Abstracts

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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 susceptible to this approach but have no immunogenic tumor antigens for presentation on MHC-I. Decitabine and other DNA methyltransferase (DNMT) inhibitors promote expression of oncogenic antigens and endogenous immune responses through epigenetic alterations. We explore 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 oncogenic antigens. PBT showed exquisite sensitivity to IFNγ, increasing expression of MHC-I/PD-L1 along with ISGs (TAPI, MX1, IFI1). Decitabine enhanced IFNγ-induced gene expression of oncogenic 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 patient material 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-quietest 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 allow us to recapitulate biological 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, transcripional profiling, and MHC1 expression. In vivo preclinical studies treated mouse models with CDK4/6i with or without immune check-point inhibitors. RESULTS: CDK4/6i (Abemecicib) reduced proliferation of DIPG cells derived from mouse models, and displayed a modest increase in immune activation by MHC1 expression and transcriptions. Pilot in vivo preclinical studies showed no significant changes in DIPG proliferation or immune changes with CDK4/6i treatment, ICT treatment, or the combination of CDK4/6 + ICT. In vivo testing of other immune-modulatory drugs identified additional candidates that can be tested in vivo. CONCLUSIONS: We have developed new mouse models of DIPG that will allow us to develop and test new therapeutic strategies.

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

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The choroidplexus (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, choroidplexus 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, a FDA-approved SHH pathway inhibitor, suppressed 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-ciliaogenesis circuit driven by Geminin/coiled-coil domain-containing protein 1 (GEMC1) are expressed in CP epithelium, GEMC1's functional 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-ciliaogenesis program. Together, these results indicate that a solitary primary cilium is crucial for CPC development, whereas multi-ciliaogenesis 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 carries 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: To develop and validate this model, we generated primary (BT245) and propagating irradiated (BT245RM) pHGG tumor cells. We developed in vitro and in vivo preclinical models with the pHGG BT245 cell line. We implanted BT245 cells subcutaneously in mice to develop an in vivo model. We isolated BT245RM cells from tumor xenografts and implanted into nude mice to develop an in vitro model. RESULTS: BT245RM 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 pHGG patient sample, including relaxation of the G2/M cell cycle checkpoint. BT245RM model recapitulates altered SHH pathway and elevated immune response genes. We tested the convergence of these immune priming mechanisms with CDK4/6 inhibition, anti-PD-L1, and combination of CDK4/6i + ICI. In vitro testing of other immune-modulatory drugs identified additional candidates that can be tested in vivo. 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 gliomas, we studied the effects of Atrx loss using four previously characterized Nft1+/+ Trp53+ murine glioma lines. Lines 130G8/3 and 130D8 (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, BIRB1352. 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-267. These cell lines all expressed ATRX and DAXX. However, STS-267 contained a TERT promoter mutation and STB8-14 had a known SNP in the TERT promoter, while NF90-8 had no alterations. ATRX RNA 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-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-ciliaogenesis circuit driven by Geminin/coiled-coil domain-containing protein 1 (GEMC1) are expressed in CP epithelium, GEMC1's functional 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-ciliaogenesis program. Together, these results indicate that a solitary primary cilium is crucial for CPC development, whereas multi-ciliaogenesis circuit possesses tumor suppressive functions and may represent a novel therapeutic target in CPC.
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 drugs of class.

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 13 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 splicing (likely mosaic) NF2. James’ pain was partially responsive to multiple oral medications, including opioids. At age 18 he 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 provided a maximal reduction of 45% in two patients at 22 & 45 months. No change of preseptal eyelid tumor in first year of medical treatment. Volumetric MRI showed decreased orbital pain after one week and another, with involvement of the palpebral conjunctiva and increased ability to chew food. Toxicities were mostly to skin and muscle, with 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 ulcerated mass of the left temporal bone found on surveillance imaging. Pathology was consistent with Langerhans cell 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 cutaneous 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 cytarbine 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 GLOBLASTOMA 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 a pathogenic MSH2 plus pathogenic MSH6 mutations in 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 GMBs, first in left midbrain biopsyed revealing somatic MSH2 and NF1 mutations underwent radiation then 7 cycles of temozolomide, then new left frontal GBM underwent re-