patient – identified and not included - was on KD < 3 months due to disease progression). Further feasibility analyses showed a duration of the KD of ≥ 3 months and less than 7 months (n=2), > 7 months and less than 1 year (n= 2), and two years (n=1), respectively. CONCLUSION: These results – based on a small patient population – suggest that the KD appears to be a feasible treatment option for children with DIPG. The potential duration of the KD is limited by the aggressive clinical behavior of DIPG. The safety analysis is currently being retrospectively assessed. These data should encourage further studies on a larger scale; ideally assessing the impact of the KD in DIPG patients in a randomized controlled trial.

DIPG-26. THERAPEUTIC EFFECTS OF RADIOTHERAPY WITH CONCOMITANT AND ADJUVANT TEMOZOLOMIDE VERSUS RADIOTHERAPY WITH CONCOMITANT TEMOZOLOMIDE ALONE IN CHILDREN WITH DIPG: A SINGLE-CENTER EXPERIENCE WITH 82 CASES

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OBJECTIVE: To retrospectively analyze the therapeutic effects of radiotherapy with concomitant and adjuvant temozolomide *versus* radiotherapy with concomitant temozolomide alone for pediatric diffuse intrinsic pontine glioma (DIPG), and to evaluate the value of temozolomide in the treatment of pediatric DIPG. METHODS: The clinical data of children with confirmed DIPG in Guangdong Sanjiu Brain Hospital between January 1, 2010 and December 30, 2019 were collected. The inclusive criteria included (1) receiving a total radiotherapy dose of 54 Gy in 27 fractions, (2) treated with concomitant temozolomide chemotherapy, and (3) with or without adjuvant temozolomide chemotherapy. RESULTS: A total of 82 pediatric patients were eligible for the study, with a median age of 7 years (range 2–16 years). The median follow-up was 8.6 months (range 2–28 months) and the median survival time was 9.4 months. The median survival time of 66 patients treated with radiotherapy with concomitant and adjuvant temozolomide was 9.8 months, longer than 7.5 months of the other 16 patients treated with radiotherapy with concomitant temozolomide alone, with statistical differences *(P*=0.010). Moreover, bevacizumab and nimotuzumab didn't bring survival benefits to patients with disease recurrence or progression. Hematological toxicity (Grade IV) was not found. CONCLUSION: Radiotherapy with concomitant and adjuvant temozolomide prolongs the survival time of children with DIPG.

DIPG-27. TARGETING FACILITATES CHROMATIN TRANSCRIPTION (FACT) AS A NOVEL STRATEGY FOR DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) THAT ENHANCES RESPONSE TO HISTONE DEACETYLASE (HDAC) INHIBITION

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Diffuse intrinsic pontine glioma (DIPG) is an aggressive and incurable childhood brain tumour for which new treatments are needed. A high throughput drug screen of 3500 pharmaceutical compounds identified anti-malarials, including quinacrine as having potent activity against DIPG neurospheres. CBL0137, a compound modelled on quinacrine, is an anti-cancer compound which targets Facilitates Chromatin Transcription (FACT), a chromatin remodelling complex involved in transcription, replication, and DNA repair. CBL0137 effectively crosses the blood-brain barrier and is currently in Phase I trials in adult cancer. CBL0137 induced apoptosis in DIPG neurospheres *in vitro* and had profound cytotoxic activity against a panel of DIPG cultures. In a DIPG orthotopic model, treatment with CBL0137 significantly improved survival. We found that treatment with CBL0137 up-regulated TP53 and increased histone H3.3 acetylation and tri-methylation in DIPG cells. We therefore examined the interaction between CBL0137 and the HDAC inhibitor, panobinostat. *In vitro* experiments showed that the two agents had profound synergistic activity against DIPG neurospheres in clonogenic assays and enhanced apoptosis. Transcriptomic analysis and immunoblotting indicated that combination treatment activated signalling pathways controlled by Retinoblastoma (RB)/E2F1 and subsequently increased phosphorylation and enzymatic activity of enhancer of zeste homolog 2 (EZH2). Consistent with the *in vitro* results, the combination of CBL0137 and panobinostat significantly prolonged the survival of two orthotopic models of DIPG, while histological analysis showed increased H3K27me3 and decreased Ki67 positive cells. Given these promising results, a paediatric trial of CBL0137 is planned to open through the Children's Oncology Group with an expansion cohort for DIPG patients.

DIPG-28. REPEATED LOW DOSE RT FOR PEDIATRIC DIPG – LESS DISEASE BURDEN WITH COMPARABLE OUTCOMES

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PURPOSE: Pediatric diffuse intrinsic pontine glioma (DIPG) is the most dismal prognosis pediatric brain tumor. Six weeks radiation therapy (RT) remains the mainstay of treatment. The aim of the current study was to compare the results of firstly reported repeated low dose RT (rLRT) with conventional RT (CRT). METHODS AND MATERIALS: This retrospective review included 24 children with DIPG, aged 3 -18 years, underwent CRT (52- 60.0 Gy in 1.8- 2.0 Gy, n = 16) or rLRT (18 - 30 Gy in 1.5–2.0 Gy per cycle for 1–3 cycles, n = 8). All children had typical symptoms and MRI features of DIPG, or biopsy-proven DIPG. RESULTS: The median overall survival (OS) was 12.6 months in rLRT group and 11.4 months in CRT group (p =0.347), progression-free survival (PFS) was 3.6 months in rLRT group and 6.5 months in CRT group (p = 0.821), no significant survival difference was observed between two groups. Temporary discontinuation or tapering of steroids rate was significantly higher in rLRT group (100% vs 60%, p = 0.028). Although not statistically significant, mean non-hospitalized days were longer in the rLRT group, 403 days versus 305 days in the CRT group, as were mean cumulative progression-free days, 276 days versus 163 days and 1-year free from CSF diversion rate was higher, 100% versus 64.9%. CONCLUSIONS: For patients with newly diagnosed DIPG, repeated low dose RT, given over 3 to 4 weeks per cycle for 1 to 3 cycles, offers comparable survival outcome with less disease burden compared with conventional RT.

DIPG-29. PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE 3-KINASE (PI3K) INHIBITION DRIVES PROTEIN KINASE C ACTIVATION (PKC) IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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Recurring somatic mutations and gene amplifications to members of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling axis are overarching contributors to the aggressive growth and survival of diffuse intrinsic pontine gliomas (DIPG). However, targeting PI3K has thus far failed to improve outcomes for patients in the clinic. To identify the mechanisms underpinning PI3K/AKT/mTOR treatment failure in DIPG, we have employed high-resolution quantitative phosphoproteomic profiling in patient-derived DIPG cell lines harboring H3K27M and PI3K mutations, +/- the blood-brain barrier permeable PI3K inhibitor, paxalisib (previously "GDC-0084", currently in Phase I trials - NCT03696355) and rapamycin. Paxalisib was significantly more potent than rapamycin at inducing PI3K/AKT/mTOR inhibition, however, both simultaneously activated protein kinase C signaling (^{pT500}PKCβ +8.2 and +4.5 fold, respectively). PKC lies directly upstream of myristoylated alanine-rich C-kinase substrate (MARCKs), which was phosphorylated at Ser170 by +9.4 and +4.7 fold, respectively; promoting actin cytoskeletal remodeling and cellular migration. Indeed, activation of PKC signaling using phorbol 12-myristate 13-acetate (PMA), increased DIPG cell growth and migration by >3 fold. Targeting PKC using midostaurin (FDA-approved for acute myeloid leukemia), and enzastaurin (blood-brain barrier penetrant inhibitor of PKCβ), in combination with paxalisib was highly synergistic (CI=<0.9), reducing proliferation and driving apoptosis. Mechanistically, compensatory activation of PKC signaling following PI3K inhibition was regulated by the accumulation of Ca^{+2} ions, as chelation using

BAPTA-AM significantly reduced PKC activity following PI3K inhibition. These data highlight the power of phosphoproteomic profiling for the rational design of drug combination strategies, which need to be tested *in vivo* prior to clinical trials for DIPG.

DIPG-31. MOLECULAR MECHANISMS AND FUNCTIONAL IMPACT OF ABERRANT SPLICING IN DIFFUSE MIDLINE GLIOMAS

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Fewer than 1% of children diagnosed with diffuse-midline glioma (DMG) survive for more than 5 years, because no effective therapies exist for these patients. Here, we sought to identify and characterize mechanisms of aberrant splicing (AS) in primary DMG tumors. We observed transcriptome-wide AS (9,805 differential splicing variations in 4,734 genes), and identified a DMG-specific splicing signature, that included known cancer genes. We hypothesize that AS of cancer genes play a role in DMG tumor formation. Assessing whether splicing factor dysregulation impacted known cancer transcripts, we discovered several splicing factors, including *SRRM4*, *SRRM3* and *RBFOX3* to be down-regulated in DMG. Additionally, we found an enrichment of binding motifs for these proteins within flanking regions of these mis-spliced exons. We also observed recurrent significant exon inclusion in tumor suppressor *SMARCA4*, an integral member of the SWI/SNF family of proteins involved in chromatin remodeling. Further, we identified AS in 16 of the 27 members of the SWI/SNF complex, including increased skipping of exon 7 in *DPF2*, representing a complete mRNA transcript switch in DMG. Since *SRRM4, SRRM3 and RBFOX3* are known regulators for neural-specific microexons, we focused on microexon splicing changes, hypothesizing that these regulators may be driving microexon mis-splicing in these tumors. We identified 245 known microexons lost or gained in DMG. Moreover, a quarter of which were observed in known cancer genes, with the most frequent splice event causing gain of a clathrin-binding site in the tumor suppressor *BIN1* with a concurrent loss of an out-of-frame microexon in the oncogene *BAK1,* presumably activating it. Altogether, our results suggest that aberrant splicing may be an alternative mechanism driving DMG tumorigenesis and we are currently molecularly validating a subset of these events with the overall goal of identifying novel therapeutic targets for DMG tumors.

DIPG-32. AKT SIGNALING DRIVES RESISTANCE TO ONC201 IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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Diffuse intrinsic pontine glioma (DIPG) is a highly aggressive, childhood brainstem cancer with a median overall survival of 10 months post diagnosis. Remarkably, 80–90% of patients harbor recurring point mutation in histone H3, which induces a lysine for methionine substitution at amino acid 27 (H3K27M) in either H3.1 (*HIST1H3B* ~25%) or H3.3 (*H3F3A* ~65%) variants. Using the blood-brain barrier (BBB) permeable DRD2 antagonist, ONC201 (in clinical trials for DIPG and H3K27M-mutant gliomas – NCT03416530), we hypothesized that DRD2 antagonism would induce TRAIL expression via indirect inhibition of AKT and ERK signaling, to drive apoptosis in both H3.1K27M and H3.3K27M patient-derived DIPG cell lines alike. For the first time, we reveal that ONC201 shows efficacy in 100% of WT-H3 and H3.1K27M mutant DIPG cell lines (n=5), compared to 50% of H3.3K27M mutant DIPGs (n=6). Investigations to identify the mechanisms of resistance to ONC201, revealed that cell lines with decreased sensitivity upregulated the PI3K/AKT/MTOR signaling axis to drive phosphorylation of AKT and increase metabolic activity. Combined administration of ONC201 and the BBB-permeable PI3K/AKT inhibitor, paxalisib (previously GDC-0084, in clinical trials for newly diagnosed DIPG – NCT03696355), showed synergistic cytotoxicity, reduced PI3K/AKT signaling and metabolic reprogramming to drive apoptosis in all DIPG cell lines tested. This combination was used to treat a 3-year-old DIPG patient, commencing 14 weeks post disease progression, completing 40 weeks of therapy prior to her passing, December 2019. These studies highlight the potential of combined administration of two safe, BBB penetrant, oral targeted therapies and supports testing under clinical trial conditions.

DIPG-33. CHARACTERIZING THE NEURO-VASCULAR UNIT IN DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse intrinsic pontine glioma (DIPG) is a childhood brainstem tumor with a median overall survival of eleven months. Lack of chemotherapy efficacy may be related to an intact blood-brain-barrier (BBB). In this study we aim to compare the neuro-vascular unit (NVU) of DIPG to healthy pons tissue. End-stage DIPG autopsy samples (n=5) and age-matched healthy pons samples (n=22), obtained from the NIH NeuroBioBank, were immunohistochemically stained for tight-junction proteins claudin-5 and zonula occludens-1 (ZO-1), basement membrane component laminin, and pericyte marker PDGFRβ. Claudin-5 stains were also used to determine vascular density and diameters. In DIPG, expression of claudin-5 and ZO-1 was reduced, and claudin-5 was dislocated to the abluminal side of endothelial cells. Laminin expression at the glia limitans was reduced in both pre-existent vessels and neovascular proliferation. In contrast to healthy pons, no PDGFRβ expression was detected. The number of blood vessels in DIPG was significantly reduced compared to healthy pons, 13.9±11.8/mm^2 versus 26.3±14.2/mm^2, respectively (P<0.01). Especially the number of smaller blood vessels (<10μm) was significantly lower (P<0.01). Distribution of larger blood vessels (≥10μm) did not differ between groups (P=0.223). Mean vascular diameter was 9.3±9.9μm for DIPG versus 7.7±9.0μm in healthy pons (P=0.016). Our study demonstrates evidence of structural changes in the NVU in end-stage DIPG. Chemotherapeutic inefficacy could be the result of reduced vascular density. However, further research is needed to determine meaning and extent of these changes and to determine whether these observations are caused by the tumor or the result of treatment.

DIPG-34. SUPER ELONGATION COMPLEX AS A TARGETABLE DEPENDENCY IN H3K27M+ DIFFUSE MIDLINE GLIOMA

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Mutations in the histone 3 gene (H3K27M) are the eponymous driver in diffuse intrinsic pontine gliomas (DIPGs) and other diffuse midline gliomas (DMGs), aggressive pediatric brain tumors for which no curative therapy currently exists. To identify specific epigenetic dependencies within the context of the H3K27M mutation, we performed an shRNA screen targeting 408 genes classified as epigenetic/chromatin-associated molecules in patient-derived DMG cultures. This identified AFF4, a component of the super elongation complex (SEC), as necessary for DMG cells to maintain growth and self-renewal. We hypothesized that aberrant SEC expression occurs as a consequence of the H3K27M mutation and that this dysregulated SEC signaling overcomes repressive transcriptional regulation in order to suppresses differentiation and promote self-renewal of DMG tumor stem cells. We interrogated the role of AFF4 in DMG using an shRNA lentiviral approach. We demonstrate a significant decrease in *in vitro* clonogenicity and stem cell maintenance following AFF4 depletion. We employed RNA-seq-based gene set enrichment analysis to delineate differentiation programs under SEC regulatory control. Finally, we sought to determine whether CDK9, the catalytic subunit of the SEC, represents a therapeutic vulnerability in DMG. Using RNA polymerase II ChIP-seq, we demonstrate that CDK9 pharmacologic inhibition restores regulatory Pol II pausing, promotes cellular differentiation, and leads to potent anti-tumor effect both in vitro and in patient-derived xenograft models. These studies present a biologic rationale for the translational exploration of CDK9 inhibition as a promising therapeutic approach.

DIPG-35. BIOLOGICAL MEDICINE FOR DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) ERADICATION: RESULTS OF THE THREE ARM BIOMARKER-DRIVEN RANDOMIZED BIOMEDE 1.0 TRIAL

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