adjunct therapy to rehabilitation for post-stroke motor deficits. TMS could be effective for postoperative rehabilitation in GBM, but its effect on GBM cells has not been evaluated. While TMS utilizes magnetic fields to induce electrical currents at low frequencies to cause neuronal excitation or inhibition, tumor-treating fields (TTF) utilize electrical currents with intermediate frequency to exert anti-mitotic effects, demonstrating promise as an adjunctive therapy in recurrent GBM. Although similarities exist between electrical and magnetic fields, the effects of magnetically induced electrical currents at low frequencies via TMS must be studied systematically *in vitro* on GBM cell lines. METHODS: We studied the effect of theta burst stimulation (TBS), a form of patterned TMS, on *in vitro* G55 cell viability using colony forming assays. We compared TMS-treated cells to controls using a combination of parameters: continuous versus intermittent TBS (cTBS and iTBS), 300 versus 600 pulses, stimulation intensity of 32% versus 60%, and no pre-TMS chemotherapy versus 100 nM or 100 µM temozolomide (TMZ). Viability measurements between controls and TMS were analyzed using analysis of variance (ANOVA). Independent t-tests were used to analyze effects of stimulation parameters on viability percent difference within each TMZ condition. RESULTS: There was no statistically significant increase in viability between control and TMS conditions for any of the stimulation parameters (+/- TMZ) while some showed decreased viability of GBM cells. CONCLUSIONS: TMS did not significantly increase GBM viability compared to controls. Future studies include validation in other cell lines and characterization of the effects of stimulation parameters in conjunction with TMZ and dexamethasone, (often administered concurrently with GBM treatment).

EXTH-48. NOVEL COMBINATION THERAPY IN PRECLINICAL GLIOMA MODELS USING THE CELL CYCLE STABILIZING COMPOUND ARGYRIN F AND CHECKPOINT INHIBITION

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Glioblastoma are incurable aggressive tumors and remain a therapeutic challenge. Glioblastoma frequently harbor alterations in the retinoblastoma pathway with subsequent cell cycle abnormalities. Here, we aimed to investigate the anti-glioma activity of the cell cycle-stabilizing compound Argyrin F and its potential treatment-induced vulnerabilities to exploit possibilities for novel combination therapies. We investigated cell viability, clonogenic survival, cell cycle status and immunoblots of human and murine glioma cells treated with Argyrin F. Moreover, we established an *ex vivo* glioma model using residual freshly resected tissue from patients, i.e. patient-derived microtumors (PDMs). Additionally, we extracted autologous tumor infiltrating lymphocytes (TILs) to perform co-culturing experiments. We performed mass spectrometry-based immunopeptidomics and used the orthotopic syngeneic SMA560/VM/Dk glioma mouse model. Argyrin F displayed anti-glioma efficacy in glioma cell lines *in vitro* and in PDM models *ex vivo*. Moreover, Argyrin F treatment induced cell cycle arrest, reduced clonogenic survival *in vitro* and prolonged survival *in vivo*. Argyrin F-treated SMA560 glioma displayed 4.6-fold more glioma-infiltrating $CD8^+$ T cells. We discovered a distinctive treatment-induced immunopeptidome. Combination of Argyrin F plus PD-1 antibody increased cellular toxicity in PDM/TILs co-cultures *ex vivo* and prolonged overall survival compared with monotherapies *in vivo*. We conclude that our experimental data suggest a novel combination of Argyrin F plus PD-1 blockade and its clinical translation.

EXTH-49. FOCUSED ULTRASOUND AND 5-ALA MEDIATED ELIMINATION OF DIFFUSE MIDLINE GLIOMA

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Diffuse midline glioma (DMG) is a devastating and incurable childhood brain cancer. With a median survival of only 9 to 11 months, over 90% of children affected by DMG die within two years of diagnosis. Despite decades of research and a growing understanding of the biology of these tumors, there have been no advancements in therapies for DMGs. Tumor heterogeneity and diffuse infiltration in inoperable brain regions make these tumors uniquely difficult to manage both surgically and pharmacologically. Therefore, there is an urgent need for the exploration of novel treatment regimens. Focused Ultrasound (FUS) is an emerging technology with significant clinical potentials. Sonodynamic therapy (SDT) is an up-and-coming treatment strategy aiming to non-invasively eliminate tumor cells by acting through compounds known as sonosensitizers, which render tumor cells sensitive to ultrasound energy. Recently, 5-Aminolevulinic acid (5-ALA), an FDA-approved molecule, has been proposed as a sono-sensitizing agent. 5-ALA mediated SDT prolonged survival in C6 rat glioma models by selective elimination of tumor cells upon sonication. Mechanistically, it is thought that 5-ALA uptake and metabolic conversion into Protoporphyrin IX (PpIX) occurs preferentially in tumor cells due to differential activity of enzymes involved in heme metabolism. Here, we investigated SDT in DMG cells treated with 5-ALA. PpIX fluorescence increased linearly up to 24 h upon 5-ALA treatment and accumulated significantly more (1.6-fold, $p < 0.01$) when compared to C6 cells. Consequently, FUS sonication of 5-ALA treated DMG cells at 250 kHz significantly ($p < 0.05$) decreased DMG cell viability compared to treatment with 5-ALA or FUS alone. Here, we show the first 5-ALA mediated sonodynamic effect in DMG cells, leading to enhanced cell death. Our findings provide a rationale for considering clinical investigation of 5-ALA mediated sonodynamic therapy in DMG.

EXTH-50. THE COMPLEXITIES OF FATTY ACID DESATURATION IN GLIOBLASTOMA

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Fatty acid desaturation is an enzymatic reaction in which a double bond is introduced into an acyl chain. Monounsaturated fatty acids (MUFA) are essential components of membrane. The most abundant MUFA-synthesizing enzyme is the delta 9 desaturate called Stearoyl Co-A Desaturase (SCD and SCD5 in humans, and SCD1-4 in mice). SCD desaturates Stearoyl-CoA (C18) and palmitoyl-CoA (C16) to oleoyl-CoA (C18:1) and palmitoyl-CoA (C16:1), respectively. SCD is often upregulated and a therapeutic target in cancer. We made an unexpected discovery that that median expression of SCD is low in glioblastoma relative to normal brain due to hypermethylation and monoallelic co-deletion with the tumor suppressor PTEN in a subset of patients. Cell lines from this subset, expressed nearly undetectable SCD yet they retained residual SCD enzymatic activity. Surprisingly, these lines evolved to survive independent of SCD through unknown mechanisms. On the other hand, cell lines that escaped such genetic and epigenetic alterations expressed higher levels of SCD and were highly dependent on SCD for survival. Finally, we identify that SCD-dependent lines acquire resistance through a previously unknown mechanism that involved drug-induced target (SCD) upregulation by the transcription factor FOSB. Accordingly, FOSB inhibition blunted acquired resistance and extended survival of tumor bearing mice treated with SCD inhibitor. Our findings reveal an intriguing feature of the cancer genome that may be used to stratify PTEN deleted cancer patients for SCD inhibitor therapy. A recent study showed that some cancer cells can use another MUFA-synthesizing enzyme FADS2 to bypass the SCD reaction. However, our data shows that the SCD inhibitor- resistant GBM lines are also FADS2-independent. Our targeted and untargeted metabolomics studies revealed unexpected findings that cannot be explained by conventional wisdom, and may lead to identification of novel lipogenic targets in GBM.

EXTH-51. DEVELOPMENT OF A NOVEL GENE THERAPY APPROACH TARGETING GLIOBLASTOMA FOLLOWING ARTIFICIAL INTELLIGENCE (AI)-DIRECTED IDENTIFICATION OF THE GBM STATE

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BACKGROUND: Profound heterogeneity has severely hampered therapeutic advancements in GBM. Remarkably, however, GBM exhibits broad clinical and histopathologic overlaps suggesting the presence of a common state. The GBM state embodies network restructuring forced by founding mutations and perpetuated in subclones of GBM stem-like cells (GSCs). Successful targeting of the GBM state promises to circumvent the heterogeneity. METHODS: To decipher the GBM state, we applied NETZEN, an AI suite integrating a deep neural network with gene network-based ranking, to first generate the reference GBM gene network from TCGA's entire GBM RNAseq collection, and then identify the altered master regulatory gene subnetwork in GBM using a dataset containing >30 diverse patient-derived GSC lines and their paired differentiated cells, 6 astrocyte and 3 neuronal precursor cell lines. To develop a gene therapy against the GBM state, we screened a rAAV capsid library through GBM patient-derived xenografts (PDX) to identify variants with specific tropism for GBM cells that can deliver targeting constructs intratumorally. RESULTS: We discovered a putative GBM state anchored by developmentally

restricted master regulators. To validate its critical role, we deconstructed it using shRNA in a panel of PDX and uniformly observed improved tumor control and survival compared to controls ($p< 0.05$ in all lines). More notably, when the core GBM state was forcibly reconstructed in astrocytes, transformation into GSC-like cells occurred, as measured by single-cell analysis, neurosphere formation, and most importantly, development of lethal infiltrating brain tumors in 15/15 mice. Finally, selected novel rAAV capsids with 10-40-fold higher specificity for GBM cells were utilized in a shRNA-based rAAV platform to target key master regulators of the validated GBM state. CONCLUSIONS: The GBM state is established by a developmental master regulator subnetwork permitting the creation of a first-of-its-kind, heterogeneity-agnostic GBM therapy. This AI-directed R&D program can be expanded to target other tumors.

EXTH-52. SYSTEMIC AND BRAIN PHARMACOKINETICS OF THE AMP-ACTIVATED PROTEIN KINASE SELECTIVE INHIBITOR SBI-0206965 AS A POTENTIAL THERAPEUTIC AGENT FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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PURPOSE: AMP-activated protein kinase (AMPK) is a molecular hub for cellular metabolic control. Recent evidence suggests that AMPK is a "druggable" novel target for the treatment of Glioblastoma Multiforme (GBM). However, AMPK-inhibitory compounds are largely limited to compound C, which has a poor selectivity profile. SBI-0206965 is a diaminopyrimidine derivative that directly inhibits AMPK with 40-fold greater potency and markedly lower kinase promiscuity than compound C. The current studies provide insights into systemic pharmacokinetics and plasma to brain partitioning of SBI-0206965. METHODS: We conducted an intracerebral microdialysis study employing jugular vein-cannulated Sprague Dawley rats (males, 6- 8 weeks). Serial brain extracellular fluid (ECF) and venous blood samples were collected up to 10 hrs following intraperitoneal administration of SBI-0206965 (25 mg/kg). These samples were then quantitated for SBI-0206965 levels using a LC/MS method (Thermo Scientific LTQ-FT™, Ionization: Electrospray Ionization; positive ion). PK analysis was performed using the Non–Compartmental Analysis (Phoenix® WinNonlin 8.2 Certara USA, Inc.). RESULTS: Plasma and ECF peak concentrations (C_{max}) were 7.15 µM and 0.68 µM, whereas the time to peak (T_{max}) were 0.5 and 1 hr, respectively. The plasma and brain ECF elimination half-lives were 1.5 and 3 hours, respectively. Plasma protein binding of SBI-0206965 was 82%. A comparison of the brain ECF C_{max} and area under the curve (AUC) to corresponding plasma values suggested that the brain partitioning of the compound was 10-18%. When corrected for unbound fraction in plasma the AUC ratio was 0.86. Thus, these studies show that SBI-0206965 has adequate brain penetration. Further studies are now in progress to assess selectivity of SBI-0206965 for AMPK expressing cell lines, efficacy against patient-derived GBM and PK in tumor-bearing mice. CONCLUSION: Results from this study will help to design optimal dosing regimen of SBI-0206965 in our efforts to explore AMPK as a GBM-specific drug target.

EXTH-53. ANTI-ROBO1 CAR T CELLS EFFECTIVELY TARGET MALIGNANT BRAIN CANCER

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No standardized treatment exists for patients with recurrent glioblastoma (GBM). Given the aggressive nature of the disease and difficulty in modeling tumor recurrence, minimal efforts have been made to design rational therapies against it. The roundabout guidance receptor 1 (ROBO1) protein is involved in axonal guidance during neurodevelopment and is aberrantly upregulated in glioma where it mediates glioma cell migration. Here, we present that ROBO1 is highly expressed on the surface of malignant and treatment-refractory brain tumor initiating cells (BTICs), prompting the development of an anti-ROBO1 CAR-T cell therapy. Using the binding region of a single-domain antibody targeting ROBO1, we developed second-generation anti-ROBO1 CAR-T cells specific and effective against ROBO1-expressing BTICs. Upon antigen exposure, anti-ROBO1 CAR-T cells upregulated markers of activation and degranulation. Additionally, treatment of orthotopic and patient-derived brain tumor xenograft models with anti-ROBO1 CAR-T cells resulted in reduced tumor burden and prolonged survival, demonstrating the therapy's therapeutic potential for treating neoplastic brain malignancies.

EXTH-54. PHOTODYNAMIC ONCOLYTIC VIROTHERAPY INCORPORATING GENETICALLY ENGINEERED PHOTOSENSITIZER KILLERRED FOR THE TREATMENT OF CENTRAL NERVOUS SYSTEM MALIGNANCIES

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BACKGROUND: Photodynamic therapy (PDT) is a targeted cancer therapy utilizing tumor-specific accumulation of photosensitizers and generation of reactive oxygen species (ROS) upon receiving specific light. The deadly CNS malignancies, high-grade gliomas and malignant meningioma, represent excellent candidates for this therapeutic method due to accessibility to light irradiation at the time of surgery. On the other hand, oncolytic virotherapy using a genetically engineered oncolytic herpes simplex virus (oHSV), has been intensively investigated as a multi-mechanistic therapy against these tumors. One of the advantages of oHSV is its ability to incorporate therapeutic transgenes. Our study aims to address our hypothesis that incorporating KillerRed, the first fully genetically encoded photosensitizing fluorescent protein, into oHSV will establish photodynamic oncolytic virotherapy that enhances tumoricidal efficacy as a novel treatment approach to CNS neoplasms. METHOD: The optical properties of the intracellular KillerRed protein expressed in cells were determined by scanning by a multi-mode microplate reader to determine the optimal irradiation wavelength. In vitro efficacy of KillerRed-mediated PDT was tested using human glioblastoma and malignant meningioma cell lines. oHSV G47delta expressing KillerRed was constructed by a bacterial artificial chromosome-based method. KillerRed-transduced cells were confirmed to express red fluorescence, followed by irradiation by an amber color LED. Cell death and viability were assessed by DAPI staining and MTS assay, respectively. ROS generation post light treatment was assessed by DCF-DA cellular ROS assay. RESULTS: KillerRed had an excitation peak at 580-585nm in transduced cells. Light irradiation by an amber LED after infection with G47delta-KillerRed induced increased cell growth inhibition and death compared with virus infection without light or light alone. Increased ROS production was observed following KillerRed PDT. CONCLUSION: G47delta-KillerRed enables a combination of oncolytic virus therapy and PDT to augment tumor killing. This approach is being tested in in vivo mouse models using potent focused laser irradiation.

EXTH-55. TARGETING RECURRENT IDH MUTANT GLIOMA WITH CDK4/6 INHIBITION

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Despite initial responsiveness to standard treatments like radiation and chemotherapy, IDH mutant gliomas inevitably recur, become more clinically aggressively and lead to untimely death. Recurrent IDH mutant tumors are less responsive to conventional treatments, highlighting the need for improved therapeutic strategies at this stage of the disease. At least 20% of recurrent IDH mutant gliomas exhibit homozygous loss of *CDKN2A*, which results in aberrant signaling through the CDK-RB pathway. We hypothesized that *CDKN2A* loss leads to enhanced sensitivity to CDK4/6 inhibitors, which are approved for use in a variety of other cancer types. We examined the relationship between CDK4/6 inhibitor sensitivity and *CDKN2A* loss using patient-derived models of IDH mutant glioma with endogenous *CDKN2A* homozygous deletion as well as with CRIPSR-mediated gene deletion. We observed enhanced cytotoxicity in glioma models with *CDKN2A* loss in vitro. Studies to examine the efficacy of CDK4/6 inhibitor treatment on slowing tumor growth in patient-derived xenograft models are ongoing. These preclinical results provide foundational data for design of a biomarker-driven clinical trial of CDK4/6 inhibitors in patients with recurrent IDH mutant glioma.

EXTH-56. ZIKA VIRUS TREATMENT SIGNIFICANTLY PROLONGS SURVIVAL IN A GLIOBLASTOMA PATIENT DERIVED XENOGRAFT MODEL

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The poor median survival for patients with glioblastoma (GBM) of 15 months has not budged for the past 15 years, when the current standard treatment was first approved. There is no standard of care chemotherapy for recurrent GBM. We previously showed that Zika virus (ZIKV) tropism for