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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

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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

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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

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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

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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

knockdown alone did not affect sensitivity to carboplatin. Our findings further support a role for ATRX loss with subsequent ALT activation in a biologic subset of NF1-associated malignancies, thereby opening an opportunity for therapeutic targeting of these aggressive tumors using specific classes of drugs.

NFB-02. TREATMENT OF PAIN AND TUMOR GROWTH IN NF2

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BACKGROUND: Neurofibromatosis Type 2 (NF2) is an autosomal dominant disorder characterized by multiple nervous system tumors. Chronic pain affects the majority of patients with NF2 and is the primary factor that contributes to decreased quality of life. There are limited therapies that effectively reduce pain in NF2, but intravenous (IV) bevacizumab has been reported to provide significant relief to patients suffering from debilitating pain. CASE STUDY: James is a 24-year-old who initially presented with manifestations of NF2 at age 10, and by 15 years old had developed daily pain affecting his neck, back, and lower extremity. He has multiple CNS schwannomas, meningiomas, neurofibromas, and meets clinical NF2 criteria. While genetic testing did not reveal a mutation in his gDNA, low level skipping of exon 4 via RNA supports (likely mosaic) NF2. James's pain was poorly controlled with multiple oral medications, including opioids. James started IV bevacizumab at age 16 that improved his pain. He was critically dependent on bevacizumab for pain control and required continuous IV pain medication when bevacizumab was held for a surgical procedure. Following five years of bevacizumab he developed worsening toxicities including hypertension, proteinuria, and elevated hemoglobin. James transitioned to therapy with trametinib, a MEK inhibitor, and was able to wean off bevacizumab six months later. Treatment of NF2 related pain with trametinib instead of bevacizumab has improved his QOL with decreased medical visits, improved pain management, and decreased side effects. FUTURE IMPLICATIONS: Treatment of NF2 tumor related pain can be managed with MEK inhibitors.

NFB-03. TRAMETINIB FOR ORBITAL PLEXIFORM NEUROFIBROMAS IN YOUNG CHILDREN WITH NF1

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Plexiform neurofibromas (PN) in NF1 are diagnosed in early childhood and may grow rapidly during this period. In 10% of patients they involve the orbital-periorbital area and may cause visual problems including glaucoma and visual loss from amblyopia (deprivational, strabismic, or refractive), optic nerve compression or keratopathy. Ptosis, proptosis and facial disfigurement lead to social problems and decreased self-esteem. Complete surgical removal is usually impossible and there is a tendency for regrowth after debulking. Recently inhibitors of the RAS/MAPK pathway have been investigated for their activity in PN. We describe 5 young children with NF1 and PN of the orbital area treated with the MEK inhibitor trametinib followed clinically and by volumetric MRI. Treatment was initiated at mean age 26.8 months (SD ±12.8) and continued for median 25 months (range 17– 48). Reasons for initiating treatment were visual compromise and progressive tumor growth. Doses were as recommended. One child reported decreased orbital pain after one week and another, with involvement of the masseters, had increased ability to chew food. Toxicities were mostly to skin and nails grades 1–2 as expected. Additionally, 60% had debulking surgery of preseptal eyelid tumor in first year of medical treatment. Volumetric MRI measurements showed reduction of 8–26% at 1 year from baseline with a maximal reduction of 45% in two patients at 22 & 45 months. No change in visual function was recorded following treatment initiation. In conclusion, trametinib may decrease tumor size in young children with orbital PN and may prevent progressive disfigurement.

NFB-04. EXAMINING DIFFUSION, ARTERIAL SPIN-LABELED PERFUSION, AND VOLUMETRIC CHANGES IN THE NEUROFIBROMATOSIS TYPE 1 BRAIN USING AN ATLAS-BASED, MULTI-PARAMETRIC APPROACH

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BACKGROUND: Neurofibromatosis Type 1 (NF1) is a multisystem disorder with wide ranging clinical implications. Patients may present with macrocephaly, stroke, and cognitive deficits, all of which may impede normal neural development. We applied atlas-based, multi-parametric MRI analysis of regional brain to evaluate diffusion, arterial spin-labeled (ASL) perfusion, and volumetric changes in children with NF1. METHODS: Children evaluated for NF1 from 2009 to 2018 at Stanford University (n=78) were retrospectively reviewed and compared to healthy controls (n=100). All patients underwent diffusion-weighted (DWI) magnetic resonance imaging at 3T, and children with brain tumors were excluded. Using atlas-based DWI analyses, we assessed volume, median apparent diffusion coefficient (ADC), and cerebral blood flow in the cerebral cortex, thalamus, caudate, putamen, globus pallidus, hippocampus, amygdala, nucleus accumbens, brain stem, and cerebral white matter. We also measured volume of the lateral ventricles. Multivariate analysis of covariance was used to test for differences between controls and NF1 patients, controlling for gender and age at time of imaging. RESULTS: Comparing NF1 to controls, we detected increased volume and decreased ASL cerebral blood flow in white matter and all subcortical and cortical structures except for brainstem volume. Median ADC was also increased in the thalamus, pallidum, hippocampus, and brainstem. CONCLUSIONS: Using a multi-parametric approach, we demonstrate quantitative measures of microstructural and physiologic changes of the NF1 brain. Atlas-based, quantitative MRI brain signatures may serve as biomarkers of neural development and further provide insight into associated cognitive dysfunction or risks for vasculopathy-related strokes in children with NF1.

NFB-05. AN UNUSUAL PRESENTATION OF RECURRENT LANGERHANS CELL HISTIOCYTOSIS OF THE CRANIOFACIAL BONES IN A PATIENT WITH NEUROFIBROMATOSIS TYPE I

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Neurofibromatosis type 1 (NF1), predisposes patients to benign and malignant tumors due to lack of suppression of the mitogen activated protein kinase (*MAPK*) signaling pathway. Langerhans cell histiocytosis (LCH) manifests in numerous ways, from localized lesions to multisystem organ involvement secondary to a constitutively active *MAPK* signaling cascade often driven by *BRAF* mutations. While both LCH and NF1 are characterized by overactive *MAPK* signaling, there are few reports of the two diseases occurring simultaneously. We report a novel case of a patient with underlying NF1 and recurrent LCH without a *BRAFV600E* mutation. She initially presented at 2 years of age with an aggressive appearing mass of the left temporal bone found on surveillance imaging. Pathology was consistent with Langerhans histiocytosis and she was treated with the LCH-III protocol for patients with high-risk LCH due to the location of her lesion. Five years after completion of therapy, MRI demonstrated development of a calvarial mass consistent with relapsed LCH in a new risk site. Lesional curettage was performed and pathology confirmed recurrence of LCH with juvenile xanthogranulomatous features. *BRAF* testing of blood and the lesion were negative for any *BRAF* alterations. Further genomic evaluation of the tumor is in progress at this time to evaluate for other known mutations associated with LCH. The patient is currently receiving monthly cytarabine treatment which she has tolerated to date. Our patient represents a unique presentation of recurrent LCH in a patient with NF1 and further molecular evaluation may help identify other drivers of LCH activation.

NFB-06. TREATMENT CHALLENGES IN PEDIATRIC GLIOBLASTOMA MULTIFORME WITH CONCURRENT SOMATIC AND GERMLINE NF1 MUTATIONS WITH GERMLINE MISMATCH REPAIR MUTATIONS: TWO UNIQUE CASES

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INTRODUCTION: We report the first known cases of pediatric glioblastoma (GBM) with prior clinical NF1 diagnoses, one with concurrent germline Lynch syndrome (LS) and NF1, and the other with somatic NF1 mutation and germline constitutional mismatch repair deficiency (CMMRD). METHODS: Two pediatric GBM cases with prior NF1 clinical diagnoses based on neurocutaneous criteria were reviewed. Next generation sequencing and immunohistochemical staining were used for somatic and germline *NF1* and MMR gene mutation detection, and for MMR protein expression, respectively. RESULTS: Sixteen year old male with prior NF1 clinical diagnosis had resection of right frontal GBM revealing somatic mutations of POLE and PMS2, but not NF1. His father had confirmed LS with MSH2 mutation and no neurocutaneous stigmata. Patient's germline testing revealed both pathogenic MSH2 plus NF1 mutations confirming LS and NF1. Treatment consisted of chemoradiation with temozolomide followed by adjuvant temozolomide with stable disease at 8 cycles. Nineteen year old male with former NF1 clinical diagnosis had 2 GBMs, first in left midbrain biopsied revealing somatic PMS2 and NF1 mutations underwent radiation then 7 cycles of temozolomide, then new left frontal GBM underwent re-