

RT response, RT treated tumors have increase in cell cycle regulatory genes such as Cdkn1a, across all clusters. In non-resident myeloid cells, compared to untreated tumor, RT is associated with a robust upregulation of interferon response genes in both macrophages (Isg15 Fold Change (FC) 2.30; Ifit1 FC 1.64; Ifit3 FC 2.02; Cxcl10 FC 2.29) and dendritic cells (Isg15 FC 2.67; Ifit1 FC 1.72; Ifit3 FC 2.06; Cxcl10 FC 1.50). We also find differential expression of immune checkpoints in RT-treated versus untreated tumor with decreased expression of Lag3, Tim3 (Havcr2), and Csf1R and increased expression of Cd47, Sirpa and Gitr (Tnfrsf18) post RT. In summary, RT stimulates a pro-inflammatory TIME response and alters immune checkpoints in DMG, highlighting the potential for combining RT and immunotherapy in these tumors.

DIPG-46. RADIATION INDUCED SENESCENCE IN DIFFUSE INTRINSIC PONTINE GLIOMA CELLS REVEALS SELECTIVE VULNERABILITY TO BCL-XL INHIBITION

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Diffuse intrinsic pontine glioma remains a devastating condition with a dismal five year survival rate less than 5%. New approaches for treating this aggressive disease are critical to driving progress. Conventional radiotherapy remains the cornerstone of treatment, with no chemotherapeutic agent found to improve survival. However, radiotherapy is often delivered as a palliative treatment, and disease often recurs 3-6 months after. Radiation causes DNA damage and oxidative stress yielding a senescent state of replicative arrest in susceptible cells. However, increasing evidence demonstrates malignant cells can escape senescence leading to tumour recurrence. Targeted ablation of non-replicating senescent tumour cells following radiation could negate tumour recurrence. It remains unknown whether DIPG undergoes senescence following radiation, and furthermore, whether senolytics can be utilised to target senescent DIPG cells. We employed radiation to induce a senescent state in primary human DIPG cell lines. Senescence was confirmed using SA- β -gal staining, lack of EdU incorporation and qRT-PCR to characterise the SASP in three primary human DIPG cell lines. RNA-sequencing on DIPG cells following radiation revealed senescence and SASP signatures. Likewise, expression of senescence markers has been detected in human tumours. Viable cells that survive radiation were then utilised to screen candidate senolytic drugs, only Bcl-XL inhibitors demonstrated reproducible senolytic activity in radiation treated DIPG cells. In addition, Bcl-XL degradation using PROTACs (proteolysis targeting chimeras) resulted in a significant increase in senolysis of susceptible tumour cells. Conversely, Bcl-2 inhibitors failed to show any consistent senolytic activity. We are currently performing preclinical studies in the mouse to test the efficiency of senolytics against DIPG. These results demonstrate future possibilities of targeting radiation induced senescence in DIPG, using novel senolytic therapies and highlight Bcl-XL dependency as a potential vulnerability of surviving DIPG cells following exposure to radiation.

DIPG-47. TSO500CTDNA SEQUENCING REVEALS ONCOGENIC MUTATIONS AND COPY NUMBER VARIATIONS IN THE LIQUID BIOME OF CHILDREN WITH DIFFUSE MIDLINE GLIOMA

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BACKGROUND: Molecular profiling of childhood CNS tumors is critical for diagnosis and clinical management, yet tissue access is restricted due to sensitive neuroanatomical locations. Moreover, CNS tumors including diffuse midline glioma (DMG) exhibit mutational heterogeneity and clonal evolution, which cannot be captured by upfront diagnostic biopsy alone. To address the lack of tumor visibility, and provide opportunity for longitudinal sampling, we validated and optimized a commercially available deep sequencing platform for analysis of circulating tumor DNA (TSO500ctDNATM). **METHODS:** In a proof-of-concept study,

we defined the sensitivity, specificity, and clinical relevance of our novel ctDNA platform via analysis of paired tissue, CSF, and blood from children with DMG (n=10). Paired samples were assessed for concordance and sequencing results were compared to digital droplet PCR (ddPCR) detection of prognostic H3K27M mutation. **RESULTS:** DMG associated mutations in genes including *H3-3A*, *H3C2*, *TP53*, and *ACVR1* were detected in ctDNA, including in CSF samples with low (<5ng) starting DNA input. Of 9 H3K27M mutations identified in tumor, 8 were present in CSF and 3 in plasma/serum, for a positive percent agreement with tumor results of 89% and 33%, respectively. Among CSF samples, H3.3K27M was detected in 6/6 cases, and H3.1K27M in 2/3 cases, with variant allele frequencies comparable to ddPCR results. CNVs including *PDGFRA*, *KIT*, and *MDM4* gains were detected in CSF and paired tumor. Low frequency events including *ACVR1*, *PIK3CA* activating mutations and *KRAS* amplification were detected in CSF but absent from paired tumor, indicating tissue heterogeneity. Strategies to optimize ctDNA detection, including optimization of ctDNA isolation and adjustment of library QC metrics, were identified. **CONCLUSION:** Targeted ctDNA deep sequencing is feasible, can inform on clinically relevant tumor mutation and CNV profiling, and provides an opportunity for longitudinal monitoring of tumor genomic evolution in the liquid biome of children with CNS tumors.

DIPG-48. MRI VOLUMETRIC AND MACHINE LEARNING BASED ANALYSES PREDICT SURVIVAL OUTCOME IN PEDIATRIC DIFFUSE MIDLINE GLIOMA

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INTRODUCTION: Diffuse midline glioma (DMG) is a fatal childhood CNS tumor. Magnetic resonance imaging (MRI) is the gold standard for DMG diagnosis and monitoring of response to therapy. Leveraging novel MRI analytical approaches, including volumetric and machine learning based analyses, may aid in the prediction of patient overall survival (OS) and help to identify high-risk cases. **METHODS:** T1- and T2-weighted MR images were retrospectively collected from children and young adults diagnosed with DMG (n=43). MRI features, including manually determined 3D tumor volume (T2), T1 contrast-enhancing tumor volume, T1 relative to T2 volume (T1/T2), tumor relative to whole brain volume, tumor average intensity, and tumor heterogeneity (i.e., intensity skewness and kurtosis), were evaluated at upfront diagnosis. MRI features were analyzed to identify significant predictors of OS outcome, which was defined as OS shorter, or longer, than one year from diagnosis. A support vector machine was used to predict OS outcomes using combinations of these features. **RESULTS:** The presence of T1 contrast-enhancing tumor at diagnosis (p=0.01), and a high T1/T2 ratio (>25%, p=0.009), predicted significantly shorter median OS. Moreover, feature selection identified T2 mean intensity (p<0.001), T2 image intensity skew (p=0.006), T1/T2 ratio (p=0.02), and T1 volume relative to whole brain (p=0.03) as significant predictors of OS outcome (short versus long). Combining T2 mean intensity, T2 image skew, T1 segment kurtosis and patient gender resulted in OS outcome prediction accuracy of 83.3% (sensitivity=85%, specificity=81.8%, n=42 cases). **CONCLUSION:** We have identified MRI volume and imaging features that significantly predict OS outcome in children diagnosed with DMG. Our findings provide a framework for incorporating MRI volumetric and machine learning analyses into the clinical setting, allowing for the customization of treatment based on tumor risk characteristics.

DIPG-49. INTERNATIONAL PRECLINICAL DRUG DISCOVERY AND BIOMARKER PROGRAM INFORMING AN ADOPTIVE COMBINATORIAL TRIAL FOR DMG

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