**DIPG-37. PREDICTING OUTCOME IN CHILDHOOD DIFFUSE MIDLINE GLIOMA USING MAGNETIC RESONANCE IMAGING BASED TEXTURE ANALYSIS**

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**BACKGROUND:** Diffuse midline gliomas (DMG) are aggressive brain tumours with 10% overall survival (OS) at 18 months. Predicting OS will help refine treatment strategy in this patient group. MRI based texture analysis (MRTA) is a novel technique that provides objective information about spatial arrangement of MRI signal intensity and has potential as an imaging biomarker. To investigate MRTA in predicting OS in childhood DMG, we employed a novel proteomics approach developed at our institution to further elucidate the impact of H3K27M mutation on glioma epigenetic signatures and treatment response. METHODS: Epitope-specific analysis was performed on tumor samples (H3K27M WT n=3, H3K27M n=9) to characterize 95 distinct Histone H3 N-terminal tail modification states. Cells were treated with JQ1 or DMSO, and collected at 0, 24h, 48h, Histones extracted from isolated nuclei and immunopurified, then analyzed by LC-MS/MS. Results were integrated with RNA-Seq and ChIP-Seq (H3.3K27M, H3.3, H3K27Ac, H3K27me3, H3K4me1, H3K4me3) from the same cell lines. Pediatric glioma tissues (H3K27M WT n=3, H3K27M n=9) were similarly analyzed to validate cell line results. RESULTS: Cell PTM profiles cluster by H3 mutation status on unsupervised analyses, significant differential PTM abundance and genomic enrichment of H3K27M. In 25% of patients, all of which can be targeted. Notably, intratumoral ad

**DIPG-38. ADDITION OF MULTIMODAL IMMUNOTHERAPY TO COMBINATION TREATMENT STRATEGIES FOR CHILDREN WITH DIPG: FINAL RESULTS OF A COHORT OF CHILDREN**

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The children with Diffuse Intrinsic Pontine Glioma (DIPG) remain dismal in spite of radio- and chemotherapy or therapies based on molecular biology diagnostics. Immunotherapy is a powerful and promising approach for improving the overall survival (OS). A retrospective analysis for feasibility, immune responsiveness and OS was performed on 41 children treated with Newcastle Disease Virus, hyperthermia and autologous dendritic cell vaccines as part of an individualized combined treatment approach for DIPG patients at diagnosis (n=28), or at time of progression (n=13). All except one patient had reduced values of at least one immune test before initiating immunotherapy and at least one PanTum Detect test was outside the normal range. Ten patients had PD-L1 mRNA expression in circulating tumor cells at diagnosis. Multimodal immunotherapy was feasible as scheduled, until progression, in all patients without major toxicities. When immunomodulation was part of primary treatment, median PFS and OS were 8.4m and 14.4m respectively. With a 2-year OS of 10.7%. When immunotherapy was given at the time of progression, median PFS and OS calculated from diagnosis were 6.5m and 9.1m respectively. T1 shift and rise in PanTum Detect test scores were linked with longer OS. Multimodal immunotherapy is feasible with major toxicity, and its value as part of a combination treatment for primary diagnosed DIPG should be elaborated in clinical trials.

**DIPG-39. NOVEL PROTEOMIC ANALYSIS REVEALS EPIGENETIC THERAPEUTIC TARGETS IN Pediatric GLIOMA**

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**INTRODUCTION:** Diffuse midline glioma is a highly morbid pediatric cancer. Higher than 80% harbor Histone H3K27M mutation. In all pathways, H3 post-translational modifications (PTMs) and genomic enrichment patterns, affecting chromatin structure and transcription. We previously identified putative epigenetic responses of H3K27Ac/bromodomod co-enrichment and the predictive efficacy of bromodomain inhibition (JQ1) in DIPG. Here, we employ a novel proteomics approach developed at our institution to further elucidate the impact of H3K27M mutation on glioma epigenetic signatures and treatment response. METHODS: Epitope-specific analysis was performed on tumor samples (H3K27M WT n=3, H3K27M n=9) to characterize 95 distinct Histone H3 N-terminal tail modification states. Cells were treated with JQ1 or DMSO, and collected at 0, 24h, 48h, Histones extracted from isolated nuclei and immunopurified, then analyzed by LC-MS/MS. Results were integrated with RNA-Seq and ChIP-Seq (H3.3K27M, H3.3, H3K27Ac, H3K27me3, H3K4me1, H3K4me3) from the same cell lines. Pediatric glioma tissues (H3K27M WT n=3, H3K27M n=9) were similarly analyzed to validate cell line results. RESULTS: Cell PTM profiles cluster by H3 mutation status on unsupervised analyses, significant differential PTM abundance and genomic enrichment of H3K27M. In 25% of patients, all of which can be targeted. Notably, intratumoral ad
predicted drug sensitivities with distinct groups of tumors predicted to re-

DIPG. DISSECTING THE MECHANISTIC BASIS FOR ACVR1 AND
PK3CA MUTATION CO-OCCURRENCE IN DIFFUSE MIDLINE
GLIOMAS USING GENETICALLY ENGINEERED MOUSE MODELS

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Diffuse midline gliomas (DMGs) are aggressive childhood brain tumors with poor survival rates. Most of these tumors carry K27M mutations in their H3-encoding genes, particularly H3.3A and HIST1H3B. In addition, activating mutations in ACVR1 and PK3CA co-occur in a subset of DMGs. To understand how these lesions drive the development of DMGs, we generated genetically modified mouse models by overexpression of ACVR1 or PK3CA with or without activating K27M mutations in the brains of newborn mice. We find that ACVR1, but not PK3CA, promotes glioblastoma formation. ACVR1 induces a glioblastoma-like transcriptome in neural progenitors and glioblastoma cells, suggesting that ACVR1 may be a therapeutic target for DMG treatment.

DIPG-42. TOWARD MULTIMODALITY THERAPY FOR DIPG/
DIPG DEVELOPMENT AND INVESTIGATION OF CRANIOSPINAL
IRRADIATION AND CONVECTION-ENHANCED DELIVERY PDX
MODELS

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BACKGROUND: Diffuse intrinsic pontine glioma (DIPG) and diffuse midline gliomas (DMGs) are aggressive childhood brain tumors with poor survival rates. Most of these tumors carry K27M mutations in the histone H3-encoding genes, particularly H3.3A and HIST1H3B. In addition, activating mutations in ACVR1 and PK3CA co-occur in a subset of DMGs. To understand how these lesions drive the development of DMGs, we generated genetically modified mouse models by overexpression of ACVR1 or PK3CA with or without activating K27M mutations in the brains of newborn mice. We find that ACVR1, but not PK3CA, promotes glioblastoma formation. ACVR1 induces a glioblastoma-like transcriptome in neural progenitors and glioblastoma cells, suggesting that ACVR1 may be a therapeutic target for DMG treatment.

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DIPG-44. A GAIN OF FUNCTION EZH2 MUTATION DELAYS
DIFFUSE INTRINSIC PONTINE GLIOMA PROGRESSION IN AN
ADULT HUMAN H3K27M+ DIPG MOUSE MODEL

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BACKGROUND: Diffuse Intrinsic Pontine Glioma (DIPG) remains an incurable pediatric brain cancer. The oncoprotein H3K27M implicated in 80% of the cases, is also predicted to target Enhancer of Zeste Homolog 2 (EZH2), the catalytic component of the Polycomb Repressive Complex 2 (PRC2). There are no reported mutations of Ezh2 and its function in DIPG is not fully determined. This work aims to address the role of Ezh2 in DIPG. METHODS: Brainstem tumors were established by intracranial injections of Nestin-εAx; Ezh2-KO/mice. Results: EZH2 expression significantly reduced in DIPG. EZH2 overexpression (Ezh2-εAx) and knockout (Ezh2-KO/mice). RESULTS: Ezh2 overexpression (Ezh2-εAx) and knockout (Ezh2-KO/mice). CONCLUSIONS: EZH2 overexpression reduces EZH2 expression significantly reduced in DIPG. EZH2 overexpression (Ezh2-εAx) and knockout (Ezh2-KO/mice). CONCLUSIONS: EZH2 overexpression reduces EZH2 expression significantly reduced in DIPG. EZH2 overexpression (Ezh2-εAx) and knockout (Ezh2-KO/mice).