response and quality of life data are incomplete, but survival appears to be lengthened with rRT. Prospective clinical trials will elucidate benefits and risks of rRT.

DIPG-75. PRECISION MEDICINE FOR PAEDIATRIC HIGH-GRADE DIFFUSE MIDLINE GLIOMAS - RESULTS FROM THE ZERO CHILDHOOD CANCER COMPREHENSIVE PRECISION MEDICINE PROGRAM

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The Australian Zero Childhood Cancer (ZERO) program aims to assess the feasibility of a comprehensive precision medicine approach to improve outcomes for patients with an expected survival <30%. ZERO combines molecular profiling (whole genome sequencing, whole transcriptome sequencing, DNA methylation profiling) with *in vitro* high-throughput drug screening (HTS) and patient-derived xenograft drug efficacy testing. We report on the cohort of patients with midline high-grade glioma (HGG), including H3-K27M DMG, enrolled on the pilot study (TARGET) and on the ongoing ZERO clinical trial (PRISM). We identified 48 patients with midline HGG. Fresh or cryopreserved samples were submitted in 37 cases and cell culture was attempted in 30/37 cases with 45% success rate. The most commonly mutated genes/pathways identified by molecular profiling include H3-K27M mutations, DNA repair pathway, and PI3K/mTOR pathway. Two targetable fusions (NTRK and FGFR1) were reported. Five patients with germline alterations were identified. Thirty-five (72%) patients received a therapeutic recommendation from the ZERO molecular tumour board and the main recommended therapies were mTOR inhibitors, PARP inhibitors or tyrosine kinase inhibitors. HTS added evidence for the recommended therapy (n=3) or identified novel potential therapy (n=1). Out of the 35 patients, 16 received a recommended drug. Response to treatment was complete response for five months (n=1), partial response for nine months (n=1), stable disease (n=4), and progressive disease (n=10). These results highlight the feasibility of the ZERO platform and the value of fresh biopsy, necessary for pre-clinical drug testing. Targetable alterations were identified leading to clinical benefit in six patients.

DIPG-76. HISTONE H3 PHOSPHORYLATION IN H3K27M MIDLINE GLIOMAS

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Diffuse midline gliomas (DMG) patients have a dire prognosis despite radiation therapy and there is an urgent need to develop more effective treatments. DMG are characterized by heterozygous mutations in select H3 genes resulting in the replacement of lysine 27 by methionine (K27M) that leads to global epigenetic reprogramming and drives tumorigenesis. We previously reported that pharmacological inhibition of aurora kinase (AKI) may represent a targeted approach for treating tumors with this mutation. Our analysis with both published dataset and patient samples showed that patients with higher aurora kinase A (AKA) expression were associated with worse survival. AKA phosphorylates H3S10 and H3S28 during mitosis. Intriguingly, phosphorylation of the H3S28 (H3S28ph) by AKA blocks PRC2 methyltransferase activity and decreases global H3K27me3 in certain stem cells. We propose that a similar mechanism occurs in H3K27M DMG tumors, where there is a reciprocal relationship between H3S28ph and H3K27me3. We found that AKI significantly decreases H3S28ph while increasing H3K27me3 specifically in H3K27M tumors. To further evaluate the link between the H3K27M mutation and H3 serine phosphorylation, we used CRISPR/Cas9-directed gene editing to silence H3S28ph by replacing serine with alanine (H3S28A) in DIPG cell lines. Ectopic expression of histone H3S28A leads to a prominent epigenetic changes in H3K27M tumors and is similar to AKA inhibition. Overall, this study highlights H3S28ph, one of the targets of AK, is a key driver of epigenetic changes in H3K27M tumors through both direct and indirect changes to H3K27me3 and H3K27ac across the genome.

DIPG-77. TREATMENT EXTENT AND THE EFFECT ON SURVIVAL IN DIFFUSE INTRINSIC PONTINE GLIOMA

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BACKGROUND: Front line radiotherapy for diffuse intrinsic pontine glioma (DIPG) remains the only standard of care. Is this still appropriate? PATIENTS AND METHODS: We examined survival outcomes across six treatment modalities including I) no treatment (n=19), II) radiotherapy alone (n=38), III) radio-chemotherapy (n=101), IV) radiotherapy and relapse chemotherapy (n=35), V) radio-chemotherapy and relapse chemotherapy (n=163), and VI) radio-chemotherapy and relapse chemotherapy, plus reirradiation (n=54). Data were collected retrospectively using the Society of Pediatric Oncology and Hematology (GPOH) and the SIOPE DIPG Registry. 410 patients were included with radiologically centrally reviewed DIPG, mostly unbiopsied. Of note, the untreated patients and radiotherapy only cohorts chose limited treatment voluntarily. RESULTS: Median overall survival (MOS) of the whole cohort was 11 months and progression free survival (PFS) 7 months. PFS was not significantly different between the treatment groups. OS and post-progression survival (PPS) were significantly different between cohorts. For the respective treatment groups, median OS was 3 months (I), 7 months (II), 8 months (III), 13 months (IV), 13 months (V), and 15 months (VI). For only front line vs at least one second line therapy, MOS was 8 months vs 14 months and PPS 2 months vs 5 months. CONCLUSIONS: Although subject to biases to some extent, it seems that additional therapies beyond radiation therapy are of benefit to extending survival in DIPG patients. This is at least partially caused by the introduction of reirradiation regimens. To what extent other therapies contribute to survival and quality of life is subject to further investigation.

DIPG-78. REVERTANCE OF THE H3K27M MUTATION RESCUES CHROMATIN MARKS NECESSARY FOR ONCOGENESIS IN DIFFUSE MIDLINE GLIOMA

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Diffuse midline glioma (DMG) is a lethal brain tumor that typically occurs in children. Numerous studies have demonstrated the central role of the H3K27M mutation and secondary loss of H3K27 trimethylation (H3K27me3) in DMG tumorigenesis. Understanding how the H3K27M mutation alters the epigenetic landscape of the cell is necessary for revealing molecular targets that are critical to tumorigenesis. To investigate the epigenetic effects of H3K27M mutation in DMG, we developed revertant DMG cell lines with the mutant methionine residue reverted to wildtype (i.e., M27K). Revertant cells were analyzed for epigenetic changes and phenotypic differences in vitro and in vivo. H3M27K DMG cells grew in culture but displayed diminished proliferative capacity. H3M27K cells demonstrated total loss of H3K27M expression and restored trimethylation of H3K27 and H3K4. Furthermore, consistent with the hypothesis that the H3K27M mutation impacts H3 phosphorylation via expression of Aurora Kinase during mitosis, H3M27K cells demonstrated reduced expression of both Aurora Kinase A and phosphorylation of H3 serine residues 10 and 28. In line with the critical role of H3S10 phosphorylation in chromatin segregation, H3M27K cells also demonstrated restored chromosome segregation compared to H3K27M cells. In vivo data will be discussed. Revertance of the H3K27M mutation reduces tumorigenesis in DMG tumors. Isogenic H3M27K cells display reversal of key epigenetic changes associated with oncogenesis in DMG. The revertant H3M27K DMG model is a useful tool to investigate the downstream epigenetic reprogramming specific to H3K27M mutation in these tumors.

DIPG-79. H3K27M INDUCES EPIGENETIC AND ONCOGENIC CHANGES THAT ARE PARTIALLY REVERSED BY SMALL MOLECULE AURORA KINASE B/C INHIBITION

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Diffuse intrinsic pontine glioma (DIPG) is a fatal pediatric brain tumor with no curative treatments. Approximately 80% of DIPGs contain an H3K27M mutation. The implications of the mutation and how they may be targeted are not fully understood. We established an H3K27M effect-isolating model by transducing H3K27-wildtype lines (HSJD-GBM-001, normal human astrocytes) with lentiviral-packaged H3K27M. We characterized H3K27M-related changes through western blot, phenotypic assays, and RNA-seq. Drug screening of H3K27-wildtype and matched H3K27M-transduced lines was used to identify targets more effective with H3K27M present. Patient-derived pediatric glioblastoma and DIPG lines (BT-245, SU-DIPG-IV, HSJD-DIPG-007, SU-DIPG-XIII*, SF7761) were used for validation. We observed increased H3K27ac and decreased H3K27me3, as well as increased proliferative and migratory abilities, with the addition of H3K27M to H3K27-wildtype lines. RNA-seq showed downregulation of cell cycle regulation and upregulation of epithelial-mesenchymal transition. GSK1070916, an Aurora kinase B/C inhibitor, was isolated from a synthetic lethality screen with H3K27M. GSK1070916 showed strong efficacy in native H3K27M lines (IC_{50}s=60nM-1250nM), superior to the Aurora kinase A inhibitor alisertib, to which all cell lines showed substantial resistance. Combination of both drugs was not synergistic. GSK1070916 treatment caused increased H3K27me3 and decreased H3S10ph and H3S28ph. GSK1070916 induced apoptosis and S-phase stall. The H3K27M mutation induces epigenetic, phenotypic, and cell cycle regulation changes resulting in relaxation of transcriptional controls and more aggressive growth. Aurora kinase B/C inhibition is a novel therapeutic modality for DIPG that appears capable of reversing some H3K27M-related epigenetic changes, inducing apoptosis, and repressing uncontrolled cellular division.

DIPG-80. CLINICAL AND RADIOGRAPHIC RESPONSE TO ONC201 IN A PEDIATRIC PATIENT WITH A THALAMIC H3K27M AND BRAFV600E MUTANT DIFFUSE MIDLINE HIGH GRADE GLIOMA

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Recent improved understanding of the molecular markers of high grade glioma has shifted the approach to these aggressive CNS tumors to increasingly use molecularly guided targeted therapies. Treatment of patients with BRAFV600E mutant high grade gliomas with BRAF inhibitors has shown efficacy, however the impact of concomitant H3K27M mutation is unknown. ONC201 targets dopamine receptor D2 (DRD2), which is shown to be broadly overexpressed in the thalamus as well as multiple tumor types; its antagonism has demonstrated anti-tumor efficacy and immunomodulatory properties in preclinical studies. ONC201 has also demonstrated clinical efficacy in patients with H3K27M mutant gliomas. We present the case of a 9-year-old male with a right thalamic H3.3K27M mutant diffuse midline glioma with a concomitant BRAFV600E mutation with an ongoing partial response to ONC201 treatment. The patient was diagnosed in May 2018. He underwent biopsy, followed by standard focal proton radiation therapy (54Gy) and subsequent treatment with dasatinib, bevacizumab and everolimus over the course of five months. After continued radiographic progression on serial imaging, in April 2019 he started ONC201 375mg orally once per week through an expanded access trial. He has tolerated the medication well with grade 1 nausea and fatigue. Over the next nine months, he demonstrated clinical and radiographic improvement with modest increased use of his left side and MRIs showing progressive decrease in size of the thalamic lesion with a 70 % decrease in the target lesion (measuring 53x62mm prior to treatment, decreased to 38x26mm in January 2020).

DIPG-82. CLINICAL EXPERIENCE OF CONVECTION ENHANCED DELIVERY (CED) OF CARBOPLATIN AND SODIUM VALPROATE INTO THE PONS FOR THE TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) IN CHILDREN AND YOUNG ADULTS AFTER RADIOTHERAPY

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PURPOSE: Effective treatment of diffuse intrinsic pontine glioma (DIPG) remains a formidable challenge due to inadequate penetration of the blood-brain barrier (BBB) by systemically administered chemotherapies. The BBB can be overcome by directly infusing drugs into pons using method of convection-enhanced delivery (CED). We describe our clinical experience and what we have learned about the safety and feasibility of treating DIPG with intermittent CED of carboplatin and sodium valproate to the pons through the Renishaw Drug Delivery System (RDDS). METHODS: Retrospective review (2017–2020) of children with DIPG, who following radiotherapy, received compassionate treatment commencing 3.3–10 months post diagnosis (median 4.9 months). They received up to 7 cycles of 3–6 weekly pontine infusions of carboplatin (0.12-0.18mg/ml) and sodium valproate (14.4- 28.8mg/ml). RESULTS: 13 children 3–19 years (mean 6.9 years) were treated. There were no surgical complications. With the exception of infusion channels blocking in one device there were no adverse device effects. Two patients developed persistent 6th nerve palsies, which led to drug concentration reduction in the combination therapy. Subsequently infusion/ drug related toxicities were transient. Tumour was controlled in pons in 11/13 patients. Median progression free survival (PFS) was 13.0 months, while median overall survival (OS) was 15.3 months. CONCLUSIONS: Use of the RDDS was safe and well tolerated in all 13 patients. Treatment improved control of pontine disease resulting in longer PFS and OS than reported for conventional therapy and merits further evaluation in a clinical trial.

DIPG-83. USING COPPER CHELATING AGENTS TO TARGET RECEPTOR TYROSINE KINASE SIGNALLING IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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DIPG is a universally fatal pediatric brain cancer. Receptor tyrosine kinase (RTK) pathway alterations are among the defining characteristics in many patients. Copper is a transition metal essential for cellular signaling, known to impact PI3K/AKT and MAPK/ERK pathways. Copper chelating agents are clinically approved for use in children with Wilson's Disease, documented to reduce brain copper levels and are cited as potential cancer therapeutics. Due to copper's wide cellular integration, we propose that targeting copper in DIPG through use of copper chelators is a viable therapeutic strategy and are strong candidates for combination therapy. Cytotoxicity assays performed in a panel of DIPG cell lines using copper chelator tetraethylenepentamine (TEPA) demonstrated a millimolar range of efficacy. To identify copper integrated pathways, western blots were performed on DIPG cell lines dosed with sub-lethal copper concentrations, which increased phosphorylated expression of AKT, ERK1/2, ERK5 and STAT3. Conversely, western blots performed after TEPA treatment demonstrated reduced phosphorylated expression of all these proteins compared to controls. Western blots investigating TEPA in combination with Everolimus and Trametinib demonstrated synergistic targeting of these proteins. Our results indicate that adding copper in the culture media initiated two RTK-mediated downstream signal transductions, including AKT and ERK and additionally STAT signaling. The use of copper chelator TEPA affected copper homeostasis and reduced DIPG cell proliferation. Our study proposes copper plays an important role in RTK-mediated signaling promoting DIPG proliferation. This implies that reducing copper with clinically available chelation agents can represent a potential anti-cancer treatment for DIPG.

DIPG-84. COMPLEMENTARY AND ALTERNATIVE MEDICINE IN DIFFUSE INTRINSIC PONTINE GLIOMA

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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is a rare and aggressive childhood brainstem malignancy with a two-year survival rate of ≤10%. In this international survey study we aim to evaluate the use of complementary and alternative medicine (CAM) in this patient population. METHODS: Parents of-, and physicians treating DIPG patients were asked to participate in a retrospective online survey with questions regarding CAM use during time of illness. RESULTS: 120 parents and 75 physicians contributed to the online survey between January and May 2020. Physicians estimated that <50% of their patients used CAM, whereas 69% of the parents reported to have used CAM to treat their child during time of illness. Cannabis was the most widely used form of CAM, followed by vitamins and minerals, melatonin, curcumin and boswellic acid. CAM was mainly used to actively treat the tumor. Other motivations were to treat side effects of chemotherapy, or to comfort the child. Children diagnosed ≥2016 were more likely to use CAM ($\chi 2$=6.08, p=0.014). No significant difference was found between CAM users and non-users based on ethnicity ($\chi 2$=*4.18*, p=0.382) and country of residence ($\chi 2$=*9.37*, p=0.154). Almost 50% of the physicians do not frequently ask their patients about possible CAM use. CONCLUSION: This survey demonstrates that worldwide a considerable number of DIPG patients use CAM. Physicians should be more aware