TRTH-29. CONTEXT-SPECIFIC TUMOR SUPPRESSIVE FUNCTION OF THE CANONICAL WNT PATHWAY IN PEDIATRIC MEDULLOBLASTOMA HIGHLIGHTS A THERAPEUTIC STRATEGY FOR TREATMENT OF PEDIATRIC BRAIN TUMORS
Branavan Manoranjan, Chitra Venugopal, Michelle Kameda-Smith, David Bakhshian, Minomi Subapanditha, Bradley Doble, and Sheila Singh; McMaster University, Hamilton, ON, Canada.

Current molecular subgroups of childhood medulloblastoma (MB) recognize distinct disease entities which of activated Wnt signaling is associated with a distinct subgroup and the best overall outcome. In contrast, non-Wnt MBs are characterized by metastatic disease, increased rate of recurrence, and poor overall survival. Given the excellent clinical outcome in Wnt-driven MB, we aimed to convert treatment-resistant MB subgroups 3 and 4 into an ostensibly benign tumor. Activated Wnt signaling by way of a functional beta-catenin was used in vitro self-renewal cells. Comparative RNA-sequencing of control and transgenic lines containing a stabilized beta-catenin mutant demonstrated a reduction in self-renewal genes following beta-catenin overexpression, including Sox2 and Bmi1. In order to validate the therapy-sensitive nature of Wnt-activated cells, we compared survival of primary patient-derived and 4 patient-derived lines containing a 7XTOPFlash reporter to determine the presence of endogenous Wnt signaling. Rare subclonal Wnt-active cells demonstrated a reduced self-renewal and tumor-initiating capacity through in vivo limiting dilution assays when compared to bulk Wnt-inactive cells from Group 3 and 4 MBs. The therapeutic potential of these findings were demonstrated with an in vivo survival advantage in mice with orthotropic injections of cells containing stabilized beta-catenin overexpression or endogenous Wnt-active cells. Resulting xenograft tumors were smaller in size, maintained a lower rate of proliferation, and reduction in MB self-renewal genes. To develop a rationale clinical therapeutic, we used a novel substrate-competitive peptide inhibitor for GSK. Treatment with our peptide inhibitor showed a significant reduction in tumor burden and metastatic disease with a corresponding increase in survival of patient-derived Group 3 and 4 tumors that were otherwise treatment-resistant. Our work establishes activated Wnt signaling as a novel treatment paradigm in childhood MB, identifies a rationale therapeutic approach for recurrent MB, and provides evidence for the context-specific tumor suppressive function of the canonical Wnt pathway.

INTRODUCTION: MLN0128, a second-generation ATP-competitive pan-mTOR kinase inhibitor, acts on both mTORC1 and mTORC2. We investigated the effects of MLN0128 monotherapy and in combination with MEK and BRAFV600E inhibition in models of pediatric low-grade glioma (PLGG). METHODS: We used human glioma cell lines expressing BRAFV600E (AM38), wild-type BRAF (LN229, TN98, SF188) and isogenic systems of KIAA1549:BRAF-expressing NIH3T3 cells. Signaling inhibitors including MLN0128, everolimus, and BRAFV600E specific inhibitor PLX4720, and MEK selective inhibitors AZD6244 and GSK1120212. Cell proliferation was determined using an ATP-based assay. Biochemical effects were assessed using western blot analysis. The DBTRG (BRAFV600E) xenograft mouse model was used to assess in vivo efficacy. RESULTS: MLN0128 monotherapy demonstrates more potent anti-proliferative effects compared to everolimus in all tumor burden and metastatic disease with a corresponding increase in survival of patient-derived Group 3 and 4 tumors that were otherwise treatment-resistant. Our work establishes activated Wnt signaling as a novel treatment paradigm in childhood MB, identifies a rationale therapeutic approach for recurrent MB, and provides evidence for the context-specific tumor suppressive function of the canonical Wnt pathway.

TRTH-31. EFFECTS OF TORC1/2 INHIBITOR MLN0128 ALONE AND IN COMBINATION WITH MEK INHIBITION IN BRAF MUTATED GLIOMA CELLS
Sabine Mueller, Roger Dirk, Xiaodong Yang, Steve DuBois, Angela Waanders, Adam Resnick, William Weiss, and Daphne Haas-Kogan; 1University of California, San Francisco, San Francisco, CA, USA, 2Children’s Hospital of Philadelphia, Philadelphia, PA, USA, 3Dana Farber Cancer Institute, Boston, MA, USA, 4VA University Medical Center, Amsterdam, The Netherlands.

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TRTH-30. PRELIMINARY EXPERIENCE WITH SERIAL WHOLE EXOME SEQUENCING OF PEDIATRIC CNS TUMORS AT DIAGNOSIS AND RECURRENCE.
Lucia Szalontay, Danielle Pendrick, Neil Feldstein, Richard Anderson, Erendira Garcia, Deedle Bender, Jennifer Hussein, Andrew Turk, Anthony Sirci, Mahesh Mansukhani, and James Garvin; Columbia University Medical Center, New York, NY, USA; 3Herbert Irving Comprehensive Cancer Center, New York, NY, USA.

INTRODUCTION: Whole exome sequencing (WES) of newly diagnosed pediatric central nervous system (CNS) tumors is quickly becoming part of routine care. Through the Precision in Pediatric Sequencing (PiPseq) program at Columbia University, we have found potential actionable mutations in more than 40% of evaluable CNS cases at diagnosis. More recently, we have integrated this approach into the management of patients undergoing surgery for CNS tumor recurrence. METHOD: After obtaining informed consent, tumor-normal WES with transcriptome analysis was performed in a CLIA-certified laboratory on fresh frozen CNS tumor samples and peripheral blood. RESULTS: 7 cases (5 male, 2 female; median age 3 years) with adequate diagnostic and recurrent tumor tissue were tested. No case had a somatic mutation of established clinical utility (tier 1). Among 3 embryonal tumors, a splice variant of TSC1 (tier 2, potential utility) was detected in a medulloblastoma, but only at recurrence and not at initial diagnosis. FOXX2 overexpression was detected at diagnosis and confirmed at early progression of a temporal lobe tumor, prompting revision of the initial diagnosis of high grade glioma to CNS nevus cell astrocytoma subtype of PNET, and treated accordingly. In a third patient initially diagnosed with medulloblastoma, overexpression of PDGFRα, MDM4, CDKN2A, EGFR, OLIG2, and GFAP supported a change in diagnosis to glioblastoma, which was confirmed on biopsy. In one patient with ependymoma, copy number gain of 1q25 (associated with poor prognosis) was seen only in the recurrence specimen, CONCLUSION: Our preliminary experience suggests that in pediatric CNS tumor patients referred for reoperation at recurrence, repeat WES may reveal a previously unrecognized treatment option, at least in embryonal tumors.

TRTH-32. COMBINATION IMMUNOTHERAPY TO ACTIVATE THE INNATE IMMUNE MICROENVIRONMENT AGAINST PEDIATRIC BRAIN TUMORS
Sharadar Ghoshal, Rogelio Esparza, Samuel Cheshier, and Siddhartha Mitra; Stanford University School of Medicine, Stanford, CA, USA.

Currently combinations of surgery, chemotherapy, and irradiation are utilized to treat pediatric malignant brain tumors; resulting in significant and permanent morbidity. Recently we published the effect of a humanized anti-CD47 antibody, HuSF9-G4, on five aggressive and etiologically distinct pediatric brain tumors: Group 3 medulloblastoma (primary and metastatic), atypical teratoid rhabdoid tumor, primitive neuroectodermal tumor, pediatric glioblastoma, and diffuse intrinsic pontine glioma. HuSF9-G4 demonstrated therapeutic efficacy in vitro and in vivo in patient-derived orthotopic xenograft models. Disabling the inhibitory signals transduced by SIRPα by blocking its receptor on embryonic brain cells results in robust activation of the innate immune system by utilizing a three-pronged approach. a) Increase the infiltration of macrophages into the tumor. b) Block the anti-phagocytic signals and c) Stimulate pro-phagocytic signals. RESULTS: To determine the in vivo efficacy of combinatorial anti-CD47 and anti-CD40 therapies, Group 3 medulloblastoma xenografts were treated by, i.p. injections with either PBS (control), anti-CD47, anti-CD40, or anti-CD40+anti-CD47 mAbs. Mice tumors, a splice variant of TSC1 (tier 2, potential utility) was detected in a medulloblastoma, but only at recurrence and not at initial diagnosis. FOXX2 overexpression was detected at diagnosis and confirmed at early progression of a temporal lobe tumor, prompting revision of the initial diagnosis of high grade glioma to CNS nevus cell astrocytoma subtype of PNET, and treated accordingly. In a third patient initially diagnosed with medulloblastoma, overexpression of PDGFRα, MDM4, CDKN2A, EGFR, OLIG2, and GFAP supported a change in diagnosis to glioblastoma, which was confirmed on biopsy. In one patient with ependymoma, copy number gain of 1q25 (associated with poor prognosis) was seen only in the recurrence specimen, CONCLUSION: Our preliminary experience suggests that in pediatric CNS tumor patients referred for reoperation at recurrence, repeat WES may reveal a previously unrecognized treatment option, at least in embryonal tumors.
anti-CD40 therapy demonstrated increase macrophage recruitment and intra-tumor macrophage distribution in comparison to both monotherapy and combination approaches. Antibody-dependent phagocytosis of solid tumors has also been demonstrated in vitro using anti-HER2 antibodies against breast cancer. To test if Herceptin (Trastuzumab) had similar opsonizing effect on MB cells, we carried out in-vitro in-vivo assays in combination with anti-CD47 and observe a synergistic effect of combination treatment.

**TRTH-33. COMPARATIVE PLASMA AND CEREBROSPINAL FLUID PHARMACOKINETICS OF BRAF AND MEK INHIBITORS IN A NONHUMAN PRIMATE MODEL**

Louis Rodgers, Cynthia M Lester McCully, Andrea Gross, Cody Peer, Rafael Cruz, William D Figg, Brigitte Widemann, and Katherine Warren; National Cancer Institute, Bethesda, MD, USA.

**PURPOSE:** BRAF 

E599EA mutations are present in several pediatric CNS tumors, particularly pilocytic astrocytomas, gangliogliomas and pleomorphic xanthoastrocytomas. Several BRAF inhibitors are under clinical investigation, alone and in combination with MEK inhibitors. To guide the clinical development of BRAF and MEK inhibitors, we evaluated CNS penetration of dabrafenib, selumetinib, vemurafenib, and trametinib (using CSF as a surrogate) in a nonhuman primate model predictive of pharmacokinetics (PK) in pediatric populations. METHODS: This study was approved by the NCI Animal Care and Use Committee. Agents were administered orally to rhesus macaques (n=4), with human equivalent dosing as follows: dabrafenib (161 mg/m²) and selumetinib (50 mg/m²). Planned studies include vemurafenib (516 mg/m²) and trametinib (1.1 mg/m²). Serial, paired plasma and CSF samples were collected from 0–24 hr for dabrafenib and 0–48 hr for selumetinib. Dabrafenib was quantified using a validated ultra HPLC-MS/MS method (lower limit of quantitation (LLOQ) = 0.5 ng/mL in plasma and CSF). PK parameters were calculated using noncompartmental methods. RESULTS: In plasma, mean half-life and dose-normalized AUC(0-∞) for dabrafenib were 3.2 ±1.2 hr and 73.9 ±34.0 hr*ng/mL/mg, respectively, and for selumetinib, 10.8 ±2.5 hr and 122 ±17.8 hr*ng/mL/mg, respectively. In CSF, 5.0 ±1.7 hr and 0.45 ±0.21 hr*ng/mL/mg, respectively. CSF levels of selumetinib were detectable in one animal, with an AUC(0-∞) of 0.39 hr*ng/mL/mg. CSF penetration of dabrafenib and selumetinib were poor (0.57 ±0.18 % and 0.4%, respectively). CONCLUSIONS: Quantifiable concentrations of dabrafenib were found in plasma and CSF for 24 hr after administration, but, as with selumetinib, CSF penetration was low. Due to inter-animal variability, an additional animal will be studied. Alternate delivery methods (intranasal and intrathecal) may be useful to evaluate in efforts to increase CNS exposure.

**TRTH-34. NOVEL ORAL PRODRUGS OF 6-DIAZO-5-OXO NORLUCINE IMPROVE BRAIN PENETRATION AND DEMONSTRATE EFFICACY AGAINST MYC-DRIVEN ORTHOTOPIC MEDULLOBLASTOMA XENOGRAFTS**

Allison Hanaford, Charles Eberhart, and Eric Raabe; Johns Hopkins Medicine, Baltimore, MD, USA.

Increased MYC levels can alter cellular metabolism, creating a reliance on glutamine. Glutamine PET and MRI spectroscopy demonstrate that aggressive brain malignancies have increased uptake of glutamine and increased glutamate relative to normal brain, suggesting that agents targeting glutamine metabolism may be active in brain tumors. The most aggressive subgroup of medulloblastoma tumors are driven by high expression of MYC, so we hypothesized that these tumors would have altered glutamine metabolism and be sensitive to 6-diazo-5-oxo norlucine (DON), a glutamine analog. Western blotting revealed that expressing MYC in human cerebellar-derived neural stem and progenitor cells (CB NSC) induced the expression of glutaminase (GLS), the enzyme that converts glutamine to glutamate—a critical step in glutamine metabolism. Human neural stem cells transformed with SV40 do not express MYC and thus do not express GLS. MYC-expressing patient-derived medulloblastoma cell lines (D425MED and D283MED) also express GLS. We treated our MYC-transformed CB NSC and medulloblastoma cell lines with DON and observed an increase in apoptosis of up to 450% as determined by cleaved caspase-3 immunofluorescence (p=0.001). DON treatment did not significantly increase apoptosis in SV40-immortablized CB NSC (p=0.65) and had no effect on normal, untransformed human NSC. DON is not orally bioavailable, so we decided to develop DON prodrugs designed for improved oral bioavailability and brain penetration. In non-human primates, our DON produgs exhibited superior 10-fold enhanced CSF/plasma ratio versus DON. In orthotopic xenograft models, our novel DON produgs increased the median survival of mice bearing D425-MED MYC-driven medulloblastoma tumors by 60 percent (22 days for vehicle treated mice compared to 35 days for produg treated mice, p<0.01 by log-rank test). DON produgs can be engineered to improve oral bioavailability and brain penetration, and these drugs have efficacy in orthotopic xenograft models of aggressive MYC-driven medulloblastoma tumors.