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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