show evidence of disease progression within months of completing radiation therapy, especially those who harbor the H3.3K27M mutation. Since chemotherapy does not provide any significant outcome improvement, it is crucial to find a suitable radiosensitizer. Our research has shown that the JMJD3 demethylase inhibitor, GSK-J4, not only exerts potent anti-tumor activity on H3K27M mutant DIPG cells, but also restores methylation while decreasing genes that encode proteins involved in DNA double-strand break (DSB) repair. Our aim is to investigate the hypothesis that GSK-J4 may inhibit radiation-induced DNA repair via chromatin modification, making it a potential radiosensitizing agent. **METHODS:** We evaluated DNA damage repair via quantitative-PCR (q-PCR), immunocytochemistry of DSB markers γ H2AX and 53BP1, and DNA repair assay. MNase digestion was used to analyze nucleosome assembly in cellular chromatin. We are currently conducting western blotting, DNA repair assay, FAIRE-seq and clonogenic survival assays to assess DIPG response to radiation therapy (RT) in combination with GSK-J4. *In vivo* response to radiation monotherapy and combination of RT + GSK-J4 will be measured by bioluminescence imaging and animal survival studies. **RESULTS:** qPCR results showed that GSK-J4 significantly reduces DNA DSB repair genes such as PARP1, PARP2, SMARCB1 and BRCA1, in irradiated DIPG cells. Immunocytochemistry results support that GSK-J4 sustains high levels of γ H2AX and 53BP1 in irradiated DIPG cells, thereby inhibiting DNA DSB repair. MNase assay indicated that GSK-J4 may cause chromatin compaction which prevents DNA DSB repair. Results from the clonogenic assays, to assess long term effect of GSK-J4 on irradiated cells, as well as western blotting, FAIRE-seq and *in vivo* studies will be reported at the meeting.

DIPG-29. GENOMIC LANDSCAPE OF DIFFUSE INTRINSIC PONTINE GLIOMA: AN ANALYSIS OF THE DIPG-BATS COHORT

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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) remains a devastating and incurable disease. The DIPG-BATs clinical trial incorporates diagnostic biopsy with molecularly determined treatment stratification. Here we present the initial genomic analysis of the DIPG-BATs cohort.

METHODS: Children enrolled on the DIPG-BATs clinical trial underwent upfront diagnostic biopsies prior to commencement of therapy. RNA and DNA were extracted from single core biopsies and subjected to whole-genome sequencing (WGS) and RNA-sequencing. Tumor samples were also collected at autopsy if there was parental consent. **RESULTS:** Fifty-three patients were enrolled on study, of whom 50 underwent biopsy. There were no biopsy-related deaths. A mean of 5ug of RNA and 10ug of DNA were extracted from single frozen cores. WGS on 41 DIPG samples (including eight autopsy samples) revealed a mean mutation rate of 0.753 mutations per Mb. We confirmed TP53, PIK3CA, H3F3A, ACVR1, PPM1D, and HIST1H3B to be recurrently mutated in DIPG. Additional mutations were found in epigenetic modifiers including ASXL1. Copy-number analysis revealed PDGFRA to meet statistical significance as a recurrent amplification peak in DIPG (q<0.25). Gene-set analysis revealed mutations in the TERT pathway, ARF pathway, AKT/PTEN pathway, TP53 pathway, cell cycle and apoptosis pathways to be statistically enriched across the cohort (q<0.10). Analysis of paired diagnosis and autopsy samples revealed evolution of tumors following treatment. Samples obtained at autopsy exhibited a significantly increased mean mutation rate compared to untreated biopsies (p<0.0001). **CONCLUSIONS:** Whole-genome sequencing of DIPG-BATs samples confirms driver mutations in multiple pathways implicated in cancer. Initial investigation of paired biopsy and autopsy samples allows the analysis of the genomic evolution of DIPG. These findings shed insight into both oncogenic and resistance drivers in DIPG. *equal contribution

DIPG-30. DIFFERENTIAL HYPOXIC RESPONSE IN HUMAN DIPG CELL LINES

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BACKGROUND: Diffuse Intrinsic Pontine Glioma (DIPG) are rare and aggressive childhood tumors, with an abysmal prognosis and limited experimental models available for study. A recent report showed that DIPG are hypoperfused compared to surrounding brain tissue, suggesting that the tumor cells are exposed to a hypoxic microenvironment. This stimulus may induce widespread transcriptional changes through activation of Hypoxia-inducible Factors (HIF), which have been associated with invasion, metastasis, angiogenesis, and resistance to radiation therapy and chemotherapy. Therefore, *in vitro* models using DIPG cells cultured in conditions of normal oxygen tension may not be representative of *in vivo* tumors because of artificial differences in HIF activation. **METHODS:** To test cellular responses to hypoxia, human DIPG cell lines with H3.1 K27M and H3.3 K27M mutations were treated with the hypoxia-mimetic compound, cobalt (II) chloride, for 24 hours. Simulated hypoxic responses were assayed using Western blot analysis, proliferation assays, and extracellular flux analysis. **RESULTS:** The H3.3 K27M cell line increased expression of HIF1 α with cobalt (II) chloride treatment and correlated with an increase in glycolytic rates and increased expression of proteins involved in glucose transport and glycolysis. The H3.1 K27M cell line increased expression of HIF2 α and not HIF1 α after cobalt (II) chloride treatment, correlating with an increase in some glycolysis-associated proteins but no change in glycolytic rate. **CONCLUSION:** These data suggest that the effects of a hypoxic microenvironment should be considered in experimental models using cultured DIPG cell lines to understand differences in cell behavior, patient prognosis, and predicted treatment responses. Activation of specific HIF isoforms may underlie differences in hypoxic responses among cell lines.

DIPG-31. CLINICO-PATHOLOGICAL REVIEW OF 26 CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA

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METHODS: Twenty-six patients with diffuse intrinsic pontine glioma (DIPG) were retrospectively analyzed at National Center for Child Health and Development, one of the largest pediatric brain tumor centers in Japan. The following information was collected from the electric medical record: clinical characteristics, surgery / pathology reports, treatments and outcomes. **RESULTS:** Between June, 2002 and April, 2016, 26 patients were diagnosed as DIPG and treated. Median age was 6 years (2–12). There were 19 boys and 7 girls. Common presenting symptoms included, cranial nerve