioblastoma (GBM) is a devastating primary malignancy of the brain, with a median patient survival of less than 15 months. One of the primary challenges to effective treatment is the inability to fully resect the primary tumor due to the invasive phenotype of GBM cells. The development and use of targeted therapies that specifically curb the invasive potential of glioma prior to resection may serve to enhance current outcomes and improve patient survival of this devastating disease. TAM (Mer, Axl, and Tyro-3) receptor tyrosine kinase (RTK) expression is increased in malignant glioma and correlates with rapid progression of the disease. Further, preliminary data suggests that TAM RTK expression is up-regulated in induced neurospheres compared to monolayer GBM cells. From this, we hypothesize that TAM RTK expression is the driving force behind the migratory phenotype and direct inhibition of TAM receptors may abrogate the invasive phenotype. The RTK inhibitor, foretinib, inhibits several RTKs, and notably all three TAM RTKs at nanomolar concentrations in vitro and is currently being studied in Phase II clinical trials for the treatment of a variety of solid tumors. In this study, the effects of foretinib treatment on the migratory and invasive phenotypes of glioblastoma derived cell lines were assessed using a transwell migration assay as well as by embedding neurospheres derived from glioblastoma cell lines in a three-dimensional (3D) collagen matrix. In both the transwell assay and 3D collagen invasion assay, treatment with 100 nM foretinib significantly inhibited invasion and migration of U251 and SF188 cells at 8, 15, and 24h time points when compared to DMSO treated controls. This finding indicates that administration of foretinib to glioblastoma patients prior to surgical resection or radiation therapy may enhance current clinical outcomes by inhibiting the ability of glioblastoma cells to invade into the normal brain tissue.

ET-071. PRECLINICAL HIGH-DOSE ACETAMINOPHEN WITH N-ACETYLCYSTEINE RESCUE ENHANCES THE EFFICACY OF CISPLATIN CHEMOTHERAPY IN ATYPICAL TERATOID RHABDOID TUMORS

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BACKGROUND: Atypical teratoid rhabdoid tumors (AT-RT) are pediatric tumors of the central nervous system with limited treatment options and poor survival rate. We investigated whether enhancing chemotherapy toxicity by depleting intracellular glutathione (a key molecule in cisplatin resistance) with high dose acetaminophen, may improve therapeutic efficacy in AT-RT in vitro. PROCEDURE: BT16 (cisplatin-resistant) and BT12 (cisplatin-sensitive) AT-RT cell lines were treated with combinations of acetaminophen, cisplatin, and the anti-oxidant N-acetylcysteine (NAC). Cell viability, glutathione and peroxide concentrations, mitochondrial damage and apoptosis were evaluated in vitro. RESULTS: Acetaminophen enhanced cisplatin cytotoxicity in cisplatin-resistant BT16 cells but not cisplatin-sensitive BT12 cells. Baseline glutathione levels were elevated in BT16 cells compared to BT12 cells, and acetaminophen decreased glutathione to a greater magnitude in BT16 cells than BT12 cells. Unlike BT12 cells, BT16 cells did not have elevated peroxide levels upon treatment with cisplatin alone, but did have elevated levels when treated with AAP + cisplatin. Both cell lines had markedly increased mitochondrial injury when treated with AAP + cisplatin relative to either drug treatment alone. The enhanced toxic effects were partially reversed with concurrent administration of NAC. CONCLUSIONS: Our

results suggest that AAP could be used as a chemo-enhancement agent to potentiate cisplatin chemotherapeutic efficacy particularly in cisplatin-resistant AT-RT tumors with high glutathione levels in clinical settings.

ET-072. EFFECTS OF LAZAROID U-74389G LIPOSOMES IN A GLIOBLASTOMA MOUSE MODEL

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BACKGROUND: LAZ is a 21-aminosteroid that has radioprotective effects against radiation-induced lipid peroxidation. Also antiproliferative effects have been reported against glioblastoma cell lines. DESIGN/METHODS: LAZ PEGylated liposomes (Lipo G) were developed at the University of Houston.. Glioblastoma cell line U87-expressing firefly luciferase reporter gene (100,000 cells in 2 μL) was injected intracranially in each SCID mouse. There were 4 treatment groups (n = 8-9, each): brain model (M) without treatment (control), radiation 2Gy weekly (M + R), Lipo G at 5 mg/kg dose intraperitoneally twice per week (M + L) and radiation with Lipo G (M + R + L). Treatment lasted three weeks. Tumor size was monitored using bioluminescence imaging (BLI), in each mouse. Mice were sacrificed after 3 weeks. Brain was harvested. Lipid peroxidation of brain tissues was quantified by measuring malondialdehyde (MDA) as a surrogate biomarker. Survival was evaluated using Kaplan Meier analysis at P= 0.05. RESULTS: BLI intensity was 4002.03 ± 1737.67, 2034 ± 737.72, 1387.36 ± 684.53 and 2498.89 ± 2521.32 % for M, M + R, M + L and M + R + L, respectively. Tumor size of the M + L group was reduced by 65% compared to control. There was no significant difference in tumor size of radiated groups compared to control group. MDA brain concentration in M + L and M + R + L groups was significantly less than in M + R group (8.27 ± 0.78 and 10.37 ± 3.30 μM/gm vs. 23.09 ± 3.79 μM/gm). The survival mean was 22.67, 25.33, 25.22 and 27.13 days for M, M + R, M + R + L and M + L groups, respectively. Mean survival of LAZ treated groups (M + L and M + R + L) was significantly longer than that of the control group. CONCLUSIONS: LAZ liposomal formulations reduced tumor growth by 65%. LAZ also protected brain tissue from radiation-induced lipid peroxidation by reducing MDA concentration by 50%. These provocative data warrant further investigation of LAZ as a radiation protectant and chemotherapeutic agent.

ET-073. COMBINING PARP INHIBITORS WITH TMZ: IMPROVED EFFICACY OBSERVED IN RECURRENT PATIENT-DERIVED CELL LINES COMPARED TO PRIMARY CELLS

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Glioblastoma (GBM) is a highly aggressive malignant brain tumor. After initial therapy fails, therapeutic options are limited and generally not effective. There is no standard of care for recurrent GBM. Median time to progression at this stage is about 10 weeks and overall survival ~30 weeks. Response to the standard therapy regime of radiotherapy (RT) combined with temozolomide (TMZ) (chemoradiotherapy) is mediated, in part, by MGMT methylation status. We observed significant sensitivity in our patient derived cell lines that are MGMT methylated in response to Temozolomide (TMZ) (n = 4, IC50 range: 11-100 μM). One patient derived cell lines tested unmethylated and the response to TMZ was considerably less with an IC50 of 384 μM). In addition we derived cells from 2 tumors from patients who had relapsed and subsequently re-operated on (post chemoradiotherapy). Both cell lines were MGMT unmethylated and demonstrated high IC50s (450 μM, 300 μM). Poly (ADP-ribose) polymerase (PARP) plays a key role in base excision repair (BER) pathway by binding to processed single strand breaks. PARP inhibitors have been used to treat several cancers including GBM with moderate efficacy reported. Herein we show that the addition of a PARP inhibitor may be efficacious in the recurrent setting. By adding the PARP inhibitor, ABT-888 (10mM) to cell lines treated with various concentrations of TMZ, we saw a dramatic improvement in sensitivity specific to the recurrent lines with IC50 shifts of 450mM to 212mM for G13 and 300mM to 100mM for G28. No synergy between ABT-888 and TMZ was observed in the primary cell lines, independent of the MGMT methylation status. We are currently treating the combination treatment in mice with orthotopically implanted recurrent and primary patient-derived cell lines.

ET-074. MiR-655 ENHANCES THE SENSITIVITY OF GLIOBLASTOMA TO TEMOZOLOMIDE THERAPY

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It is well known that O^6-methylguanine-DNA methyltransferase (MGMT) is related to resistance of aggressive brain tumor glioblastoma (GBM) to DNA alkylating agent Temozolomide (TMZ) therapy. In this study, we searched for microRNA that modulate MGMT expression and give better sensitivity to TMZ. We picked up 6 microRNAs out of tens of candidates from several binding site prediction softwares, which have complementary seed region with 3'-UTR of MGMT. One out of 6 microRNAs down regulated more than 40% MGMT mRNA and protein level in both T98G glioma cell line and primary GBM cell line (GBM30). Direct interaction between miR-655 and 3'-UTR of MGMT mRNA was suggested by reporter assay. MicroRNA transfected T98G cells showed increased sensitivity to TMZ as well as U87MG which cell line is not expressing MGMT and the T98G cells transfected with siRNA of MGMT. We will also show that microRNA expressing GBM30 transplanted in nude mice showed better survival with injection of TMZ in vivo. Thus, this endogenous mechanism of suppressing drug resistance gene MGMT will increase chemo-sensitivity to TMZ. Further study of TMZ effecting cell proliferation and survival related to miR-655 will be discussed. This study will provide a new target to enhance efficacy of chemotherapy.

ET-075. INFLUENCE OF TIME OF TREATMENT ON THE OUTCOMES FOR IN VIVO THERAPEUTIC EFFICACY STUDIES

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A frequently overlooked factor that influences the outcome of in vivo therapy-response experiments, involving orthotopic brain tumor engraftment models, is the time of treatment initiation. We hypothesized that extent of survival benefit, to animals receiving treatment, is increased by initiating treatment sooner to the time of tumor cell implantation. To test this we conducted experiments involving cytotoxic and cytostatic therapies for treating mice with intracranial xenograft tumor. For the former, cohorts of mice with intracranial U251 tumor were treated with single administration of temozolomide (50 mg/kg) on day d7 or d28-post tumor cell implantation. Median survival for untreated control mice was 39d, in comparison to 57d and 77d median survivals for mice treated on d28 and d7, respectively. Whereas both treatment groups survived significantly longer than control group mice ($p < 0.001$ for each comparison), survival extension for mice treated on d7 was significantly greater than for mice treated on d28 ($p = 0.049$). To further investigate the influence of time of treatment on survival, we conducted additional experiments using EGFR amplified GBM cells for intracranial implantation, beginning continuous daily treatment of mice with EGFR inhibitor on d7 or d23. Median control group survival for the experiment involving d7 treatment was 23.5d, with median survival for treatment group mice reached at 63d-post implantation. Median survival for control group mice involving treatment initiation on d23 was 34d, with median survival of treatment group mice reached at 62d-post tumor cell implantation. As with the prior experiment, each treatment group of mice survived significantly longer than their corresponding controls ($p < 0.001$), though median survival benefit for mice receiving treatment beginning d7 (39.5d) was substantially more than for mice receiving treatment beginning day 23 (28d). Thus for each agent investigated, earlier initiation of treatment was associated with significantly increased survival benefit.

ET-076. DOWN-REGULATION OF H-FERRITIN AS AN ADJUVANT THERAPY IN HUMAN GLIOMA

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Elevated iron metabolism is observed as one of the key characteristics of highly proliferative cancer cells. As the major iron storage protein, ferritin expression is up-regulated in cancer cells and we have previously shown that ferritin down-regulation in human glioblastoma cells increases their sensitivity to chemotherapeutic agent. In this study, we evaluated the sensitization of H-ferritin down-regulation in glioma cells against radiation and suggest the potential of H-ferritin down-regulation as an adjuvant therapy in human

glioma. Down-regulation of H-ferritin was performed in human glioma cell line U251 through a nanotechnology-based transfection platform. A transient decrease in transferrin receptor level was observed, suggesting a release of intracellular iron with loss of ferritin. This iron release in turn produced intracellular oxidative stress, demonstrated by protein oxidation damage, as well as a decrease in the stability of hypoxia-inducible factors (HIFs), which is an indicator of radioresistance. Besides, exposure to radiation resulted in protein oxidation which could be exacerbated by H-ferritin down-regulation. Previously we have demonstrated a DNA protection role for H-ferritin. Here we report that the down-regulation of H-ferritin is associated with an impaired activation of DNA repair mechanisms induced by radiation when H-ferritin was absent. The combination of radiation and H-ferritin down-regulation led to a decrease in cell viability and an increase in apoptosis level also suggests a synergistic effect. Additionally, we expand our research into CD133-positive glioma stem cells (GSCs), which are notoriously resistant to anti-cancer treatment. Down-regulation of H-ferritin in these cells inhibited cell proliferation in vitro. With an intracranial glioma model established by GSCs implantation, we demonstrated that the survival was significantly prolonged by H-ferritin siRNA transfection through intravenous injection, in tumor bearing mice treated with first-line drug Temodar. In summary, our current study supports the potential of H-ferritin siRNA as an adjuvant therapy in glioma treatment.

ET-077. SMALL MOLECULES KILL PROLIFERATING AND NON-PROLIFERATING GLIOMA CELLS BY ALTERING THE INTRACELLULAR TRAFFICKING OF PAI-1:uPA PROTEIN COMPLEXES

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High-grade gliomas (HGGs) are an aggressive group of infiltrative brain cancers characterized by hypoxic and necrotic regions resulting from inefficient tumor vascularization. Hypoxic tumor regions harbor subpopulations of glioma-initiating cells (GICs) having self-renewal capacity and thought to contribute to the extremely high recurrence rate of HGGs. Hypoxic GICs have been shown to be resistant to radiation therapy (RT), anti-mitotic therapies, and anti-angiogenic agents. HGGs, demonstrate elevated expression of urokinase plasminogen activator system components (uPAS), notably urokinase plasminogen activator (uPA), the uPA receptor (uPAR), and the serpine chaperone PAI-1. In particular, uPA and PAI-1 have been demonstrated to be predictive biomarkers of cancer recurrence in HGGs and other aggressive cancers, most notably lymph node negative metastatic breast cancers. Our Neurotherapeutics group designed a new class of small molecules that target and selectively kill proliferating and non-proliferating GICs residing in perinecrotic and hypovascularized tumor microenvironments. The lead small molecules are cytotoxic to glioma cell lines in the low micromolar range, are transported across the blood brain barrier and appear to target chronically hypoxic GICs in intracerebral human glioma xenografts in NGS mice. These small molecules kill proliferating and non-proliferating hypoxic GICs by a newly identified drug mechanism caused by relocalization of intracellular uPAS. "Druggable" cell-permeant small molecules disrupt intracellular PAI-1: uPA protein complexes expressed only in chronically hypoxic GICs within the glial tumor. This disruption causes relocalization of uPAS to perinuclear mitochondria that triggers mitochondrial AIF release and nuclear translocation. AIF initiates irreversible caspase-independent necrotic cell death; independent of cell cycle progression. We identified the same drug-induced cytotoxicity and mechanism of action in triple receptor negative breast cancer implanted into the CNS. This new class of anti-cancer cytotoxic agents are envisioned as being paired with current RT and current chemotherapeutic agents targeting normoxic, proliferating GICs.

ET-078. SYNERGISTIC EFFECT OF SUPERCRITICAL EXTRACTS OF *CURCUMA* SPECIES WITH CANCER DRUGS IN GLIOMA CELL LINES

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Pediatric brain tumors are the most common form of solid tumors in children accounting for about 20-25% of all pediatric cancers. Chemotherapy options for brain tumor treatment are very much limited because of the blood brain barrier and emergence of drug resistance in brain tumor cells. Combining nutraceuticals or botanical drugs with cancer drugs is one of the ways to improve the efficiency of chemotherapy and quality of life in an integrative oncology setting. In the present investigation, cytotoxicity of anticancer drugs [Etoposide(ETP), Temozolomide, TMZ)] and supercritical extracts of *Curcuma amada* (CA-CO), *C. xanthorrhiza* (CX-CO) and *C. longa* (CL-CO), curcumin and Turmeric Force™ either as single agent or their combinations in glioma cell lines (U87MG, U188 MG) were analyzed MTT assay. Synergism, additiveness or antagonism between cancer drugs and supercritical extracts were determined using CompuSyn analysis of cytotoxicity data. Apoptosis and necrosis induced by different agents or their combinations were analyzed using Roche Annexin-V-FLUOS staining kit in a flow cytometer. The expression of genes associated with apoptosis and cell proliferation (p53, p21, Bcl-2, Bax, and P10) were determined by RT-PCR assay. Both glioma cell lines are generally resistant to cancer drugs such as TMZ. CA-CO showed superior cytotoxic effects as compared to CX-CO and CL-CO. CA-CO also had significantly better cytotoxic effects than curcumin and Turmeric Force™. Compusyn analysis of cytotoxic data showed that the combination of ETP and/or TMZ with CA-CO produced very high synergistic effects on cytotoxicity. The combination of cancer drugs with CA-CO induced higher percentage of apoptosis and necrosis than single agents. Gene expression studies showed that CA-CO down regulated the expression of P10 and P53 genes and increased the ratio of Bax/Bcl-2 mRNA. These positive results suggest the need for continuous evaluation of CA-CO in xenograft models and clinical trials in brain tumor patients.

ET-079. ABT-414: AN anti-EGFR ANTIBODY DRUG CONJUGATE FOR THE TREATMENT OF GLIOBLASTOMA PATIENTS

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More effective therapeutics are urgently needed for those diagnosed with Glioblastoma multiforme (GBM). To this end, we have developed ABT-414, an antibody drug conjugate (ADC) comprised of an anti-EGFR antibody (ABT-806) conjugated to the potent cytotoxic monomethylauristatin F. ABT-414 targets a unique epitope exposed only in activated wild-type EGFR and in the EGFRde2-7 (EGFRvIII) deletion mutant, both of which are frequently amplified in GBM. EGFRde2-7 generally confers a worse prognosis for those patients with GBM. The absence of binding to normal tissue and the ability to target both EGFR wild-type and de2-7 expressing tumors makes ABT-414 an attractive therapeutic agent for GBM. ABT-414 exhibited potent cytotoxicity against GBM derived xenograft models, including those models expressing EGFR wild-type only or EGFRde2-7, with sustained tumor regressions and cures observed at clinically relevant doses. Combination of ABT-414 with the current post resection standard of care treatment of radiation and temozolomide provided significant additive benefit in these models. The indium-111 labeled parental antibody (ABT-806i) has also been developed and shown to effectively target tumors in both an orthotopic GBM model and in patients with brain cancer. Additional platforms are under evaluation for their potential as companion diagnostics with the goal of identifying patients likely to respond to treatment with ABT-414. These include fluorescent in situ hybridization (FISH) and IHC to detect EGFR amplification and overexpression respectively and QPCR for detection of EGFRde2-7. Phase 1/2a trials are ongoing to evaluate the safety, efficacy and PK profile of ABT-414 in combination with temozolomide or gradiation and temozolomide in patients with GBM.

ET-080. INTRAVENOUS ADMINISTRATION OF TOCA 511 RESULTS IN UNIFORM DISTRIBUTION OF VIRAL SPREAD WITHIN THE TUMOR AND INCREASED SURVIVAL IN A SYNGENEIC, ORTHOTOPIC MOUSE GLIOMA MODEL

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Toca 511 (vocimagene amiretrorepvec), a retroviral replicating vector (RRV) that replicates by budding from infected cells, can successfully and safely deliver an optimized yeast cytosine deaminase gene to tumors when administered intratumorally (IT) in orthotopic glioma models. Toca 511 is highly tumor specific due, in part, to its inability to infect non-dividing cells and to defects in innate immunity in tumors. Toca 511, in conjunction with subsequent oral extended-release 5-fluorocytosine (Toca FC), is currently under

investigation delivered IT (NCT01156584) or to the surgical bed (NCT01470794) in patients with recurrent High Grade Glioma (HGG). Intravenous (IV) administration may circumvent or complement methods of delivery that require surgery to deliver vector to brain tumor. IV administration may theoretically: 1) deliver to multiple tumor areas with disrupted vasculature common with tumor growth, 2) deposit vector to the most mitotically active and invasive regions at the tumor periphery, and 3) when repeated, deliver vector over time, increasing the potential for contact of the vector with a greater number of dividing tumor cells. To assess the therapeutic potential of IV administration of Toca 511, biodistribution and general safety, vector spread, and survival studies were performed in a syngeneic, orthotopic mouse glioma model. No vector and/or 5-FC related toxicity was observed in this model. IV administration resulted in successful trafficking and spread in intracranial tumors. Immunohistochemical detection of viral protein showed uniform distribution throughout the tumor. Survival after 5-day IV administration of Toca 511 was significantly improved compared to control, and equivalent to 1-day IT administration. These results provide support for intravenous administration of Toca 511 in patients with malignant glioma. Tocagen is planning to initiate a Phase 1 ascending dose trial of the safety and tolerability of Toca 511 administered intravenously, in subjects undergoing subsequent resection for recurrent HGG, followed by treatment with Toca FC.

ET-081. TUMOR DERIVED MUTATIONS OF PROTEIN TYROSINE PHOSPHATASE RECEPTOR TYPE K AFFECT ITS FUNCTION AND ALTER SENSITIVITY TOCHEMOTHERAPEUTICS IN GLIOMA

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INTRODUCTION: Using mapping arrays to screen for genomic alterations in gliomas, we recently identified alterations of the protein tyrosine phosphatase receptor type kappa gene (PTPRK) which correlate to patient outcomes. These PTPRK alterations are very relevant to glioma biology as PTPRK can directly sense cell-cell contact and is a dephosphorylation regulator of tyrosine phosphorylation signaling, which is a major driving force behind tumor development and progression. Subsequent sequencing of the full length PTPRK transcripts revealed novel PTPRK gene deletion and missense mutations in numerous glioma biopsies. METHODS: Six clinical glioma samples and two cell lines were used in this experimental study. PTPRK mutations were cloned and expressed in PTPRK-null malignant glioma cells. Effect of these mutations on PTPRK anti-oncogenic function and their association with response to anti-glioma therapeutics, such as temozolomide and tyrosine kinase inhibitors, were subsequently analyzed using in vitro cell based assays. RESULTS: These genetic variations altered PTPRK activity and its post translational processing. Reconstitution of wild-type PTPRK in malignant glioma cell lines suppressed cell growth and migration by inhibiting EGFR and ß-catenin signaling, and improved effect of conventional therapies for glioma. However, PTPRK mutations abrogated tumor suppressive effects of wild-type PTPRK and altered sensitivity of glioma cells to chemotherapy. CONCLUSIONS: In summary, our findings provide compelling evidence in support of tumor-suppressive properties of PTPRK and its prognostic significance in glioma along with discovery of several mutations leading to altered functionality of the PTPRK protein which subsequently affect therapeutics. The results demonstrate biological relevance of PTPRK loss in glioma pathogenesis. In future, with data herein being confirmed using in vitro and in vivo models, PTPRK may be used as a therapeutic predictive marker for tyrosine kinase inhibitors and other anti-glioma agents.

ET-082. SYNERGISTIC ANTIGLIOMA EFFECT OF TEMOZOLOMIDE AND TRIPTOLIDE THROUGH INHIBITION OF NF-kappaB PATHWAY

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Temozolomide (TMZ) is currently the standard care for patients with glioblastoma multiforme (GBM). But the chemoresistance to TMZ in GBM is frequently observed. Therefore, strategies to enhance the antiglioma effect of TMZ are needed to explore. In this study, we found triptolide (TPL), a compound from Chinese traditional medicine, synergize with TMZ in a glioma initiating cell (GIC) model in vitro. Administration of TPL and TMZ significantly decreased the proliferation and capability to form tumor spheres of GIC-1 (a GIC line) compared to those of TPL or TMZ alone. With flow cytometry, we demonstrated the combination of TPL and TMZ dramatically increased the percentage of apoptotic cells in GIC-1. The expression of anti-apoptotic factor XIAP was found inhibited at protein level in the combined treatment of TPL and TMZ. The phosphorylation of IκB and p65 was found suppressed by the combination of these 2 agents. In addition, the luciferase assay demonstrated that combined treatment of TPL and TMZ significantly inhibited the binding ability of NF-kappaB component to the promoter of downstream genes. Therefore, our preliminary results indicated that TMZ and TPL can synergize to kill GIC cells. The mechanism underlying the synergism is probably through the inhibition of NF-kappaB pathway.

ET-083. CONVECTION-ENHANCED DELIVERY OF CHEMOTHERAPEUTIC AGENT FOR BRAINSTEM MALIGNANT GLIOMA: FROM BENCH TO BEDSIDE

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OBJECTIVES: Convection-enhanced delivery (CED) is a technique that delivers therapeutic agents directly and effectively into the brain parenchyma. Application of CED is now under investigation as new treatments for various diseases. Glioma affecting brainstem is one of the important candidates that could be targeted with CED. We describe our recent effort to develop novel CED based therapeutic approach for gliomas affecting brainstem. METHODS: Direct local CED of nimustine hydrochloride (ACNU) monitored with real-time MRI is our strategy. First, the efficacy of CED of ACNU against rodent intracranial glioma model was evaluated. Subsequently, in vivo experiments infusing gadolinium solution mixed with ACNU to the non-human primate brainstem were analyzed. Magnetic resonance (MR) images acquired during infusion to develop a real-time monitoring strategy. Using these images, computational simulation was constructed for the brainstem infusion. We then proceeded to clinical trial. Pilot feasibility study recruited three patients. Consequently, we are now in Phase I study. RESULTS: CED of ACNU was effective against intracranial xenografted rodent glioma model. Safety and feasibility of CED of ACNU with real-time MRI monitoring were confirmed with non-human primate study. The simulations we developed corresponded well with the in vivo experiments. Clinically, CED of ACNU was effective for a patient suffering recurrent glioblastoma at brainstem. Succeeding phase I study have recruited 6 patients so far, and is now under investigation. CONCLUSIONS: CED of ACNU can be safely performed with real-time MRI monitoring. This strategy can be a novel therapeutic approach for gliomas affecting brainstem in future.

ET-084. CROSSING THE BLOOD-BRAIN BARRIER: BRAIN DELIVERY OF UNMODIFIED CANCER THERAPEUTICS VIA INTRAVENOUS ROUTE MEDIATED BY A PEPTIDE TRANSPORTER

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Rapid pre-clinical evaluation of anti-cancer drugs against brain cancers remains largely unattained due to the presence of the blood-brain barrier (BBB), which seriously limits transport of most therapeutic compounds to the brain. Present efforts to deliver drugs across the BBB include physical (nanoparticles) and/or chemical modification (cross-linking to a linker) of the drugs and reliance on a leaky BBB resulting from brain cancers. However, receptors present at the BBB allow cognate ligands cross the barrier. Previously, a synthetic peptide carrier, K16ApoE, was developed that mimics the ApoE-LDLR (apolipoprotein E-low-density lipoprotein receptor) ligand-receptor system (Sarkar et al. PloS One, 6, e28881, 2011). This peptide enables transport of target proteins to the brain. Realizing that the peptide carrier creates transient BBB permeability, we performed experiments to learn if anti-cancer drugs such as cisplatin, methotrexate and other compounds can be delivered to the brain via the peptide in a non-covalent manner. Brain delivery of the drugs and other agents was achieved either by separate or combined injection of K16ApoE and a given drug/small molecule. A modification of the method comprised of injection of K16ApoE pre-mixed with cetuximab, followed by injection of a drug such as cisplatin. Brain-uptake of cisplatin and methotrexate was ~40-fold greater with K16ApoE, amounting to ~1% of the injected dose, than without. Brain-wide-uptake of Evans Blue, Light Green SF and Crocein scarlet was

also achieved. 60% higher brain uptake of I-125 with insulin (a natural hormone having receptor on the BBB) suggests that ligand-receptor signaling intrinsic to the BBB provide a natural means for passive transport of small molecules across the BBB. The results suggest that the carrier peptide can non-covalently transport small molecules throughout the brain. Thus, the method offers a reasonable approach for rapid pre-clinical evaluation of small-molecule anti-cancer drugs against brain cancers and other neurological disorders.

ET-085. DENDRITIC CELL THERAPY AGAINST VIRALLY DELIVERED TARGET ANTIGENS IS EFFECTIVE IN A MURINE MODEL OF GLIOBLASTOMA

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INTRODUCTION: We hypothesize that a novel combination therapy against malignant glioma employing a replicating retroviral vector (RRV) to deliver a highly immunogenic viral epitope to glioma cells, followed by dendritic cells (DC) vaccination will result in extended survival in a tumor-bearing mouse model system. METHODS: We used a murine glioma GL26 cell line transduced with an RRV harboring a minigene for the lymphocytic choriomeningitis virus epitope gp33 (GL26-gp33). Cytotoxic T cells (CTL) derived from the transgenic P14 mouse are specific for gp33. We co-cultured GL26-gp33 cells with the gp33-specific P14 CTLs and quantified cytokine release and glioma cell lysis. We assessed survival in C56BL/6 mice harboring intracranial GL26-gp33 implants after treatment with either intravenous adoptive cell transfer (ACT) of P14 CTLs or subcutaneous injection of gp33-pulsed DCs. RESULTS: P14 CTLs co-cultured with GL26-gp33 cells exhibited dose-dependent tumor-cell killing and released multiple pro-inflammatory and chemotactic factors. These effects are absent in control co-cultures with GL26 transduced with RRV lacking the gp33 minigene, indicating specific recognition of GL26-gp33 cells. C56BL/6 mice bearing intracranial GL26-gp33 tumors showed an increase in median survival from 26 to 35 days ($p < 0.02$) with P14 ACT and from 29 to 47 days ($p < 0.0013$) with gp33-pulsed DC therapy. CONCLUSIONS: RRV-mediated transduction of gp33 in murine glioma GL26 cells results in selective recognition by gp33-specific P14 CTLs. This recognition is manifested in culture by selective release of pro-inflammatory cytokines and lysis of glioma cells only when they express the gp33 viral epitope. There is a significant survival benefit with either P14 ACT or gp33-pulsed DC therapy over RRV-only controls in a murine intracranial tumor model, demonstrating the feasibility and potential efficacy of a combination virotherapy/immunotherapy approach for the treatment of intracranial tumors.

ET-086. TARGETING NOTCH AND mTOR IN GLIOBLASTOMA: CROSSTALK IN A NEW DIRECTION

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Activation of the PI3K-mTOR pathway has been implicated in glioblastoma (GBM) and many other cancers. Indeed, almost 90% of GBMs have aberrations that affect this pathway, making it an attractive drug target. However, it will likely be necessary to combine mTOR blockade with other targeted therapies to eradicate aggressive brain tumors. We therefore investigated the affects of mTOR/TORC1 blockade using the small molecule inhibitor MK8669 on three GBM neurosphere lines, alone and in conjunction with Notch pathway inhibition. mTOR/TORC1 pathway blockade was assessed by examining phospho-S6 protein levels, which were decreased by 50-80% following 24 hours of 1nM MK8669 treatment. Phospho-AKT protein levels increased in a dose dependent manner in response to mTOR inhibition, likely reflecting a feedback loop affecting the TORC2 complex. Monotherapy with the gamma-secretase inhibitor (GSI) MRK003 was able to deplete phospho-AKT protein levels by roughly 90% in GBM neurospheres, and concurrent treatment using both MK8669 and MRK003 caused a decrease in both phospho-S6 and phospho-AKT protein levels. A significant decrease in growth was observed at doses of 1nM MK8669 or higher in our neurosphere lines. Combinatorial treatment with both MK8669 and MRK003 drugs appears to have a modest additive effect on growth inhibition and clonogenicity in some lines. Interestingly, MK8669 alone was able to decrease the protein levels of the Notch target Hes1 by approximately 80% after 24 hours of treatment. The repression of Notch output following mTOR blockade suggests a novel mechanism of crosstalk between the two pathways. MK8669 did not suppress Hes1 mRNA levels, suggesting that this crosstalk does not occur at the transcriptional level. We therefore hypothesize that mTOR is able to regulate Hes1 at the level of protein translation or stability in glioblastoma.

ET-087. GBM PERIVASCULAR NICHE DISRUPTING AGENTS IDENTIFIED THROUGH A NOVEL HIGH THROUGHPUT COMPOUND LIBRARY SCREEN

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A diagnosis of Glioblastoma multiforme (GBM) carries a dismal prognosis and new approaches to treatment are needed. Among the potential high impact targets in GBM is the tumor peri-vascular niche (PVN), which acts as a protective sanctuary for the highly tumorigenic and invasive glioma "stem" cells. In this domain, GBM stem-like cells exhibit enhanced growth and relative resistance to the effects of radiation and chemotherapy. To identify the pathways that mediate the intercellular cross-talk between endothelial and glioma cells in the PVN, and to identify novel agents for disrupting these pro-tumor interactions, we performed an *in vitro* high throughput compound library screen for drugs that disrupted the niche effect of the peri-endothelial domain. In order to perform this screen we developed a co-culture model of the PVN that incorporated extracellular matrix (Matrigel), primary human brain microvascular endothelial cells (HBMECs) and primary GBM specimens or established GBM cell line cultures. Co-culture of GBM cells with HBMECs resulted in their co-localization and enhanced GBM cell growth. To identify pathways that mediate trophic effect of endothelial cells we used this co-culture system to screen the Spectrum Collection compound library. Primary screens using a glioma cell line (U87) revealed while most compounds in this 2000 component library were without effect, a small but diverse group of drugs blocked the trophic effects of HBMECs on U87 cell growth. The positive hits were further characterized through secondary screens using primary GBM specimens acquired from the Washington University Childrens Discovery Institute tumor bank. Three of the top anti-trophic compounds tigogenin, iridin and triacetylresveratrol were also effective in primary GBM cells. Maximal efficacy as well as toxicity of these compounds was determined using dose response experiments. The anti-tumor effects of these compounds are currently being tested *in vivo* using intracranial xenografts models with patient derived GBM specimens.

ET-088. PREFERENTIAL ACCUMULATION OF A MULTIMERIC H-NOX OXYGEN CARRIER PROTEIN IN MULTIPLE INTRACRANIAL GLIOBLASTOMA MODELS

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BACKGROUND: H-NOX is a novel class of oxygen carrying proteins designed to enhance radiotherapy (RT) by decreasing tumor hypoxia. To develop a therapeutic H-NOX candidate with optimal biodistribution into intracranial tumors, this study compared the tumor accumulation of two H-NOX variants: the 23 kDa monomer (OMX-4) and a 80 kDa trimer (OMX-4.80). METHODS: Distribution of H-NOX was studied in vivo using fluorescently-labeled OMX-4 and OMX-4.80, and using anti-H-NOX immunohistochemical analysis of unlabeled protein. Proteins were injected IV via tail vein into mice bearing intracranial tumors generated from patient-derived primary GBM tumor cells expanded in vivo (GBM43) or the well characterized human GBM cell line (U251). RESULTS: Both monomer and trimer demonstrate highly preferential accumulation in intracranial tumors within 30 minutes of injection. While the 23 kDa monomer (OMX-4) was cleared rapidly from tumors, with decreasing intracranial signal noted as early as 2h post-injection, the larger 80kDa trimeric H-NOX (OMX-4.80) continued to accumulate in brain tumors for over 4h. In resected mouse brains from 30min-2h time points, fluorescent signal was localized to the tumor-bearing hemisphere, indicating tumor-specific accumulation and retention. Immunohistochemical data in U251 tumors confirmed that the monomeric OMX-4 protein is more rapidly cleared from tumors compared to OMX-4.80 H-NOX trimer, and that both proteins penetrate deep into tumor tissue but not normal brain parenchyma. Preferential tumor accumulation of OMX-4.80 over multiple hours was further confirmed in additional human GBM models (GBM6, GBM39) and a murine GBM allograft model (GL261). CONCLUSIONS: OMX-4.80's preferential accumulation in intracranial tumors for therapeutically relevant timeframes is striking and supports

a clinical development for direct enhancement of radiotherapy in hypoxic GBM tumors. These promising biodistribution and PK data are compatible with clinical radiotherapy schedules and resulted in OMX-4.80 selection as the lead H-NOX candidate for future clinical development.

ET-089. ZINC ENHANCES TMZ CYTOTOXICITY IN GLIOBLASTOMA MULTIFORME MODEL SYSTEM

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Temozolomide (TMZ) is an alkylating agent that has become a mainstay of the treatment of malignant brain cancer, glioblastoma multiforme. Unfortunately only a limited number of patients benefit from it. We hypothesized that p53 inactivation has a role in TMZ response and zinc (Zn) can restore this p53 critical function. Accordingly, we tested if addition of zinc to TMZ enhances its cytotoxicity in a model system using 3 brain cancer cell lines. Overexpressed and active O^6-methylguanine-DNA methyl transferase (MGMT) enzyme imparts resistance to the cytotoxic effects of TMZ. Therefore, we used 3 glioblastoma cell lines exhibiting different status of both MGMT and p53: U87 (MGMT-/ p53 wt), U251 (MGMT-/ p53 mutant) and T98G (MGMT + / p53 mutant). Cytotoxicity was determined by colony and viability assays after 7 days of treatment. As expected, TMZ + /-Zn had no effect on T98G since it has an active MGMT. Conversely, in U251 cells, cellular viability was significantly reduced when cells were treated with TMZ and zinc (72% cell death) compared to 44% cell death during TMZ treatment alone. Comparable results were obtained with U87 cells. Interestingly, immunofluorescence studies detected p53 to be in its inactive conformation in U87 cells shifting to an active form after TMZ + Zn treatment. Detection of cell proliferation by plate colony formation assay revealed a 4 fold decrease between TMZ treatment alone and TMZ + Zn for U87 cells and 2.5 fold for U251 cells. In summary, zinc enhances the cytotoxicity of TMZ in the GBM U251 and U87 cell lines. Thus, zinc may be a promising addition to TMZ therapy. Zinc being an essential mineral can be easily added to clinical protocols.

ET-090. PENAO, A NOVEL MITOCHONDRIA-TARGETED AGENT, SYNERGIZES WITH DICHLOROACETATE TO TARGET ABERRANT GLUCOSE METABOLISM IN GLIOBLASTOMA

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New therapeutic strategies are urgently needed for patients with glioblastoma (GBM) as almost all tumors recur and result in disease-related death. PENAO, (4-(N-(S-penicillaminylacetyl)amino) phenylarsonous acid) is a novel mitochondria-targeted agent that inactivates adenine nucleotide translocator (ANT) located on the inner-membrane of mitochondria. PENAO blocks ANT delivery of ATP to mitochondrial-bound hexokinase 2, thus inhibiting glucose metabolism and triggering the mitochondria-mediated apoptotic pathway. PENAO demonstrated potent anti-proliferative activity in GBM. Low micro-molar concentrations of PENAO inhibited oxygen consumption rate (OCR), induced oxidative stress and depolarized the mitochondrial membrane potential (MMP), which in turn activates mitochondria-mediated apoptosis in GBM cells. PENAO increased caspase 3/9 activity levels, PARP cleavage and the proportion of apoptotic cells, which indicates PENAO triggered the intrinsic apoptotic pathway. Dichloroacetate (DCA) is a pyruvate dehydrogenase kinase inhibitor that can redirect ATP synthesis from glycolysis to oxidative phosphorylation, thus inhibiting aerobic glycolysis of tumor cells. DCA alone showed modest anti-tumor effects in vitro and in vivo models of GBM. However, we herein describe strong synergism when DCA is combined with PENAO. When GBM cells were treated with the DCA-PENAO combination, both OCR and glycolysis were blocked simultaneously. A synergistic inhibition of cell proliferation was observed (combination index, CI = 0.6) when the two drugs were combined. DCA did not induce apoptotic cell death, while DCA-PENAO combination increased the proportion of apoptotic cells by more than 2-fold compared to PENAO treatment alone. Further investigation demonstrated that DCA enhanced the apoptotic effect of PENAO by increasing oxidative stress and depolarization of the MMP. The combination is currently being tested in an orthotopic model of GBM. In conclusion, PENAO and DCA worked together to target glucose metabolism of GBM cells, inhibit proliferation of GBM cells synergistically and induce apoptosis more efficacious than PENAO alone.

ET-091. ANALYSIS OF COMBINATION THERAPY OF THE ADENOVIRUS VECTOR CARRYING REIC/Dkk-3 (Ad-REIC) AND THE INTEGRIN ANTAGONIST CILENGITIDE

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INTRODUCTION: Reduced Expression in Immortalized Cells/Dickkopf-3 (REIC/Dkk-3) was identified as a gene whose expression is reduced in many human cancers. REIC/Dkk-3 expression is also down-regulated in malignant glioma and the gene regulates cell growth through caspase-dependent apoptosis. Cilengitide (EMD121974), an inhibitor of integrins, has demonstrated preclinical efficacy against malignant glioma. In this study, we investigated the anti-glioma effect of combination therapy of the adenovirus vector carrying REIC/Dkk-3 (Ad-REIC) and the integrin antagonist cilengitide. MATERIALS AND METHODS: Cilengitide was generously provided by Merck KGaA & Cancer Therapy Evaluation Program (CTEP), the National Cancer Institute, NIH. We used malignant glioma cell lines U251, Gli36Δ5, and U87ΔEGFR and normal human astrocyte (NHA). REIC/Dkk-3 mRNA expression was determined with QRT-PCR and REIC/Dkk-3 protein expression was confirmed with western blot analysis. We conducted the cytotoxic assay to assess the combined treatment with Ad-REIC and cilengitide. Seven days after implantation of U87ΔEGFR cells into nude mouse brain, Ad-REIC or Ad-LacZ (control) was injected stereotactically at the tumor inoculation site and treated with either cilengitide or saline intraperitoneally. The survival of mice in each group was analyzed. RESULTS: QRT-PCR revealed a reduction of REIC/Dkk-3 mRNA levels in the malignant glioma cell lines. Lack of expression of REIC/Dkk-3 protein in the malignant glioma cell lines was also confirmed with western blot analysis. After the treatment of Ad-REIC with cilengitide, the number of the malignant glioma cells attaching to the bottom of culture wells was significantly reduced in time-dependent manner. In vivo, there was a statistically significant increase in survival of mice treated with combination therapy of Ad-REIC and cilengitide compared to Ad-REIC monotherapy. CONCLUSIONS: We revealed that cilengitide augmented anti-glioma efficacy of Ad-REIC. These results may lead to a promising approach to the treatment of malignant glioma.

ET-092. PATHOGEN-INSPIRED DRUG DELIVERY TO THE BRAIN

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Rabies virus glycoprotein (RVG) is a 29 amino acid peptide that is known to interact with nicotinic acetycholine, P75, and NCAM receptors to enable invasion of the central nervous system (CNS) by rabies virus. Here, we develop CNS-penetrating nanoparticles composed of poly(lactic-co-glycolic acid) (PLGA) for the purpose of improving the targeted delivery and controlled release of chemotherapeutics to brain tumors. Nanoparticles were prepared by single-emulsion and surface-modified with RVG or control sequence. The uptake of nanoparticles containing the fluorescent tracking dye Nile Red into human glioblastoma cells was evaluated by confocal microscopy and in cell lysates, and delivered via tail vein to mice for quantification of biodistribution at 1/2, 2, and 24 hours. Nanoparticles exhibited diffuse fluorescence in cell cytoplasm, indicative of effective endosomal escape, and RVG-targeted formulations achieved an up to three-fold higher concentration in U118 cells compared to RV-MAT and unmodified nanoparticle controls, with the highest specificity observed at early (<1 hr) time points. When RVG-modified nanoparticles were delivered by tail vein to healthy mice (4mg/kg PLGA), significantly improved brain-penetration was observed for targeted nanoparticles over non-targeted controls (36% increase at 2 hours, $p = 0.039$). 12% of the total dose reached the brain, achieving an average concentration of 1.98uM of hydrophobic payload in tissue, with heterogeneous patterns of uptake that depended on the region of interest. Evidence of nanoparticle delivery to the spinal cord was also observed. Taken in sum, we have

demonstrated the accumulation of systemically delivered, solid polymer nanoparticles in the CNS, and we also present evidence that RVG modification may enhance uptake in glioblastoma cells specifically. Our current studies are focused on evaluation of treatment efficacy of highly drug loaded nanoparticles in an orthotopic brain tumor model.

ET-093. TRAIL IN COMBINATION WITH PROTEASOME INHIBITION SHOWS SYNERGISTIC CYTOTOXICITY IN GLIOBLASTOMA SUBSETS DEFINED BY RNA EXPRESSION PROFILING

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The dismal prognosis of patients bearing high-grade gliomas warrants alternative treatments. Small molecule inhibitors targeting specific pathways have potential in treatment of various forms of cancer, however, for glioblastoma results from clinical trials are disappointing. Combination therapies are required to achieve therapeutic benefit and molecular characteristics of tumors should dictate the choice of treatment. Here the apoptosis-inducing effector molecule TRAIL was examined alone and in combination with the conventional treatment -Temozolomide and radiotherapy- as well as with a number of targeted drugs such as HDAC- and proteasome inhibitors. Therapeutic efficacy of these combinations was assessed on a panel of twenty patient-derived glioma cellcultures in different combinations and concentrations. In this drug-screening assay, cells are cultured in serum-free medium. Treatment with TRAIL in monotherapy conferred variable responses and resistance to this treatment was seen in 12 of the 20 cell lines tested. FACs analysis for TRAIL receptor DR5 revealed a correlation between DR5-expression and TRAIL sensitivity. The level of apoptosis-inhibiting protein Bcl-2 expression is inversely correlated to TRAIL response. In the combination studies with TRAIL, synergistic cell killing was observed with Daidzein and the proteasome inhibitors Bortezomib and G5. Combination with radiotherapy and TMZ showed additive or synergistic effects in only few cases, and even antagonistic effects in some cases. A clear distinction between responders and non-responders was noted with the various combinations. To gain insight into the molecular characteristics of these subgroups, differences in RNA-expression were analysed using DASL, which showed differences in expression patterns. Targeting apoptosis via TRAIL is a promising treatment in Oncology in which overcoming resistance to this treatment is key. We show that TRAIL and G5 form a potent synergistic combination in treating glioblastoma cellcultures. RNA-expression, Bcl-2 level and DR5 expression are good candidates to serve as molecular markers to predict treatment response.

ET-094. CHLOROTOXIN MODIFIED MAGNETIC NANOPARTICLE FOR SPECIFIC TARGETING OF BRAIN TUMORS AND DELIVERY OF O^6-BENZYLGUANINE

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Glioblastoma multiforme (GBM) tumors are malignant brain tumors that are among the most lethal cancers, striking 14,000 individuals in the U.S. each year. Prognosis remains dismal with only 2% of patients surviving 5 years. Current clinical treatments involve surgical resection followed by chemo radiation therapy. Temozolomide (TMZ), a DNA methylating agent, has shown promise in post-operative chemo radiation therapy of GBMs, yet its effects are mediated greatly by the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT). The addition of DNA repair inhibitor O6-benzylguanine (BG) as part of a concurrent treatment with TMZ has shown promise, but suffers from unacceptable toxicity due to BGs poor pharmacokinetics. Improved BG delivery methods are needed to improve clinical outcome. We have developed superparagmagnetic iron oxide nanoparticles (SPIONs) that efficiently cross the blood brain barrier (BBB) and are modified with a BG payload. These nanoparticles are comprised of a magnetic core and biocompatible chitosan-PEG (CP) copolymer surface coating. The NPs are conjugated with BG (NPCP-BG) by covalent attachment to the polymer surface. NPCP-BG is further modified with chlorotoxin (NPCP-BG-CTX), a tumor targeting peptide, through a polyethylene glycol linker. NPCP-BG-CTX demonstrated GBM specificity *in vitro* and showed a reduction in MGMT activity of SF767 cells treated with nanoparticles. Clonogenic assays confirmed increased sensitivity of SF767 cells to concurrent treatment with NPCP-BG-CTX/TMZ on par with BG/TMZ. BBB permeability by NPCP-BG-CTX was verified in wild type mice, and NPCP-BG-CTX/TMZ treatments showed a reduction in myelosuppression when compared to an equivalent dosage of BG/TMZ. In addition, the magnetic core allows for real-time monitoring of drug delivery by MRI, facilitating patient tailored treatment regimens. These tumor specific BG delivery nanovectors show potential as a less toxic treatment option that could lead to improved clinical outcomes.

ET-095. PRE-CLINICAL REFINEMENT OF A HIGHLY-PENETRATIVE POLYMERIC NANOPARTICLE DRUG DELIVERY SYSTEM FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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Survival statistics for glioblastoma multiforme (GBM) have remained static despite years of research. Two challenges must be overcome to extend survival: drug delivery and resistance to current therapies. Recently, we have developed a highly-penetrative polymeric nanoparticle system capable of being safely delivered to brain parenchyma using convection-enhanced delivery (CED). We have previously shown that this system is capable of protecting and releasing in a controlled manner agents with activity against glioma stem cells (GSCs), significantly improving survival in a rat xenograft model of GSC-derived GBM. We describe here our efforts to further refine this potential therapeutic. First, utilizing high-throughput small molecule screening of a library of generic and FDA- and internationally-approved compounds against GSCs of varying morphologies and genetic composition, we describe the discovery of a novel class of compounds with profound anti-proliferative activity against GSCs but with only very limited activity against normal glial cells. Identified compounds are notably small and lipophilic, making them ideal candidates for translation in our highly-penetrative polymeric nanoparticle system. Second, building upon recent advances in magnetic resonance imaging technology, we describe the incorporation of supraparamagnetic iron oxide (SPIO) in our highly-penetrative polymeric nanoparticle system. We show that incorporation of SPIO does not alter size or in vitro properties of these nanoparticles. Further, we show that SPIO-loaded nanoparticles can be: safely delivered to brain parenchyma using CED and stably release their contents in a controlled fashion over a matter of weeks. These results represent important steps toward clinical application of a promising drug delivery technology.

ET-096. PRODRUG ACTIVATOR GENE THERAPY WITH A NON-LYTIC RETROVIRAL REPLICATING VECTOR (TOCA 511) RADIOSENSITIZES GLIOMA CELLS AFTER INTRACELLULAR GENERATION OF 5-FLUOROURACIL

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Toca 511 (vocimagene amiretrorepvec), a retroviral replicating vector (RRV) expressing yeast cytosine deaminase (CD) prodrug activator gene, is being investigated in patients with recurrent high grade glioma (HGG). Toca 511 can spread through gliomas and express CD, converting 5-FC, an oral prodrug, into the cytotoxic drug 5-fluorouracil (5-FU) locally within infected cancer cells. Since 5-FU can act as a radiosensitizer, and therapy for HGG includes radiation and temozolomide, we investigated whether 5-FU generated in situ could achieve effective radiosensitization in intracranial xenograft models of radioresistant U87EGFRvIII glioma. The interaction of temozolomide with Toca 511/5-FC treatment is reported separately (Huang et al., this meeting). U87EGFRvIII cells either infected with Toca 511 or untransduced, were exposed to 5-FU or 5-FC, followed by irradiation at 0, 1, 2, 4 or 6 Gy. In vitro clonogenic survival assays showed identically reduced survival fraction at high radiation doses after 5-FU in both cell lines, hence RRV transduction did not alter intrinsic radiosensitivity to 5-FU. Only Toca 511 infected

U87EGFRvIII cells showed significant radiosensitization with 5-FC at every radiation dose tested. In vivo bioluminescence imaging showed significantly delayed intracranial tumor establishment kinetics by luciferase-marked, Toca 511 infected, U87EGFRvIII cells implanted intracranially into athymic mice after a 1-hour 5-FC pulse followed by 2 or 4 Gy irradiation ex vivo, compared to either irradiated uninfected cells ($p < 0.05$) or non-irradiated infected cells ($p < 0.001$); thus exposure to even a short 5-FC pulse could achieve radiosensitization. Further, significantly prolonged survival was obtained after direct intratumoral injection of Toca 511 into naïve intracranial U87EGFRvIII gliomas followed by 5-FC combined with radiation, as compared to controls ($p < 0.001$). In conclusion, Toca 511 and 5-FC can potentially mediate clinically meaningful localized radiation sensitization in HGG. Therefore clinical investigation of Toca 511 and Toca FC (investigational extended release 5-FC) in combination with radiation therapy is planned.

ET-097. GEOGRAPHIC VARIABILITY OF DRUG PENETRATION INTO THE CENTRAL NERVOUS SYSTEM

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INTRODUCTION: The lack of efficacy of chemotherapeutic agents for some CNS tumors, particularly diffuse intrinsic pontine gliomas (DIPG), has been attributed to inadequate drug delivery to the tumor site. CSF penetration is often used as a surrogate of CNS tissue penetration in drug delivery studies, but this model assumes homogeneous permeation of agents across the blood:-brain barrier (BBB). We hypothesized that a super-BBB exists to protect critical pontine functions and performed concurrent microdialysis (MD) in the cortex and pons to determine site-specific drug penetration. METHODS: The nonhuman primate model consists of rhesus macaques with chronically indwelling lumbar ports, central venous lines, and MD cannulas in both the cortex and pons. Temozolomide (TMZ) was administered intravenously over 60 minutes. CSF and plasma were simultaneously collected for pharmacokinetic (PK) analysis at various time points, and MD was performed with concurrent cortical and pontine extracellular fluid (ECF) collection over a 3-4 hour period. The initial 4 animals were non-survival, with planned pathology and histology necropsy. Survival animals (recovered with permanent and indwelling MD cannulas) underwent repeat MD at 2 months after healing of the BBB, with the MD probe placed through the existing cannulas. RESULTS: Animals tolerated MD probe placement with no acute effects, no significant imaging changes on diffusion tensor imaging, and no histologic abnormalities. The survival animal ($n = 1$ to date) had no evidence of procedure-related neurologic damage. TMZ area under the concentration x time curve (AUC) ratios compared to plasma (AUC/AUC_{plasma}) for CSF, cortical ECF, and pontine ECF were 0.47, 0.24, and 0.06, respectively. CONCLUSIONS: CNS tissue penetration of TMZ varies geographically, with lower penetration in the pons. This may account for the lack of chemotherapeutic efficacy for DIPG. We are continuing to validate this in additional animals, and also using alternate chemotherapeutic agents.

ET-098. NONINVASIVE SYNERGISTIC TREATMENT OF GLIOMAS IN MICE BY TARGETED CHEMOTHERAPEUTIC DELIVERY AND AMPLIFIED HYPERTHERMIA

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The prognosis of brain glioma remains grim due to the difficulty of targeting drugs to tumor cells. Current chemotherapies are often limited by poor retention, insufficient local accumulation, and serious side-effects of drugs, resulting from their inability to traverse the blood-brain barrier (BBB). Here we report a magnetic nanomedicine based on mPEGylated nanomagnetic graphene oxide (NMGO-mPEG) that efficiently accumulated in tumor tissues for targeted chemotherapy. In addition, we accurately controlled the focus point of low-power focused-ultrasound (LFUS) to irradiate the accumulated nanomedicine, thereby causing hyperthermia and inducing drug release in deep tumors. This synergistic treatment simultaneously enhanced the local drug concentration and raised the local temperature to achieve ultra-efficient tumor ablation in a mouse model, while lowering the risk of side-effects and hemorrhage. A pilot toxicity study involving histology, blood chemistry, and immunology did not reveal any obvious systemic toxicity of NMGO-mPEG in mice.

ET-099. THE KETOGENIC DIET ALTERS ANGIOGENIC PROCESSES AND EDEMA IN A MOUSE MODEL OF MALIGNANT GLIOMA

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Patients with malignant brain tumors have a median survival of approximately one year following diagnosis, regardless of currently available treatments which include surgery followed by radiation and chemotherapy. Improvement in the survival of brain cancer patients requires the design of new therapeutic modalities that take advantage of common phenotypes. One such phenotype is the metabolic dysregulation that is a hallmark of cancer cells. It has therefore been postulated that one approach to treating brain tumors may be by metabolic alteration such as that which occurs through the use of the ketogenic diet (KD). The KD is high-fat, low-carbohydrate diet that induces ketosis and has been utilized for the non-pharmacologic treatment of refractory epilepsy. We and others have shown that this diet enhances survival and potentiates standard therapy in mouse models of malignant gliomas, yet the anti-tumor mechanisms are not fully understood. It has been previously shown that caloric restriction, which induces ketosis, reduces microvessel density in mouse and human brain tumor models, suggesting an anti-angiogenic effect. We now report that in animals fed KetoCal® (KC)(4:1 fat:protein/carbohydrates) ad libitum, peritumoral edema is significantly reduced early in tumor progression when compared to those fed a standard rodent diet. Gene expression profiling demonstrated that KC decreases the expression of the gene encoding vascular endothelial growth factor B (VEGFB) and angiopoetin 1 receptor (TEK). Furthermore, protein analysis showed a reduction of platelet endothelial cell adhesion molecule 1 (PECAM1/CD31) in tumors from animals maintained on KC. Taken together our data suggests that KC alters the angiogenic processes involved in malignant progression of gliomas. A greater understanding of the effects of the ketogenic diet as an adjuvant therapy will allow for a more rational approach to its clinical use.

ET-100. PRECLINICAL EVALUATION OF NT-113, A NOVEL ERBB INHIBITOR OPTIMIZED FOR CNS BIODISTRIBUTION

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Deregulated ERBBB signaling is associated with the development of solid tumors including glioblastoma (GBM), of which approximately 45% have EGFR amplification and/or mutation. Unfortunately, small molecule inhibitors targeting ERBB receptors have not demonstrated significant anti-tumor activity against GBM in clinical settings, irrespective of tumor EGFR status, with clinical efficacy undoubtedly compromised by poor CNS penetration of ERBB inhibitors used to date. NT113 is an irreversible pan-ERBB inhibitor that has excellent CNS penetration with accumulation in brain. Oral administration of NT113 results in significant brain exposure in pharmacokinetic (PK) studies, with brain/plasma ratios >4, at two, four and twenty-four hr time points post administration, whereas erlotinib levels in brain, following oral administration, are <50% that of plasma at the same times of analysis as for NT113. If NT113 rodent PK results are realized in patients, it may promote significant clinical activity in treating intracranial tumors with ERBB signaling dependency. Consistent with this possibility, results from a preliminary study involving the treatment of mice with intracranial EGFRvIII mutant GBM xenograft tumor, NT113 completely suppressed tumor growth for a two-week period of NT113 administration and significantly prolonged the lifespan of treated mice. A second intracranial xenograft experiment, involving the use of a tumorigenic GBM cell source with amplification of wild-type EGFR, again showed potent NT113 anti-proliferative activity against tumor, and significantly extended animal survival. In this second experiment, NT113 also significantly outperformed both erlotinib and lapatinib, with each therapeutic administered at its daily MTD for athymic mice. Furthermore, results from in vitro studies indicate that NT113 has activity against GBM cell lines lacking amplification of EGFR. In total, our data support the possibility of NT113 being a leading "best in-class" inhibitor for the treatment of ERBB driven GBM, and additionally suggest that NT113 has activity against GBM lacking EGFR amplification.

ET-101. COMBINED BRAFV600E AND MEK INHIBITION FOR BRAFV600E-MUTANT ASTROCYTOMAS

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INTRODUCTION: BRAFV600E inhibition shows pre-clinical efficacy against BRAFV600E-mutant astrocytomas. However, BRAF monotherapy is only modestly efficacious preclinically, and can induce secondary skin malignancies in patients. One of the important reasons for this limited response to BRAFV600E inhibition in BRAF-mutant astrocytomas may be the feedback activation of upstream RTKs. METHODS: Immunoblotting was used to confirm the feedback activation of RTK/RAS/RAF/MEK/ERK pathway. Furthermore, combination of selective oncogenic BRAF inhibitor (PLX4720 or PLX4032) and MEK inhibitor (PD0325901 or GDC0973) was used to test their efficacy against BRAF-mutant astrocytomas, as well as the effect on secondary malignancies. RESULTS: In BRAFV600E-mutant GBM cell lines treated with PLX4720, we observed initial inhibition and then recovery of ERK phosphorylation. We found that this was the result of activation feedback of upstream RTKs, which then activated RAS, which then activated c-RAF/MEK/ERK pathway. We hypothesized that combined BRAF and MEK inhibition would lead to sustained MAPK pathway inhibition and our results showed that combined treatment using a BRAF and MEK inhibitor decreased cell viability, augmented cell cycle arrest, and prolonged intracranial and flank xenograft survival, when compared to either drug alone. In addition, to test the effect of combined therapy on the progression of a secondary malignancy, we used the RAS-mutant cutaneous squamous cell cancer cell line B9 to generate flank xenografts in mice also harboring BT40 BRAFV600E-mutant astrocytoma xenografts. We found that while PLX4720 decreased the growth of BT40 tumors, it increased the growth of B9 tumors. In contrast, PLX4720 + PD0325901 combination therapy decreased the growth of both tumors. CONCLUSION: BRAFV600E inhibition can cause the feedback activation of RTK/RAS/RAF/MEK/ERK pathway in BRAFV600E-mutant glioma and lead to MAPK pathway recovery. Combined BRAFV600E and MEK inhibition prevents MAPK pathway recovery, increases pre-clinical efficacy, and prevents the growth of secondary malignancies in animal models.

ET-102. LIPOSOMES FOR THE DRUG DELIVERY OF CARBORANES

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Aimed towards the development and effective delivery of antitumour agents, this programme of research targets the evaluation of new boronated lipophilic cations for use in the neutron capture therapy of cancer through the development of delivery vehicles that will facilitate the transport of these agents to their site of action. The work will involve the formulation of a series of carborane-modified molecular structures that possess subcellular (mitochondrial and/or lysosomal) targeting properties and high tumour/normal cell selectivity. Integral to this aspect of the work is the study of the transport properties of boronated agents through hydrophobic barriers, such as cell membranes and membranes of subcellular organelles. The second facet of the project will involve the preparation and evaluation of new carriers that can overcome reticulo-endothelial phagocytosis to facilitate drug delivery through the blood-brain barrier via liposome-mediated transport; this study will be centred on biodegradable materials that possess moderately hydrophilic surfaces, and which can be used for the transport of boronated chemotherapeutic agents across the blood-brain barrier. Successful completion of the programme of work will yield a range of well-characterised formulations, the performance and toxicity of which will have been evaluated in vitro. The datasets generated by this study will be of benefit to those working towards the development of effective drug targeting technologies that are not impeded by the blood-brain barrier. The implications in the treatment of brain cancer are significant: agents will be developed that show high tumour selectivity and increased ease of administration, at a low toxicity cost. In the long term, the concept could be extended to several cancer therapy strategies involving the selective destruction of other subcellular organelles (endoplasmic reticulum, Golgi apparatus).