

Children's Hospital, Seattle, WA, USA, ²Fred Hutchinson Cancer Research Center, Seattle, WA, USA, ³University of Washington, Seattle, WA, USA

Systemic interferon- γ (IFN γ) has been shown to induce major histocompatibility complex class I (MHC-I) and T cell infiltration in solid tumors in adult patients, demonstrating a potential strategy to abrogate tumor-intrinsic mechanisms of immune escape. Pediatric brain tumors (PBT) may be particularly sensitive to this approach but have a paucity of immunogenic tumor antigens for presentation on MHC-I. Decitabine and other DNA methyltransferase (DNMT) inhibitors promote expression of oncofetal antigens and endogenous immune responses through epigenetic alterations. We tested the convergence of these immune priming mechanisms using a novel combination of IFN γ and decitabine across a spectrum of PBT. Primary human cell lines (Med-411FH, PBT-05FH, GBM-511FH, CCHMC-GBM-1, CCHMC-GBM-4, ATRT-310FH) and murine transgenic models were treated with IFN γ alone or in combination with decitabine and evaluated expression of cell surface MHC-I and PD-L1, interferon response genes (ISGs), and oncofetal antigens. PBT showed exquisite sensitivity to IFN γ , increasing expression of MHC-1/PD-L1 along with ISGs (*TAP1*, *MX1*, *IRF1*). Decitabine enhanced IFN γ -induced gene expression of oncofetal antigens NY-ESO-1 and MAGE-A1. In a medulloblastoma flank tumor model, MHC-I was increased by 40-fold following intraperitoneal IFN γ treatment ($p=0.01$), with a 3-fold increase in PD-L1 ($p=0.005$) compared to untreated controls. Effect on CD8+ T cell killing and validation in humanized models is ongoing. Immune priming of PBT with IFN γ is feasible and results in more substantial MHC-I upregulation compared to hypomethylating agents alone. These results provide a strong rationale for priming prior to checkpoint inhibition as a compelling therapeutic strategy in immunologically-quiet PBT.

MODL-29. EVALUATING TUMOR-IMMUNE INTERACTIONS IN MOUSE MODELS OF DIFFUSE INTRINSIC PONTINE GLIOMA

Robin Furnish¹, Heather Bear¹, Xin Wei¹, and Timothy Phoenix^{1,2};
¹University Of Cincinnati, Cincinnati, OH, USA, ²Cincinnati Children's Medical Center, Cincinnati, OH, USA

BACKGROUND: While adult gliomas show some level of immune cell infiltration, diffuse intrinsic pontine glioma (DIPG) is characterized as having an "immune cold" state. We have developed new immunocompetent mouse models of DIPG. These models faithfully recapitulate the pathological hallmarks of DIPG and provides a unique platform to investigate immune modulatory therapies and potential therapeutic benefits of checkpoint inhibitor combination therapies. **METHODS:** To evaluate the effects of CDK4/6 inhibition (CDK4/6i) on cell proliferation and immune interactions we performed a series of in vitro and in vivo studies using DIPG mouse models. In vitro assays included dose response curves, transcriptional profiling, and MHC1 expression. In vivo preclinical studies treated mouse models with CDK4/6i with or without immune check-point inhibitors (ICI). We also examined other candidate immune modulatory therapies in vitro. **RESULTS:** CDK4/6i (Abemeciclib) reduced proliferation of DIPG cells derived from mouse models, and displayed a modest increase in immune activation by MHC1 expression and transcriptome. Pilot in vivo preclinical studies did not show any significant changes in DIPG proliferation or immune changes with CDK4/6i treatment, ICI treatment, or the combination of CDK4/6i + ICI. In vitro testing of other immune-modulatory drugs identified additional candidates that can be tested in vivo. **CONCLUSION:** CDK4/6i displayed in vitro action, but lacked efficacy in DIPG mouse models in vivo. Further use of spontaneous DIPG mouse models will provide a rapid preclinical platform to evaluate in vivo tumor-immune interactions, drug efficacy, and mechanisms of resistance.

MODL-30. DISSECTING THE ROLE OF MULTI-CILIOGENESIS NETWORK IN CHOROID PLEXUS TUMOR

Haotian Zhao, and Tasneem Zahran; New York Institute of Technology, Old Westbury, New York, USA

The choroid plexus (CP) in brain ventricles consists of a fibro-vascular core encapsulated by epithelial cells that possess clusters of primary cilia on cell surface. CP tumors are rare primary brain neoplasms that most commonly occur in young children. Compared to the benign CP papilloma, choroid plexus carcinoma (CPC) is poorly understood and highly lethal with few treatments available. Molecular, cytogenetics and genomics studies uncovered complex alterations in CPC including frequent chromosomal loss and recurrent focal aberrations, whereas abnormal NOTCH signaling is observed in many CP tumors. We showed that activation of both NOTCH and Sonic Hedgehog (SHH) signaling in mice drives the formation of aggressive CP tumor. Molecular and histology analyses demonstrated that these murine CP tumors closely resemble their human counterparts, which also display aberrant SHH and NOTCH signaling, suggesting they may represent potential therapeutic avenues. Indeed, treatment with vismodegib, an FDA-approved SHH pathway inhibitor, suppresses CP tumor growth. Un-

like multi-ciliated CP epithelial cells, tumor cells in these animal models are characterized by a solitary primary cilium. Though key genes of the multi-ciliogenesis circuit driven by Geminin coiled-coil domain-containing protein 1 (GEMC1) are expressed in CP epithelium, GEMC1-dependent transcriptional program is suppressed in NOTCH-driven CP tumors. Importantly, CPCs in humans consist of tumor cells with a solitary primary cilium and exhibit profound defects multi-ciliogenesis program. Together, these results indicate that a solitary primary cilium is crucial for CPC development, whereas multi-ciliogenesis circuit possesses tumor suppressive functions and may represent a novel therapeutic target in CPC.

MODL-31. RADIATION-DERIVED TREATMENT-RESISTANT PDX AND CELL CULTURE MODELS RECAPITULATE THE CHARACTERISTICS OF MATCHED PRIMARY/RECURRENT PEDIATRIC HIGH-GRADE GLIOMA

Aaron J Knox, Patrick Flannery, Anjali Zukosky, John DeSisto, Bridget Sanford, Benjamin van Court, Andrew Donson, Rakeb Lemma, Hannah Chatwin, Sana D Karam, Rajeev Vibhakar, Ken Jones, and Adam L Green; The Morgan Adams Foundation Pediatric Brain Tumor Research Program, University of Colorado School of Medicine/Children's Hospital Colorado, Aurora, CO, USA

BACKGROUND: Pediatric high-grade glioma (pHGG) is the most common cause of childhood cancer death. Recurrence after therapy is a major challenge, since recurrent pHGG proliferates aggressively and resists therapy. We developed and validated preclinical models of matched primary and recurrent tumors, providing a method to study recurrence and potential therapies. **METHODS:** We irradiated H3K27M thalamic pHGG cells (BT245) (8 Gy/week, 2Gy fractions x3 weeks) and propagated the surviving cells (BT245R). We developed a murine recurrence model by orthotopically implanting BT245 cells, irradiating the resultant tumors (4 Gy/day x2d) and propagating irradiated (BT245RM) or control (BT245CM) tumor cells at endpoint. We performed phenotypic analyses, RNA-Seq, and drug testing. **RESULTS:** BT245R cells were more stemlike than BT245, with an 8-fold greater rate of neurosphere formation ($p<0.03$). Geneset enrichment analysis showed similar molecular changes in BT245RM cells and primary/recurrent H3K27M pHGG patient sample pair, including relaxation of the G2M/cell cycle checkpoint (Hallmark_G2M_Checkpoint: BT245RM NES=-5.95, FDR=0.0; patient NES=-5.86, FDR=0.0), downregulation of MYC targets (Hallmark_MYC_Targets_V1: BT245RM NES=-7.43, FDR=0.0; patient NES=-5.86, FDR=0.0), and decreased differentiation (Go_Regulation_of_Stem_Cell_Differentiation: BT245RM NES=-3.35, FDR=0.0; patient NES=-3.15, FDR=0.0). Enrichment of the protein_kinase_C_signaling in BT245RM (NES=2.18, FDR=0.03) suggested response to MAPK pathway inhibition. BT245R cells were twice as sensitive as BT245 cells to the MEK inhibitor trametinib ($p<0.05$). **CONCLUSIONS:** Our neurosphere and murine orthotopic patient-derived xenograft models recapitulate gene expression changes of matched primary/recurrent pHGG. RNA-Seq analysis validated the model against patient samples and identified trametinib as potentially effective in recurrent pHGG.

NEUROFIBROMATOSIS

NFB-01. FUNCTIONAL CHARACTERIZATION OF ATRX LOSS IN NF1-ASSOCIATED GLIOMA AND MPNST

Ming Yuan¹, Karlyne Reilly², Christine Pratilas¹, Christopher Heaphy³, and Fausto Rodriguez¹; ¹Johns Hopkins University School of Medicine, Baltimore, MD, USA, ²National Cancer Institute, Bethesda, MD, USA, ³Boston University School of Medicine, Boston, MA, USA

To identify the biologic relevance of ATRX loss in NF1-associated gliomagenesis, we studied the effects of *Atrx* loss using four previously characterized *Nf1*^{-/-}*Trp53*^{+/-} murine glioma lines. Lines 130G#3 and 158D#8 (corresponding to grade IV and III gliomas, respectively) displayed preserved ATRX protein expression compared to NIH-3T3 cells. We studied the effects of *Atrx* knockdown in these two lines in the presence and absence of the TERT inhibitor, BIRBR1532. Using a telomere-specific FISH assay, we identified increased signal intensity after *Atrx* knockdown, only in the presence of the TERT inhibitor. These features are reminiscent of ALT, although there were no significant alterations in cell growth. Next, we studied the effect of ATRX loss in MPNST lines ST88-14, NF90-8, STS-26T. These cell lines all expressed ATRX and DAXX. However, STS-26T contained a TERT promoter mutation and ST88-14 had a known SNP in the TERT promoter, while NF90-8 had no alterations. ATRX siRNA knockdown showed no significant effects in cell proliferation or apoptosis. However, ATRX knockdown resulted in rare ultra-bright foci, indicative of ALT. Next, we studied the *in vitro* effect of the ATR inhibitor VE-821 in MPNST cell lines. Only NF90-8 (lacking TERT alterations) demonstrated a decrease in growth after ATRX knockdown and VE-821 treatment. However, ATRX