Phenotypic assessment of the models in vitro by high-throughput imaging demonstrated significantly increased invasion and migration in association with either KMT5B or KMT5C loss, but not both. Quantitative proteomic analysis of the secretome identified factors by which a minority of KMT5B-deficient cells may signal to promote motility of the neighbouring populations. These data suggest a previously unrecognised trans-histone (H4/H3) interaction in DIPG cells with a potentially profound effect on their diffusely infiltrating phenotype.

DIPG-64, INTERNATIONAL PRECLINICAL DRUG DISCOVERY AND BIOMARKER PROGRAM INFORMATION AN ADAPTIVE COMBINATORIAL TRIAL FOR DIFFUSE MIDLINE GLIOMAS
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INTRODUCTION: DMG-ACT (DMG- multi-arm Adaptive and Combinatorial Trial) aims to implement a highly innovative clinical trial design of combinatorial arms for patients with diffuse midline gliomas (DMGs) at all disease stages that is adaptive to pre-clinical data generated using extensive molecular assays including CRISPR, RNAseq, ELISA, FACS, and IHC. RESULTS: Inhibitory concentrations (IC50) were determined for: ONC201 (p=0.01), ONC206 (p=0.01), ONC201+ONC206 (p=0.02), and combination drug assays confirmed drug efficacy as increased survival in vivo (n=8) models of DMGs across seven institutions were used to assess single and combination treatments with ONC201, ONC206, marizomib, panobinostat, Val-083, and TAK228. In vivo pharmacokinetic assays using clinically relevant dosing of ONC201, ONC206, and panobinostat were performed. Predictive biomarkers for ONC201 and ONC206 were identified using extensive molecular assays including CRISPR, RNAseq, ELISA, FACS, and IHC. RESULTS: Inhibitory concentrations (IC50) were established and validated across participating sites. In vivo validation of single and combination drug assays confirmed drug efficacy as increased survival for: ONC201 (p<0.01), ONC206 (p<0.01), ONC201+ONC206 (p=0.02), and ONC201+panobinostat (p<0.01). Marizomib showed toxicity in murine/ezebrafish PDx models. Murine pharmacokinetic analysis showed peak brain levels of ONC201 and ONC206 above pre-clinical IC50. Molecular testing and analyses of existing drug screen across 537 cancer cell lines identified a small set of potential combinatorial drug targets identified by ONC201/6. CONCLUSION: Thorough preclinical testing in a multi-site laboratory setting is feasible and identified ONC201 in combination with ONC206 as promising therapeutics for DMGs. Preclinical and correlatve-clinical studies are ongoing.

DIPG-66, FEASIBILITY AND APPLICABILITY OF MOLECULAR GUIDED THERAPY IN HIGH GRADE GLIOMA/DIFFUSE MIDLINE GLIOMA: RESULTS FROM BEAT CHILDHOOD CANCER NMTRC-009 MOLECULAR GUIDED THERAPY STUDY
Virginia Harre1, Abhinav Nagulapally1, Elizabeth Lewis1, and Giselle GLIOMA: RESULTS FROM BEAT CHILDHOOD CANCER NMTRC-66. FEASIBILITY AND APPLICABILITY OF MOLECULAR GUIDED THERAPY IN HIGH GRADE GLIOMA/DIFFUSE MIDLINE GLIOMA: RESULTS FROM BEAT CHILDHOOD CANCER NMTRC-009 MOLECULAR GUIDED THERAPY STUDY
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Diffuse Intrinsic Pontine Glioma (DIPG) is a rare pediatric brain tumor for which no cure or efficacious therapies exist. Previous discoveries have revealed that DIPG harbors distinct genetic alterations, when compared with adult high-grade glioma (HGG) or even with non-DIPG pediatric HGGs. ATRX alteration is found in 9% of clinical cases of DIPG, and significantly overlaps with H3.K27M mutation and p53 loss, the two most common genetic changes in DIPG, found in 80% and 77% clinical cases, respectively. Here we developed genetically engineered mouse model of brainstem glioma using the RCAS-Tas-Tva system by targeting p53 loss, H3.K27M mutation and ATRX loss-of-function to Nestin-expression brainstem progenitor cells of the neonatal mouse. Specifically, we used Nestin-Tas-α: p53 floxed; ATRX heterozygous female and Nestin-Tas-α: p53 floxed; ATRX heterozygous females (n=6), and ATRX WT (n=10). Median survival of the three groups are 65 days, 88 days and 51 days, respectively. Also, ATRX null mice is lower in tumor incidence (44.4%), compared with ATRX WT (80%). We evaluated the pathological features of DIPG with or without ATRX alteration, RNA-seq is performed to identify differentially expressed genes between ATRX WT and loss-of-function. In conclusion, this study generated the first genetically modified mouse model studying the genetic alterations associated with DIPG and suggested that ATRX loss-of-function in DIPG may slow down tumorigenesis and decrease tumor incidence.

DIPG-70, DISORDERED DNA METHYLATION IN DIPG UNDERLIES PHENOTYPIC PLASTICITY
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Diffuse intrinsic pontine glioma (DIPG) is a childhood brainstem tumor with a dismal prognosis and no effective treatments. Recent studies point to a critical role for epigenetic dysregulation in this disease. Nearly 80% of DIPG harbor mutations in histone H3 encoding replacement of lysine 27 with methionine (K27M), leading to global loss of the repressive activity of H3K27M and p53 loss, the two most common genetic changes in DIPG, overlaps with H3.K27M mutation and p53 loss, the two most common genetic changes in DIPG, found in 80% and 77% clinical cases, respectively. Here we developed genetically engineered mouse model of brainstem glioma using the RCAS-Tas-Tva system by targeting p53 loss, H3.K27M mutation and ATRX loss-of-function to Nestin-expression brainstem progenitor cells of the neonatal mouse. Specifically, we used Nestin-Tas-α: p53 floxed; ATRX heterozygous female and Nestin-Tas-α: p53 floxed; ATRX heterozygous females (n=6), and ATRX WT (n=10). Median survival of the three groups are 65 days, 88 days and 51 days, respectively. Also, ATRX null mice is lower in tumor incidence (44.4%), compared with ATRX WT (80%). We evaluated the pathological features of DIPG with or without ATRX alteration, RNA-seq is performed to identify differentially expressed genes between ATRX WT and loss-of-function. In conclusion, this study generated the first genetically modified mouse model studying the genetic alterations associated with DIPG and suggested that ATRX loss-of-function in DIPG may slow down tumorigenesis and decrease tumor incidence.

DIPG-71, SELECTIVE HDAC INHIBITOR RG2833 INDUCES DIPG CELL DEATH VIA DOWNREGULATION OF THE NRF2 PATHWAY
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Histone deacetylase (HDAC) inhibitor panobinostat demonstrated activity against diffuse intrinsic pontine glioma (DIPG) in vitro, but its efficacy in vivo was limited by toxicity and poor blood brain barrier penetration. RG2833 (RGP109) is a selective HDAC1/3 inhibitor that has established brain penetration. In clinical trials, the Cmax (plasma) of RG2833 was 12μM. RG2833 demonstrated cytotoxicity against temozolomide-resistant
glioblastoma and downregulated the NFkB 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 suppressed cell proliferation in the 5–10% range (MTS assay for HSJD007 p=0.0004 10μM vs DMSO, JHH-DIPG1 p=0.001 10μM vs DMSO, SU-DIPG13 p=0.001 10μM vs DMSO by t-test); RG2833 induces apoptosis in the NIH 3T3 cell line as measured by flow cytometry. We found that RG2833 killed a wide range of DIPG cell lines with a morphology characteristic of senescence, and that the senescent cells were able to reactivate to form new neospheres in vitro and tumor growth in vivo. RNA-seq, ChiP-Seq and immuno-proteomic analysis revealed that the senescent cells initiate the expression of the Senescence Associated Secretory Phenotype (SASP) cytokines in an increasing occupancy of activated histone marks by SASP factor promoters. The SASP results in increased expression of anti-apoptotic BH3 proteins including BCL-xl and BCL-2. Treatment of the PTC205 treated senescent DIPG cells with BH3 mimetics induces apoptosis and clears the senescent cells. Combining RG2833 with a BH3 mimetic synergistically and significantly prolongs survival of DIPG bearing mice compared to BM1 inhibition alone. CONCLUSION: These data inform the current trial of BM1 inhibition as a mono-therapy 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 who 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 after rRT were recorded. In addition, 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 rRT has been shown to improve OS with respect to BM1ing and dose. Toxicity,

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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 an atypical imaging and/or unusual clinical course. Although most patients harbor hotspot mutations in H3.1/K27M, a proportion of patients have alternate mutations, despite a typical clinical/radiologic course. Herein we describe a long-term survivor with a histone mutation, treated with nimotuzumab, highlighting the challenges associated with such cases. CASE REPORT: A 3 year old male, diagnosed in 2012 with a 10 day history multiple cranial neuropathies and a right hemiparesis. Cranial MRI revealed a poorly delimited diffuse pontine tumor. Initial treatment and surgery were 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. MRI showed tumor progression at week 12 of treatment revealed 47.5% volume increase, 4 weeks after radiotherapy completion. Nevertheless, subsequent imaging at 24th, 36th, 60th, 90th 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 report presents a progressive tumor progression over 3.5 years diagnosed in the era prior to the discovery of the K27M mutation, highlighting 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 BM1 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 RNA screen using H3K27M and H3F3B histone mutants and genotypes. Analysis of DIPG cell cultures with BM1 resulted in inhibition of clonogenicity and cell self-renewal consistent with previous studies. We show for the first time that clinically relevant BM1 inhibitors attenuates growth of orthotopic DIPG xenografts as measured by MRI and prolong survival in vivo. We found that NIH 3T3 cell line treated with BM1 inhibitor in vitro resulted in formation of senescent BM1 cells. Combined with previous studies, our data suggest a new pharmacodynamic role for BM1 inhibitors: BM1 inhibits phenotypic cellular senescence and that the senescent cells were able re-activate to form new neospheres in vitro and tumor growth in vivo. RNA-seq, ChiP-Seq and immuno-proteomic analysis revealed that the senescent cells initiate the expression of the Senescence Associated Secretory Phenotype (SASP) cytokines in an increasing occupancy of activated histone marks by SASP factor promoters. The SASP results in increased expression of anti-apoptotic BH3 proteins including BCL-xl and BCL-2. Treatment of the PTC208 treated senescent DIPG cells with BM1 mimetics induces apoptosis and clears the senescent cells. Combining BM1 with BH3 mimetic synergistically and significantly prolongs survival of DIPG bearing mice compared to BM1 inhibition alone. CONCLUSION: These data inform the current trial of BM1 inhibition as a mono-therapy and predict the need for adding BH3 mimetics to achieve efficacy.