collected tumor tissue from pediatric ACP was cut into volumes of approximately 3 mm^3 and rested over a semi-permeable insert placed in the wells of a 6-well plate. Specimens were cultured in (1) Control media, media containing (2) Tocilizumab, (3) Trametinib, and (4) combination of Tocilizumab and Trametinib, for 24 and 96 hours. Specimens were harvested for paraffin embedding, protein and gene expression assays. Supernatants were collected to assay secreted components. Paraffin embedded specimens were sectioned and stained for H&E, Pan-CK, Beta-Catenin, cleaved Caspase-3, Ki-67, and Phospho-ERK. RESULTS: H&E staining revealed characteristic histologic features of ACP with epithelial cells with palisading nuclei, wet keratin and ghost cells. Tumor sections were markedly positive for epithelial cell markers, Pan-CK and Beta-Catenin. Ki-67 and cleaved Caspase-3 were restricted to a small fraction of cells, indicating low index of proliferation and apoptosis under the culture conditions. The response to drug treatments shall be determined using gene expression assays and evaluation of the secreted components. CONCLUSION: The organotypic chunk culture technique appears to maintain the viability and integrity of ACP tumors for several days and may serve as an appropriate model for pre-clinical studies to develop targeted therapeutics for pediatric ACP.

MODL-25. REPLICATION REPAIR DEFICIENT MOUSE MODELS PROVIDE INSIGHT ON HYPERMUTANT BRAIN TUMOURS, MECHANISMS OF IMMUNE EVASION, AND COMBINATORIAL IMMUNOTHERAPY

Melissa Galati[1], Li Li[1], Sumedha Sudhaman[1], Tatiana Lipman[1], Lucie Stengs[1], Dana Elshaer[1], Taylor Bridge[1], Dar'ya Semenova[1], Melissa Edwards[1], Karl Hodel[2], Victoria J. Forster[1], Nuno M. Nunes[1], Alberto Martin[3], Eric Bouffet[4], Zachary Pursell[2], Cynthia Hawkins[1], and Uri Tabori[1,4]; [1]The Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, ON, Canada, [2]Department of Biochemistry, Tulane University, New Orleans, LA, USA, [3]Department of Immunology, University of Toronto, Toronto, ON, Canada, [4]Division of Hematology and Oncology, The Hospital for Sick Children, Toronto, ON, Canada

Replication repair deficiency (RRD) is the leading cause of hypermutant brain tumours in children. RRD is caused by defects in one of four mismatch repair (MMR) genes and mutations in *POLE* or *POLD1*. Such tumours are resistant to common therapeutic agents and animal models are needed to study RRD in vivo and test novel therapies like immune checkpoint inhibitors (ICIs). To model RRD brain tumours specifically, we engineered a *Pole* mutant mouse model harbouring the S459F mutation (*PoleS459F*). We combined *PoleS459F* mice with conditional *Msh2* knockout (*Msh2LoxP*) and *Nestin-cre* mice. All *Nestin-cre+Msh2LoxP/LoxPPoleS459F/+* mice rapidly succumbed to posterior fossa brain tumours between 8.6 and 12.4 weeks. Importantly, tumours exhibited hallmark "ultrahypermutation" (~350 mutations/Mb) and the corresponding signatures characteristic of human combined MMR and *POLE*-proofreading glioblastoma. Interestingly, *Nestin-cre+Msh2LoxP/LoxPPoleS459F/S459F* mice failed to establish normal cerebella, suggesting such mutational loads may not support normal brain development. Furthermore, *OLIG2-cre+Msh2LoxP/LoxPPoleS459F/+* mice failed to develop tumors. Tumors transplanted into syngeneic vs immunocompromised animals egrafted well orthotopically in the mouse hindbrain but significantly less efficiently when engrafted subcutaneously. Furthermore, immunocompromised and subcutaneous tumors revealed striking differences in mutational burden and clonal architecture, suggestive of nonautonomous immunoediting. Finally, anti-PD1 was sufficient to treat subcutaneously engrafted tumors in immunocompetent animals. This first mouse model of immunocompetent, hypermutant brain tumors can be used to uncover unique characteristics of RRD tumour evolution and allow for immune based therapeutic preclinical testing. Experiments to assess combinational ICIs and other therapeutic interventions in orthotopically transplanted tumors will also be presented.

MODL-26. CHILDREN'S BRAIN TUMOR NETWORK: ACCELERATING RESEARCH THROUGH COLLABORATION AND OPEN-SCIENCE

Jena Lilly[1], Jennifer Mason[1], Elizabeth Appert[1], Allison Heath[1], Yuankun Zhu[1], Bo Zhang[1], Mateusz Koptyra[1], Mariarita Santi[1], Ian Pollack[2], Stewart Goldman[3], Sarah Leary[4], Anna Buccoliero[5], Mirko Scagnet[5], David Haussler[6], Derek Hanson[7], Jiangguo Zhang[8], Weiqing Wan[9], Chunde Li[9], Ron Firestein[10], Jason Cain[10], Joanna Phillips[11], Nalin Gupta[11], Sabine Mueller[11], Gerald Grant[12], Michelle Monje-Deisseroth[12], Sonia Partap[12], Jeffrey Greenfield[13], Brian Rood[14], Javad Nazarian[14], Eric Raabe[15], Eric Jackson[15], Stacie Stapleton[16], Robert Lober[17], David Kram[18], Phillip Storm[1], Rishi Lulla[19], Michael Prados[11], Adam Resnick[1], and Angela Waanders[3]; [1]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [2]UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA, [3]Ann and Robert H Lurie Children's Hospital, Chicago, IL, USA, [4]Seattle Children's Hospital, Seattle, WA, USA, [5]Meyer Children's Hospital, Florence, Italy, [6]Genomic Institute, UCSC, Santa Cruz, CA, USA, [7]Joseph M, Sanzari Children's Hospital at Hackensack University Medical, Hackensack, NJ, USA, [8]Genebank, Beijing Genomics Institute, Shenzhen, China, [9]Beijing Tiantan Hospital Neurosurgery Center, Beijing, China, [10]Hudson Institute of Medical Research, Melbourne, Australia, [11]University of California San Francisco Benioff Children's Hospital, San Francisco, CA, USA, [12]Stanford University/Lucile Packard Children's Hospital, Palo Alto, CA, USA, [13]Pediatric Brain and Spine Center, Weill Cornell Medicine, New York, NY, USA, [14]Children's National Health System, Washington DC, USA, [15]Johns Hopkins, Baltimore, MD, USA, [16]Johns Hopkins All Children's Hospital, St Petersburg, FL, USA, [17]Dayton Children's Hospital, Dayton, OH, USA, [18]Wake Forest Baptist Health- Brenner Children's Hospital, Winston-Salem, NC, USA, [19]Hasbro Children's Hospital, Providence, RI, USA

The Children's Brain Tumor Network (formerly known as Children's Brain Tumor Consortium- CBTTC) is a global organization pioneering a model of open-science medical research to improve treatment and discover cures. Started in 2011, our objective was to utilize a regulatory, agreement, and governance architecture to remove existing research barriers that slowed down the pace of research and collaboration. Our network now includes 17 institutions working together to empower research. As of December 2019, over 3,600 subjects have been enrolled resulting in collection of over 45,000 specimens. Clinical data collection is longitudinal and includes medical history, diagnosis, treatment, pathology slides and reports, radiology imaging and reports, and outcome data. The tissue is collected flash-frozen, in freezing media, and fresh for the generation of pre-clinical models including cell lines. Blood is collected from the subject, with blood or saliva collected from the parents for germline comparison. Additionally, the Children's Brain Tumor Network- Pediatric Brain Tumor Atlas has generated 952 WGS and RNAseq, 221 proteomics, with annotated clinical data. All of this data, both generated raw and processed data, has been made available broadly to the scientific community via cloud-based platforms, including the Gabriella Miller Kids First Data Resource Portal, Cavatica, and PedCbioportal. As of January 2020, we have 45 approved biospecimen requests and 80 genomic/molecular data requests. In summary, the Children's Brain Tumor Network's goal is to accelerate the pace of discovery by providing resources and expanding the network of scientists working towards a cure.

MODL-27. MEK INHIBITION WITH TRAMETINIB SLOWS PROGRESSION OF MEDULLOBLASTOMA AND ATYPICAL TERATOID RHABDOID TUMOR IN ORTHOTOPIC XENOGRAFT MURINE MODEL

Sujata Mushrif[1], Long Hung[2], Sakunthala Muthugounder[2], and Shahab Asgharzadeh[2]; [1]SRCC Children's Hospital-Managed by Narayana Health, Mumbai, Maharashtra, India, [2]Children's Hospital of Los Angeles, Los Angeles, CA, USA

BACKGROUND: Combination of surgery, chemotherapy, autologous transplantation, irradiation constitutes treatment of CNS embryonal-cell tumors (Medulloblastoma-MBL, atypical teratoid rhabdoid tumor-AT/RT). Targeted agents to improve survival and decrease side effects are necessary. We hypothesize that inhibiting MAPK pathway in MBL and AT/RT may be beneficial. METHODS: IHC(pERK) was performed on clinical tumors. *Trametinib*(MEK inhibitor) was tested on MBL(UW228, D283, DAOY); AT/RT(CHLA06, BT12) cell-lines. Luminescent cell-viability assay was done(72 hrs) and with crystal violet assay(10 days). Orthotopic, xenografts of MBL and AT/RT were made in *NOD-Scid gamma* mice. Mice were given *Trametinib* daily by gavage for 6 weeks(0.6mg/kg b.w). Western blot was performed on protein from cell lines and tumor xenografts incubated with *Trametinib*. H&E staining was done on murine tumors. RESULTS: AT/RT(48%) and MBL(57%); Anaplastic(50%), Desmoplastic(40%), Classic(38%); Group 4(66%), Group 3(20%), SHH(55%), WNT(0%) showed presence of pERK(clinical samples). In-vitro, *Trametinib* completely abrogated the phosphorylation of ERK at 125nM in AT/RT and 50nM in MBL. The IC50 after 10 days exposure was 10nM for AT/RT and 35nM for MBL. *Trametinib* treated mice showed delay in tumor growth and significant survival advantage in both AT/RT (p=0.00336) and MBL (p=0.0069). Murine tumors showed decreased proliferation (H&E). CONCLUSION: *Trametinib* decreased cell proliferation, increased survival in our murine model in both MBL and AT/RT. Pre-clinical results indicate benefits in subgroups of AT/RT and MBL with active MAPK pathway.

MODL-28. IMMUNE PRIMING WITH INTERFERON-Γ COMBINED WITH EPIGENETIC MODULATION IN PEDIATRIC BRAIN TUMORS

Erin Crotty[1,2], Shelli Morris[2], Ken Brasel[2], Emily Girard[2], Alyssa Noll[2,3], Andrew Mhyre[2], and James Olson[1,2]; [1]Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of Washington, Seattle

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

Systemic interferon-γ (IFNγ) 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 IFNγ 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 IFNγ 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 IFNγ, increasing expression of MHC-1/PD-L1 along with ISGs (*TAP1, MX1, IRF1*). Decitabine enhanced IFNγ-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 IFNγ 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 IFNγ 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-quiescent PBT.

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

Robin Furnish[1], Heather Bear[1], Xin Wei[1], and Timothy Phoenix[1,2]; [1]University Of Cincinnati, Cincinnati, OH, USA, [2]Cincinnati 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 check point 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. Unlike 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 G2/M 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 Yuan[1], Karlyne Reilly[2], Christine Pratilas[1], Christopher Heaphy[3], and Fausto Rodriguez[1]; [1]Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]National Cancer Institute, Bethesda, MD, USA, [3]Boston 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