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

The Australian Zero Childhood Cancer (ZERO) program aims to study the responses to precision medicine 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 gliomas (HGG), including H3-K27M DMG, enrolled on the pilot study (TARGET) and on the ongoing zero clinical trial (PRISM). We identified 48 patients with medulloblastoma or non-small cell glioblastoma with an expected survival of <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 (NTRK1, and FGFR1) were identified. Five patients with germline alterations were identified. Thirty-five (72%) patients received a therapeutic recommendation from the ZERO molecular tumor 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=9), 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.

**Discussion**

Diffuse midline glioma (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 inhibiting cell cycle progression (H3S28ph) by AKI blocks. Ectopic expression of histone H3S28A leads to a prominent epigenetic changes in H3K27M tumors and is similar to AKA inhibition. Overall, this study highlights H3S28A, one of the targets of AKI, is a key driver of epigenetic changes in H3K27M tumors through both direct and indirect changes to H3K27me3 and H3K27ac across the genome. 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 inhibiting cell cycle progression (H3S28ph) by AKI blocks. Ectopic expression of histone H3S28A leads to a prominent epigenetic changes in H3K27M tumors and is similar to AKA inhibition. Overall, this study highlights H3S28A, one of the targets of AKI, 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=103), IV) radiotherapy and relapse chemotherapy (n=35), V) radio-chemotherapy and relapse chemotheraphy (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 SIOPÉ DIPG Registry. 410 patients were included with radiologically centrally reviewed DIPG, mostly unbiased. Of note, the untreated patients and radiotherapy only cohorts chose limited treatment voluntarily. RESULTS: Median overall survival (MOS) of the whole cohort was 7 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 8 months (II), 7 months (III), 8 months (IV), 13 months (V), and 15 months (VI). For only front line vs at least 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, the therapy 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 (H3M27K) in DMG tumorigenesis. Understanding the role of H3M27K 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 H3K27 phosphorylation via expression of Aurora kinase during mitotic events, 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 chomatin segregation, H3M27K cells also demonstrated restored chromosome segregation compared to H3K27M cells. In vivo data will be discussed. Revertant 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-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

Elsheba Saeed1, Syed Adil1, Christof Kramm2,4, and Orazio Hollingworth

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 convection-enhanced delivery (CED) of Carboplatin and Sodium Valproate in the treatment of children and young adults with DIPG.

MATERIALS AND METHODS: We retrospectively reviewed records of children and young adults with DIPG treated with CED therapy at participating centers. We included patients who received CED therapy in combination with standard radiotherapy and chemotherapy. CED therapy was delivered using the Royal Marsden Drug Delivery System (RDDS) or the Renishaw Drug Delivery System (RDDS). Responses to CED therapy were classified as complete, partial or no response. Data was analyzed using descriptive statistics and correlation tests.

RESULTS: A total of 13 patients were included in the study, 11 of whom were diagnosed with DIPG. The median age at diagnosis was 5 years (range 2-13 years). All patients received standard radiotherapy and chemotherapy. Ten patients received CED therapy using the RDDS and three patients using the Renishaw Drug Delivery System. The median number of CED treatments per patient was 2 (range 1-14). The most common chemotherapy agents used in combination with CED therapy were Carboplatin and Sodium Valproate. Twenty-four percent of patients achieved a complete response, 31% a partial response and 45% showed no response to CED therapy.

CONCLUSIONS: CED therapy with Carboplatin and Sodium Valproate appears to have a significant impact on the survival and quality of life of children and young adults with DIPG. Further research is needed to evaluate the long-term effects of CED therapy in combination with standard radiotherapy and chemotherapy for the treatment of DIPG.

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

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INTRODUCTION: Diffuse intrinsic pontine glioma (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 Disease, a 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.

MATERIALS AND METHODS: Primary DIPG cell lines were generated using copper chelator TEPA. Western Blots performed in a panel of DIPG cell lines using copper chelator demonstrated significant induction of all RTKs tested in the presence of TEPA. Cytotoxicity assays performed on DIPG cell lines showed synergistic effect of TEPA on cell viability, suggesting potential for combination therapy.

RESULTS: Western Blots showed significant induction of RTKs in the presence of TEPA. Cytotoxicity assays performed in a panel of DIPG cell lines using copper chelator demonstrated significant induction of all RTKs tested in the presence of TEPA. Cytotoxicity assays performed on DIPG cell lines showed synergistic effect of TEPA on cell viability, suggesting potential for combination therapy.

CONCLUSIONS: Copper chelating agents have the potential to target RTKs in DIPG and may offer a novel therapeutic strategy for the treatment of this disease.

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 use of CAM during childhood and adolescence. 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. CAMs were 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 (p=0.03). A significant difference was found between CAM users and non-users based on ethnicity (p=0.038) and country of residence (p=0.037). 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 of the possible benefits and risks of CAM.