glioblastoma and downregulated the NFκB pathway. Because this pathway is overexpressed in DIPG and may play a role in DIPG cell growth and survival, we hypothesized that RG2833 would kill DIPG cells. Treatment of DIPG cell lines with RG2833 as a single agent suppresses cell proliferation in the 5–10μM range (MTS assay for HSJD007 p=0.0004 10μM vs DMSO, JHH-DIPG1 p=0.001 10μM vs DMSO, SF-7761 p=0.04 10μM vs DMSO, SU-DIPG13 p=0.01 10μM vs DMSO by *t-test*). RG2833 induces apoptosis by 48 hours as measured by Western blot for cPARP and cleaved caspase 3 immunofluorescence (HSJD007 p<0.003 8μM vs DMSO, JHH-DIPG1 p=0.0026 10μM vs DMSO by *t-test*). RG2833 also slows cell proliferation as measured by Western blot for pRb and immunofluorescence for BrdU (HSJD007 p=0.008 8μM vs DMSO, JHH-DIPG1 p=0.0002 10μM vs DMSO by *t-test*). Western blot confirmed a dose-dependent increase in histone 3 acetylation with RG2833 treatment at 5 hours. We detected increased acetylated p65 and decreased expression of the NFκB regulated pro-survival genes BCL2, BCL-xL, and XIAP with RG2833 treatment. Together, this data shows that HDAC inhibitor RG2833 may be a promising therapeutic candidate for DIPG via downregulation of the NFκB pathway.

DIPG-72. LONG-TERM SURVIVAL OF A CLASSIC DIFFUSE INTRINSIC PONTINE GLIOMA TREATED WITH NIMOTUZUMAB

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BACKGROUND: Long-term survival in diffuse intrinsic pontine glioma is rare, and typically associated with atypical imaging and/or atypical clinical course. Although most patients harbor hotspot mutations in H3.1/3-K27M, a proportion of patients have alternate mutations, despite a typical clinicoradiological course. Herein we describe a long-term survivor with a classical presentation, treated with nimotuzumab, highlighting the challenges associated with such cases. CASE REPORT: A 5 year old male, diagnose in 2012 with a 10 day history multiple cranial neuropathies and a right hemiparesis. Cranial MRI revealed a poorly delimited diffuse pontine tumor and secondary hydrocephalus. Tumor biopsy was not performed due to the classic clinical presentation, and he received 54Gy/30 of radiation plus concomitant weekly nimotuzumab 150mg/m2. Initial tumor dimensions were 43x31x28mm. Nimotuzumab 150mg/m2 was continued every 2 weeks. Image assessment at week 12 of treatment revealed 16.9% volume increase, 4 weeks after radiotherapy completion. Nevertheless, subsequent neuroimaging at 24th, 36th, 60th, 96th and 108th weeks of nimotuzumab therapy showed a sustained and progressive tumor cytoreduction of 47.5%, 59%, 62.2%, 63.8% and 67%, respectively, when compared with post-radiotherapy dimensions. Currently, the patient is 13y old, good school performance, no neurologic disabilities. The last MRI at 394 weeks of nimotuzumab revealed dimensions of 21x19x14mm which corresponds to 70% of reduction compared with initial volume. CONCLUSIONS: Our case of progressive cytoreduction over two years of a classic DIPG, diagnosed in the era prior to the discovery of the K27M mutation, highlights the challenges associated with long-term survival of this devastating entity.

DIPG-73. SENESCENCE ASSOCIATED SECRETORY PHENOTYPE AS A MECHANISM OF RESISTANCE AND THERAPEUTIC VULNERABILITY IN BMI1 INHIBITOR TREATED DIPG

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BACKGROUND: Diffuse intrinsic pontine gliomas (DIPGs) driven by mutations in the histone 3 (H3) gene (H3K27M) are aggressive pediatric brain tumors for which there is no curative therapy. METHODS: To identify novel therapeutic targets we performed a high throughput drug screen combined with an epigenetically targeted RNAi screen using H3K27M and H3.3 WT DIPG cells. RESULTS: Chemical and genetic depletion of BMI1 *in vitro* resulted in inhibition of clonogenicity and cell self-renewal consistent with previous studies. We show for the first time that clinically relevant BMI1 inhibitors attenuates growth of orthotopic DIPG xenografts as measured by MRI and prolong survival *in vivo*. We found that BMI1 inhibition drives phenotypic cellular senescence and that the senescent cells were able reactivate to form new neurospheres *in vitro* and tumor growth *in vivo*. RNA-seq, ChIP-Seq and immuno-proteomic analysis revealed that the senescent cells induced the expression of the Senescence Associated Secretory Phenotype (SASP) cytokines by increasing occupancy of activated histone marks at SASP factor promoters. The SASP results in increased expression of anti-apoptotic BH3 proteins including BCLxl, and BCL2. Treatment of the PTC028 treated senescent DIPG cells with BH3 mimetics induces apoptosis and clears the senescent cells. Combining BH3 mimetics with BMI1 inhibition attenuates tumor growth *in vivo* synergistically and significantly prolongs survival of DIPG bearing mice compared to BMI1 inhibition alone. CONCLUSION: These data inform the current trial of BMI1 inhibition as a monotherapy and predict the need for adding BH3 mimetics to achieve efficacy.

DIPG-74. RE-IRRADIATION OF DIPG: DATA FROM THE INTERNATIONAL DIPG REGISTRY

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PURPOSE: To review data from DIPG Registry patients recorded to have received a second course of radiation therapy (rRT). METHODS: The International DIPG Registry was searched for patients with DIPG who were treated with a known dose of rRT. Doses of rRT, timing from initial diagnosis and primary radiation therapy (pRT), radiographic response to rRT and survival from diagnosis (OS) were evaluated. RESULTS: Sixty (11.2%) of 535 Registry patients underwent rRT; dose was provided for 44 patients. Median (range) data from those 44 revealed that rRT was given at 12 (2–65) months from initial diagnosis of DIPG and at 9.6 (1–61) months from completion of pRT at a dose of 26.7 (1.8–74) Gy. After completion of rRT, MRI showed response, progression, stable disease or was not available in 19, 8, 3 and 14 patients, respectively. Median PFS and OS were 11 and 18.1 months, respectively. 475 Registry patients did not undergo rRT; their ages, duration of symptoms, and primary treatment with or without chemotherapy were not significantly different from the rRT cohort. Median PFS and OS for the non-rRT patients were 6.9 and 10 months, respectively. rRT patients were more likely to have had radiographic evidence of tumor necrosis at diagnosis than non-rRT patients. CONCLUSIONS: Administration of rRT to patients with DIPG has been inconsistent with respect to timing and dose. Toxicity, 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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