

and others have identified that acetylation of H2A.Z (H2A.Zac) can be an oncogenic driver in adult cancer types through mislocalization of H2A.Z at promoters and enhancers of cancer-associated genes loci. However, the role of H2A.Z in H3.3K27M+ DIPG has never been studied. Thus, we hypothesized that H2A.Zac cooperates with H3.3K27M to drive DIPG oncogenesis. Here we aim to unravel the molecular relationship between H2A.Zac and H3.3K27M in DIPG and their link to oncogenesis. First, using a histone mass spectrometry dataset, we found that the level of H2A.Zac is significantly higher in samples with H3K27M compared to H3.3WT. In addition, the comparison between H2A.Zac with H3.3K27M ChIP-seq data in several DIPG cell lines showed that around 30% of H3.3K27M peaks overlapped with H2A.Zac marked regions, a similar proportion found between H3.3 and H2A.Z under physiological conditions. Interestingly, active enhancers are the most enriched regulatory regions for H3.3K27M/H2A.Zac overlapping regions and those enhancers are associated with genes involved in pathways commonly altered in H3.3K27M gliomas. These data suggest H2A.Zac levels are altered in DIPG and H2A.Zac may be involved in aberrant enhancer activation in DIPG and thus constitute a novel therapeutic target for H3.3K27M+ DIPG.

DIPG-38. SIGNIFICANT TUMOR REGRESSION OF H3K27M-MUTATED DIFFUSE MIDLINE GLIOMA OF THE BRAINSTEM WITH PANOBINOSTAT: A CASE REPORT.

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INTRODUCTION: Diffuse midline glioma is a fatal CNS tumor that chiefly occurs in children. To date, standard of care has been limited to local field radiation. A histone mutation in H3K27M is seen in 80% of diffuse midline gliomas of the brainstem, also called diffuse intrinsic pontine glioma (DIPG). Panobinostat, a potent inhibitor of histone deacetylases, has shown moderate efficacy in DIPG/DMGs in preclinical models, and a dose-finding clinical trial (PBTC-047) is ongoing. We report on a case of a child with biopsy-proven H3K27M-mutated DMG of the brainstem treated off-trial with panobinostat monotherapy after radiation. **METHODS:** This is a case report of a 12-year-old female with H3K27M-mutated DMG of the medulla and cerebellum that exhibited gadolinium-enhancement on MRI. The patient was treated with 54 Gy focal photon radiation to the tumor field over six weeks after biopsy demonstrating the H3.3K27M mutation (H3F3A), as well as mutations in TP53, PIK3R1 and AXSL1 genes. She was not eligible for PBTC-047 due to uncontrolled hypertension. She received panobinostat as per the ongoing PBTC protocol at a dose of 28 mg/m² given on Monday, Wednesday, and Friday on alternating weeks. She received three 28-day cycles over three months. **RESULTS:** The patient tolerated the therapy well, with minimal adverse reactions of low-grade abdominal pain and constipation. MRI imaging after three cycles revealed an 80% reduction in tumor volume. Together with this radiographic response, she exhibited improvement in left-sided motor strength and improved left facial sensation. Due to an unrelated need for oral surgery, panobinostat was held. MRI imaging six weeks after cessation of panobinostat revealed extensive relapse with supratentorial and spinal disease. **CONCLUSION:** Systemic panobinostat may demonstrate anti-tumor efficacy in some cases of H3K27M-mutated DMG. Future work is required to determine factors predictive of favorable response.

DIPG-39. NEW PRECLINICAL MODELS FOR DIFFUSE MIDLINE GLIOMA

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Malignant brain tumors are the leading cause of childhood death in Germany, with Diffuse Midline Glioma (DMG) being the most lethal of all paediatric brain tumors. Current treatment strategies are limited to irradiation which prolongs survival only by a few months. Preclinical studies have identified effective drug candidates, but translation into the clinic remains a major obstacle. It is known that interactions between tumor cells and components of the TME (tumor microenvironment), such as cell to cell contacts between malignant and non-malignant cells or secreted factors, can increase therapy resistance and progression of brain tumors. However, these important factors are not present in most conventional cell culture models for drug testing. Consequently, there is a need for more realistic DMG models to improve the relevance and translational potential of current drug screening. Therefore, the goal of this study was to develop a new DMG model for drug testing, consisting of induced pluripotent stem cell (iPSC) derived human brain cells and patient derived DMG cells to better mimic the complex tumor microenvironment. We co-cultured three-dimensional cerebral organoids with DMG tumor spheres resulting in the formation of DMG-Brain-Organoids (DBO). Preliminary results show that co-culture induces distinct tumor cell subpopulations corresponding to those detected

in DMG tumors by single cell RNA sequencing (Filbin et al., 2018). These subpopulations mainly differ in their proliferative capacity and their differential response to clinical interventions may be critical for therapeutic success. DBOs subjected to drug treatments (single or combination) were sectioned and individual therapy effects on tumor cell subpopulations and proliferative capacity were monitored using multiplexed immunofluorescence imaging. By observing drug effects in a realistic setup, we hope to improve the predictive power of our preclinical drug screens and to find new combination therapies for DMG.

DIPG-40. COMBINED PHARMACOLOGICAL AND GENETIC SCREENING TO IDENTIFY DEPENDENCIES AND COMBINATIONS IN ACVR1-MUTANT DIFFUSE MIDLINE GLIOMA

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Somatic mutations in ACVR1, which encodes the serine/threonine kinase ALK2, are found in 20-25% of DMG-H3K27 patients. Treatment of ACVR1-mutant patient-derived models with multiple chemotypes of ALK2 inhibitors (ALK2i) results in reduced cell viability in vitro and extended survival in orthotopic xenografts in vivo but, as single agents, these inhibitors were unable to achieve a complete anti-tumour response. Recently we reported that combinatorial treatment of ACVR1-mutant DIPG cells with vandetanib (RTK inhibitor) and everolimus (mTOR/ABC transporter inhibitor) was synergistic both in vitro and in vivo and was shown to be a feasible combination to trial clinically in this setting. To identify specific dependencies in ACVR1-mutant cells which may be translatable with novel synergistic drug combinations alongside ALK2i, we have implemented both candidate and unbiased drug and genetic screening approaches. Using a panel of patient-derived ACVR1-mutant and wild-type models, we identified synergy between multiple chemotypes of ALK2i (M4K2009/LDN-214117) and PI3K/mTOR (AZD8055/everolimus) and MEK inhibitors (trametinib), reflecting the common co-segregation of PIK3CA/PIK3R1 alterations in these tumours. Whole-genome CRISPR/Cas9 screening of ACVR1-mutant SU-DIPG-IV cells in combination with two ALK2i (M4K2009/LDN-193189), confirmed a specific MTOR genetic dependency, as well as for the protein phosphatase regulatory subunit PPP2R1A, known to play a role in MAPK pathway activation. Additional hits include the serine/threonine kinase PKMYT1, a negative regulator of the G2/M checkpoint via a functionally redundant phosphorylation of CDK1/CNNB1 alongside WEE1; confirmatory drug assays with the WEE1 inhibitor AZD1775 resulted in a synergistic interaction with ALK2i in ACVR1-mutant cells. Hits were integrated with DepMap using 'gene-effect' scores (Chronos) enabling filtering of common essential genes. Preliminary pathway enrichment analysis (MAGECKFlute) identified ALK2i-specific vulnerabilities involving TGFβ1/SMAD signalling and histone deacetylation. These data highlight functionally rational and novel combinatorial possibilities for children with ACVR1-mutant DMG, with systematic preclinical assessment required for prioritisation for the clinic.

DIPG-41. MULTI-OMIC PROFILING OF PATIENT-DERIVED SUBCLONES IDENTIFIES AGGRESSIVE CELLULAR SUBPOPULATIONS IN PAEDIATRIC DIFFUSE HIGH-GRADE GLIOMAS (PDHGGs)

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Paediatric-type diffuse high-grade gliomas are classified into distinct subgroups based upon their location and defining molecular alterations, with very poor clinical outcomes in patients >3yrs. This extensive inter-tumour heterogeneity is further complicated by a wide diversity of genotypically- and phenotypically-distinct subclonal populations within individual tumours, providing a substantial barrier to developing effective treatments. We have sought to understand the dynamic cellular make-up of PDHGGs such that novel strategies aimed at targeting specific subpopulations based upon their contribution to disease progression as whole may be employed. Two complementary approaches have been undertaken to address this – first by carrying out single-cell profiling of bulk specimens, and the second isolation and propagation of single-cell-derived stem cell-like cultures in vitro. To-date we have studied 10 cases and a total of 218 subclonal colonies from both DMG-H3K27 and DHG-WT. In a spinal metastatic case of DMG-H3K27, lpWGS-FISH highlighted subpopulations driven by mis-segregation of amplified oncogenic cDNA, and mutually exclusive subpopulations defined by MYCN, PDGFRA and CCND1. Through integrated analysis of scRNA-seq and scATAC-seq, we show distinct chromatin accessibility profiles to underlie gene expression signatures defining unique subpopulations of cells. In add-