EXTH-24. INCREASING THERAPEUTIC INDUCED NEURAL STEM CELL PERSISTENCE IN THE GLIOBLASTOMA TUMOR RESECTION CAVITY

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BACKGROUND: Induced neural stem cells (iNSCs) have emerged as a promising therapeutic platform for glioblastoma (GBM). iNSCs have the innate ability to home to tumor foci, making them ideal carriers for anti-tumor payloads. However, iNSC persist for only two weeks in the murine GBM tumor resection cavity. We hypothesized that, by encapsulating iNSCs in a scaffold matrix, we could increase both the persistence of the cells the therapeutic durability. **METHODS:** iNSCs expressing TRAIL were encapsulated in a gelatin-thrombin matrix; fibrinogen was used to polymerize the matrix. SEM was used to explore interactions between iNSCs and the scaffold matrix. To evaluate persistence, iNSCs encapsulated in the matrix were implanted into mock resection cavities of athymic nude mice and followed via BLI. To study the impacts of encapsulation on iNSC efficacy, athymic nude mice were implanted with U87 or GBM8 tumors. Tumors were then resected, and iNSCs encapsulated in the matrix were implanted; tumor volume was monitored via BLI. **RESULTS:** SEM images showed homogeneous distribution of iNSCs throughout the matrix; iNSCs were completely encased in the fibrin clot component of the matrix and did not adhere to gelatin. *In vivo*, encapsulated iNSCs persisted for nearly 100 days whereas iNSCs directly injected into the brain parenchyma persisted < 20 days. Using mice bearing GBM8 tumors, animals treated with a high dose of therapeutic encapsulated iNSCs survived ~60 days longer than animals treated with non-therapeutic cells. A similar trend was observed in animals inoculated with U87 tumors. While not statistically significant, 25% of mice treated with iNSCs encapsulated in the gelatin-thrombin matrix survived longer than those treated with iNSCs encapsulated in a fibrin-only matrix, suggesting additional benefit due to the gelatin component. **FUTURE DIRECTIONS:** Prospective experiments will explore the impact of the scaffold on iNSC phenotype, including proliferation, differentiation, and migration markers.

EXTH-25. THE CSF PENETRATION OF THE PROCASPASE-ACTIVATING COMPOUND (PAC-1) IN COMBINATION WITH TEMOZOLOMIDE (TMZ) IN A NONHUMAN PRIMATE CSF ACCESS MODEL

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BACKGROUND: The synergistic activity of temozolomide (TMZ) administered in combination with procaspase-activating compound (PAC-1) has been reported in pre-clinical mouse models and canine patients, leading to clinical trials in adults with glioblastoma. To optimize pediatric clinical trial design, a translational pharmacokinetic CSF penetration study was conducted using a pre-clinical nonhuman primate non-tumor bearing CSF access model with TMZ and PAC-1 administered alone and in combination. **METHODS:** Four male rhesus macaques with CSF lateral ventricular reservoirs received PAC-1, 15 mg/kg orally [Human Equivalent Dose (HED) 558 mg/m²/day] or TMZ, 1-hr IV infusion, 7.5 mg/kg (HED 150 mg/m²) as single and combination agent administration. Paired plasma and CSF samples were collected for 0–96 hours. PAC-1 and TMZ were quantified by LC-MS/MS. Pharmacokinetic parameters were calculated using noncompartmental methods. Statistics were determined via Mann-Whitney test. **RESULTS:** (Mean ± Standard Deviation): For TMZ: Plasma AUC₀₋₂₄ (hr*ng/ml) single agent (n=4): 28590 ± 4888 and in combination (n=4): 32736 ± 10147. CSF AUC₀₋₂₄ (hr*ng/ml) single agent (n=4): 14406 ± 1279 and in combination (n=4): 15614 ± 1767. CSF penetration (% AUCCSF: AUCPLASMA) single agent: 50.9% ± 2.2 and in combination: 49.6% ± 8.4. For PAC-1: Plasma PAC-1 AUC₀₋₂₄ (hr*ng/ml) single agent (n=4): 2556 ± 2157 and in combination (n=4): 1947 ± 1311. CSF PAC-1 AUC₀₋₂₄ (hr*ng/ml) single agent (n=2): 8.7 ± 1.1 and in combination (n=3): 12.9 ± 10.2. CSF penetration single agent: 0.2% ± 0.03 and in combination: 0.4% ± 0.38. CSF PAC-1 t_{1/2} and T_{max} pharmacokinetic parameters decreased with concurrent TMZ administration. **CONCLUSIONS:** In this non-tumor bearing pre-clinical nonhuman primate model the CSF penetration of PAC-1 was low and not notably affected by the concurrent administration of TMZ. TMZ CSF penetration for single administration was within previously reported ranges and also appeared unaffected when administered in combination with PAC-1.

EXTH-26. SECONDARY STRUCTURE ANALYSIS OF THE EGFR PRE-MRNA TRANSCRIPT TO IDENTIFY TARGETABLE REGIONS AND OPTIMIZE RNA THERAPY

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Individuals diagnosed with glioblastoma multiforme (GBM) have a short life expectancy of 12–15 months. Current strategies are often limited by the blood-brain barrier. This project is to develop therapies to bypass challenges to effective and continuous drug delivery to the brain, targeting cancer-driving genes. Epidermal growth factor receptor (EGFR) is dysregulated in 57% of all GBM. Our approach uses an adeno-associated virus gene transfer vector encoding RNA therapeutics targeting critical elements of the EGFR pre-mRNA transcript. The 'pre-mRNA structure' can be used to uncover and determine the accessibility of targetable regions. Our approach has the potential to deliver one single dose of gene therapy directly to the GBM tumor environment and block the production of EGFR and activate the expression of a stable therapeutic isoform of EGFR. To advance our therapeutic strategy, we have analyzed the EGFR secondary structure using selective 2' hydroxyl acylation and primer extension followed by mutational profiling (SHAPE-MaP). SHAPE-MaP reactivity profiles were generated revealing the structure of splicing and cryptic polyadenylation signal (PAS) elements within the targeted region. We identified enhancer binding motifs surrounding the 5' splice site and hidden elements of the cryptic PAS. Based on these structural profiles, we generated RNA therapies to unravel the hidden PAS to activate expression of the short therapeutic isoform.

EXTH-27. YAP/TAZ TRANSCRIPTIONAL CO-ACTIVATORS CREATE THERAPEUTIC VULNERABILITY TO VERTEPORFIN IN EGFR MUTANT GLIOBLASTOMA

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Glioblastoma (GBM), neoplasms derived from glia and neuro-glial stem/progenitor cells, are the most common and lethal malignant primary brain tumors diagnosed in adults, with a median survival of 14 months with current treatments. GBM tumorigenicity is often driven by genetic aberrations in receptor tyrosine kinases (RTKs), such as EGFR, and the PI-3 kinase (PI3K) signaling pathway. Using a *Drosophila* glioma model and human patient-derived GBM cells, we identified the YAP and TAZ transcription co-activators, effectors of the Hippo pathway that promote gene expression via TEAD co-factors, as key drivers of GBM tumorigenicity downstream of oncogenic EGFR signaling. YAP and TAZ are highly expressed in EGFR mutant human GBMs, and their knockdown in EGFR mutant GBM cells inhibits proliferation, blocks neuro-glial stem/progenitor cell fate, and elicits apoptosis. Our results indicate that YAP/TAZ-TEAD directly regulate transcription of SOX2, C-MYC, and EGFR itself to create a feedforward loop to drive survival and proliferation as well as stem/progenitor cell fate in human GBM cells. Moreover, the benzoporphyrin derivative verteporfin, a disruptor of YAP/TAZ-TEAD mediated transcription, specifically induced apoptosis of cultured patient-derived GBM cells, suppressed expression of YAP/TAZ transcriptional targets, including EGFR, and conferred significant survival benefit in an orthotopic xenograft GBM model. Our efforts led us to design and initiate a phase 0 clinical trial of Visudyne, an FDA-approved liposomal formulation of verteporfin, where we used intraoperative fluorescence to observe and verify verteporfin uptake into tumor cells in GBM tumors in human patients. Together, our data indicate that verteporfin is a promising therapeutic agent for EGFR amplified and mutant GBM. On the basis of these results, we have initiated as phase 1/2 clinical trial of Visudyne in patients with recurrent GBM characterized by EGFR amplification and/or mutation.

EXTH-28. MOLECULAR CHARACTERIZATION OF HUMAN GLIOMAS AND IDENTIFICATION OF NEOPLASMS RESPONSIBLE FOR NEW BIOPHARMACEUTICALS OBTAINED FROM ANIMAL VENOM: A TRANSLATIONAL APPROACH

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Gliomas correspond to approximately 80% of primary malignant brain tumors in adults. Associate histopathological classification to the identifi-