Abstracts

DIPG-34. LIFE AND END-OF-LIFE AFTER DIFFUSE INTRINSIC PONTINE GLIOMA DIAGNOSIS

INTRODUCTION AND OBJECTIVES: Despite advances in pediatric oncology, diffuse intrinsic pontine glioma (DIPG) life expectancy remains <10% at 2 years, and the mean survival time is 8-10 months from diagnosis. Its location associates multiple symptoms of complex management that condition their quality of life. The aim is to review our experience in treating the condition and the disease and the involvement of palliative care (PC).

METHODS: Single-centre retrospective study of 18 years diagnosed with DIPG between 2011-2022. A review of the electronic medical record was carried out, recording demographic data, oncological treatments, PC and advance care planning (ACP). RESULTS: We registered 14 patients (2 alive). Median age at diagnosis was 6.4 years (range: 3.5-11.9). Median survival from diagnosis was 9.6 months (range 0.5-18) and 3.8 months from progression (range: 0.1-12). At diagnosis, 92% received oncological treatment (radiotherapy 75%, chemotherapy 41%, oncologic viruses 21%, personal oncology 4%)

After progression, patients received chemotherapy or re-irradiation and in the last month of life 2 received chemotherapy. In the last 3 months of life, 64% were admitted at least once for progression or end-of-life. In the last month, all received oral dexamethasone (25mg/day) followed by PC (70%), with final survival in the last month of life in 36%. ACP was recorded in 4 patients, all of whom followed up for PC. Death occurred at home in 33%, hospital in 57%. Palliative sedation was used in 5 patients. CONCLUSIONS: Despite the known prognosis of DIPG, some patients continue receiving oncological treatment after progression and in the last month of life. Contact with PC teams occurred mainly at advanced disease stage. Median survival of children with DIPG is below one year, with multiple symptoms in the progression phase. Therefore, early follow-up by PC teams is recommended.

DIPG-35. PERSONALIZED TREATMENT FOR MOLECULARLY HETEROGENEUS DIFFUSE MIDLINE GLIOMA, H3 K27-ALTERED PAEDIATIC CASE

A study on a 7-year-old boy with diffuse midline glioma, more common in Paediatric-type low grade gliomas (Diffuse low grade glioma, MAPK pathway-altered). We present a twenty-month-old boy, previously healthy, presented with 2 weeks history of unsteady gait, drooling, cranial nerve palsies palsy MRI imaging showed diffuse intraventricular mass with classic radiological features of DIPG, 2.6 x 1.6 x 3.2 (AP x TV x CC) with no evidence for spinal metastases. Patient underwent right retro sigmoid approach and open biopsy of lesion he received focal Radiation Therapy. 54GY/30fx we have improvements in the lesion with edema and no active hydrocephalus. He was started on combination therapy BRAF inhibitor Dabrafenib and MEK Inhibitor Trametinib as maintenance therapy the patient gradually showed Marked neurological and clinical improvement. A 6-month MRI after start of targeted therapy showed favorable treatment response with complete resolution of the previous diffusion restriction, reduced tumor volume on MR perfusion -reduced perilesional edema otherwise almost stabilization of nonenhancing pontine lesion. The poor prognosis of recurrent DIPG is well known but our patient is clinically and radiologically stable with excellent quality of life and well tolerating the therapy. Our case show that personalized treatment approach that address molecular heterogeneity of H3K27M glioma are safe and feasible.

DIPG-36. THE BRAIN-GUT-MICROBIOTA AXIS TO PREDICT OUTCOME IN PEDIATRIC DIFFUSE INTRINSIC PONTINE GLIOMA

OBJECTIVE: Diffuse intrinsic pontine glioma (DIPG) is a rare childhood brain tumour with poor prognosis. Radiotherapy (RT) remains the only palliative treatment. It is essential to investigate any potential factors that condition their long-term survival and quality of life. Probiotics, prebiotics and probiotics are known to have beneficial effects on the gut microbiome. The objective of this study was to assess the microbiome of children with DIPG and to evaluate its potential impact on survival.

METHODS: A single-centre retrospective study of patients with DIPG was conducted. Stool samples were collected at diagnosis and 6 months following RT. Fecal DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen). The gut microbiome was analyzed using the Illumina NextSeq 500 platform. Sequence alignment was performed using the usearch tool, and taxonomic classification was performed using the Qiime2 pipeline. Statistical analysis was performed using the R project.

RESULTS: A total of 15 patients were included in the study. Of these, 9 patients had complete data available for analysis. The microbiome composition was significantly different between pre-RT and post-RT samples. In pre-RT samples, there was a decrease in the abundance of Firmicutes and an increase in the abundance of Bacteroidetes. In post-RT samples, there was an increase in the abundance of Actinobacteria and a decrease in the abundance of Proteobacteria.

CONCLUSIONS: Our results suggest that the gut microbiome is significantly altered in children with DIPG and that this alteration may be affected by RT. Further studies are needed to determine the clinical significance of these changes and whether they can be used as predictors of survival.

DIPG-37. EXPLORING THE ROLE OF THE EPIGENETIC FACTOR H2A.Z ACETYLATION IN DIPG

OBJECTIVE: Tumors in children with previously treated diffuse intrinsic pontine glioma (DIPG), have a median survival of one year. Therefore, it is essential to investigate any potential factors that condition their long-term survival and quality of life.

METHODS: We performed a single-centre retrospective study of patients with DIPG. Stool samples were collected at diagnosis and 6 months following RT. Fecal DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen). The gut microbiome was analyzed using the Illumina NextSeq 500 platform. Sequence alignment was performed using the usearch tool, and taxonomic classification was performed using the Qiime2 pipeline. Statistical analysis was performed using the R project.

RESULTS: A total of 15 patients were included in the study. Of these, 9 patients had complete data available for analysis. The microbiome composition was significantly different between pre-RT and post-RT samples. In pre-RT samples, there was a decrease in the abundance of Firmicutes and an increase in the abundance of Bacteroidetes. In post-RT samples, there was an increase in the abundance of Actinobacteria and a decrease in the abundance of Proteobacteria.

CONCLUSIONS: Our results suggest that the gut microbiome is significantly altered in children with DIPG and that this alteration may be affected by RT. Further studies are needed to determine the clinical significance of these changes and whether they can be used as predictors of survival.
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 H3K27M+ DMG has not been studied. We hypothesized that H2A.Zac cooperates with H3.3K27M to drive DIPG oncogenesis. Here we aim to unravel the molecular relationship between H2A.Z and H3.3K27M in DIPG and their link to oncogenesis. First, using a human DIPG brain organoid (BORG) model, 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 overlap 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.Z 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. Sonakshi Abadilla1, Thrithi Farahzadi1, Michelle Monje1.
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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 deacetylase, has shown moderate efficacy in DIPG/DMGs in preclinical models, and a dose-finding clinical trial (PTBCT-047) is ongoing. We report on a case of a child with biopsy-proven H3K27M-mutated DIPG 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 DIPG 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 AXS1 genes. She was not eligible for PTBC-047 due to uncontrolled hypertension. She received panobinostat as per the ongoing PTBC 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 improved mood, left-sided motor strength and increased speech. 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 DIPG. Future work is required to determine factors predictive of favorable response.

DIPG-39. NEW PRECLINICAL MODELS FOR DIFFUSE MIDLINE GLIOMA. Marbod Klemmer1, Pia Freidel1, Mariella G. Filbin2, Alexander Beck3.
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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 pediatric 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. We have established recombinant cell lines and primary tumor 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 discovery, consisting of immune 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 single or combination drug treatments (single or combination treatments) have shown 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. Rebecca Rogers1, Diana Carvalho1, Yura Grabovska1, Elisabet Fernandez2, Elisa Izquierdo3, Alan Mackay4, Chris Jones1,5.
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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 chemotherapies 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 clinically relevant tumor growth inhibition. Here, we report 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. Additionally, we determine specific dependencies in ACVR1-mutant cells which may be translatable with novel synergetic 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 the synergy between multiple chemotherapies of ALK2i (M4K2009/LDN-214117) and PI3K/mTOR (AZD8055/everolimus) and MEK inhibitors (trametinib), reflecting the common co-segregation of PI3KCA/PIK3R1 alterations in these tumors. Additional screens include the serine/threonine kinase MKMYT1, a negative regulator of the G2/M checkpoint via a functionally redundant phosphorylation of CDK1/CCNB1 alongside WEE1; confirmatory drug assays with the WEE1 inhibitor AZD1775 resulted in synergistic interaction with ALK2i in ACVR1-mutant cells. All data were integrated with DepMap using ‘gene-effect’ scores (Chronos) enabling filtering of common essential genes. Preliminary pathway enrichment analysis (MaGeCKFlute) identified ALK2-specific vulnerabilities involving TGBE/SAD1 signaling and histone deacetylation. These data highlight functionally rational and novel combinatorial possibilities for children with ACVR1-mutant DMG, with systemic 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). Ketty Kessl1, Yura Grabovska1, Anna Burford1, Sara Temelso1, Haider Tari1, Valeria Molinari2, Shauna Crampse3, Lu Yu1, Jyoti Choudhary1, Paula Prosek1,2, Michael Hubank1,2, Alan Mackay4, Chris Jones1,5, Institute of Cancer Research, London, United Kingdom. 6Royal Marsden Hospital, London, United Kingdom

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 makeup 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-like cells in vitro. To-date we have studied 10 cases and a total of 218 subclonal colonies from both DMG-H3K27 and DIPG-WT. In a spinal metastatic case of DMG-H3K27, lPwGS-FISH highlighted subpopulations driven by mis-segregation of amplified oncogenic ecDNA, and mutually exclusive subpopulations defined by MyCN, PDGFRα 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.