munoglobulin and mucin domain protein family. It is expressed on multiple immune cell types, including T cells, NK cells, myeloid populations, and microglia, regulating adaptive and innate immunity. In silico assessment of TIM-3 expression in DIPG datasets showed a robust expression of this gene. Single-cell sequencing analyses of DIPG biopsies uncover TIM-3 expression, especially in microglia. In vivo efficacy studies showed that treatment with AbTIM-3 significantly increased overall survival in two DIPG immunocompetent orthotopic models, led to long-term survivors (50%), and showed immune memory. TIM-3 treatment led to a significant increase in the tumor microenvironment of microglia, granulocytes, NK, and CD8+ cells and higher levels of IFNγ, GrzB and TNFα corresponding with an NK and T-cell activate phenotypes. Interestingly, there was a decrease in the Treg population which causes an increase in the pro-inflammatory CD8/Treg ratio. CD4, CD8 or NK cell depletion leads to a significant but not a total loss of treatment efficacy. CD4+ and CD8+ cells were aumented in treated draining lymph nodes and expressed higher amounts of pro-inflamattory cytokines than control-mice. Population analysis and depletion experiments demonstrated the relevance of NK, CD4, CD8 and myeloid populations in the response to anti-TIM-3 therapy. Interestingly, the depletion of the different immune populations combined or using immunodeficient Rag2 mice, did not completely abrogate the treatment efficacy. These results suggest the concurrence of an additional mechanism of action that together with the immune response leads to a robust anti-DIPG effect. In conclusion, these data demonstrate that TIM-3 is a potential target for the treatment of DIPG.

DIPG-23. ARTIFICIAL INTELLIGENCE FOR DETECTING ACVR1 MUTATIONS IN PATIENTS WITH DIPG USING MRI AND CLINICAL DATA

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INTRODUCTION: ACVR1 mutations are found in about 25% of patients with diffuse intrinsic pontine glioma (DIPG). Recent work has identified the combination of vandetanib and everolimus as a promising therapeutic approach for these patients. We investigate the predictive power of an AI model integrating clinical and radiomic information to predict ACVR1 mutation. METHODS: This retrospective monocentric study includes 65 patients with known ACVR1 status. Patients were scanned at the diagnosis time with at least one of the four structural MRI modalities (pre- and post-contrast T1, T2, FLAIR) and basic clinical information (age and sex) was collected. Radiomic features were extracted within the tumor region from each modality. For each modality, a recursive feature elimination method was used to select the most relevant features. Inside a leave-one-out framework, up to five logistic regression models were built: one per MRI modality and one for the clinical information. The final prediction for each patient was computed as the mean of the probabilities of ACVR1 mutation for the up to 5 different models. Assigning a different weight to clinical data according to age, (more or less than 10 years old) was also tested. RESULTS: Out of the 65 patients (mean age 7.9±3.7, 15 patients older then 10 years), ACVR1 mutations were identified with a 78% accuracy (sensitivity = 92% and specificity = 75%) in the leave-out-out process. Accounting for the clinical data in the model increase the accuracy to 82% (resp. sensitivity = 86% and specificity = 80%). CONCLUSION: The proposed multi model approach compensates for missing MR modalities while taking advantage of all the available information. Our first results suggest that a dedicated model could be developed for younger patients to improve the prediction. The different models will now be tested using additional data coming from the ongoing multicentric BIOMEDE trials.

DIPG-24. NEUROLOGICAL SYMPTOM IMPROVEMENT AFTER RE-IRRADIATION IN PATIENTS WITH DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG): A RETROSPECTIVE ANALYSIS OF THE SIOP-E-HGG/DIPG PROJECT.

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PURPOSE: To investigate the spectrum of neurological triad improvement in patients with diffuse intrinsic pontine glioma (DIPG) treated by re-irradiation (re-RT) at first progression. METHODS: Re-analysis of the SIOP-E retrospective DIPG cohort by investigating clinical benefits after re-RT with focus on the neurological triad. Patients were divided as "responding" or "non-responding" to re-RT. To assess the interdependence between patients' characteristics and clinical benefits we used a Chi-Square or Fisher's Exact test. Survival according to clinical response to re-RT was calculated by the Kaplan-Meier method. RESULTS: As earlier reported, 77% (n = 24/31) of patients had any clinical benefit after re-RT. Among 25/31 well documented patients, 44% (n=11/25) had improvement in cranial nerve palsies, 40% (n=10/25) in long-tract signs, 44% (11/25) in cerebellar signs. Clinical benefits were observed in at least 1, 2 or 3 out of 3 symptoms of the DIPG triad, in 64%, 40% and 24% respectively. Patients irradiated with a dose ≥ 20 Gy versus < 20 Gy may improve slightly better with regards of ataxia (67% *versus* 23%; *P*-value = 0.028). The survival from the start of re-RT to death was not different between responding and non-responding DIPG patients (*P*-value = 0.871). CONCLUSION: A median re-irradiation dose of 20 Gy provides a neurological benefit in two-third of patients with an improvement of at least one symptom of the triad. DIPG patients receiving ≥20 Gy appear to improve slightly better with regards of ataxia, however we need more data to determine whether dose escalation up to 30 Gy provides additional benefit.

DIPG-25. PATTERNS OF CEREBROSPINAL FLUID DIVERSION AND SURVIVAL IN CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA: A REPORT FROM THE INTERNATIONAL DIFFUSE INTRINSIC PONTINE GLIOMA REGISTRY

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BACKGROUND: There are no standard practice guidelines for cerebrospinal (CSF) diversion for diffuse intrinsic pontine glioma (DIPG), nor clear understanding of potential for palliation and life-prolongation. We evaluated CSF diversion characteristics in children with DIPG to determine incidence, indications, symptom effects, and survival. METHODS: Data

were extracted from subjects registered in the International DIPG registry (IDIPGR). Univariable analyses was performed using the Fisher's exact test or Wilcoxon rank sum test. Survival was estimated using the Kaplan-Meier method. RESULTS: Evaluable patients (n=542) met criteria for DIPG diagnosis by central radiologic review; of those, 126 (23%) had permanent CSF diversion. Median time from diagnosis to diversion was 0.5 months (IQR 0.1-4.5 months). Those with permanent diversion were significantly younger (median 5.4 years vs 7.0 years, p<0.001) and had higher incidence of hydrocephalus at diagnosis (65.3% vs 11.9%, p<0.001). Permanent CSF diversion did not significantly impact overall survival (OS) (p=0.4), even amongst the 124 patients with hydrocephalus at presentation (p=0.20). Those with permanent diversion prior to radiation therapy demonstrated longer median OS than those in whom diversion was placed after radiation (14.3 vs 9.6 months, p=0.001). Patients reported significantly less headache and vomiting at last follow up after permanent CSF diversion compared to pre-diversion (p<0.0001 and p=0.001, respectively), however steroid use was also significantly higher at last follow-up after CSF diversion (p<0.001). CONCLUSIONS: Amongst an international cohort, DIPG patients who had permanent CSF diversion were significantly younger and had higher rates of hydrocephalus at initial presentation than those without permanent diversion. Symptoms of increased intracranial pressure improved in those with CSF diversion, although a direct effect may be confounded by increased steroid use. Permanent CSF diversion did not prolong overall survival in this large cohort of patients, even amongst those who presented with hydrocephalus.

DIPG-26. TARGETED PROTEIN DEGRADATION OF LSD1 SYNERGIZES WITH HDAC INHIBITORS IN DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse intrinsic pontine glioma (DIPG) remains one of the most lethal brain tumors in all of childhood with no effective treatments besides radiation, which only extends survival a few months. Against this backdrop, our lab recently executed a focused CRISPR negative selection screen in DIPG cell lines after treatment with the histone deacetylase (HDAC) inhibitor panobinostat and discovered a strong co-dependence with the histone demethylase LSD1. To further explore the therapeutic potential of this synergistic interaction, we tested a drug library of HDAC- and LSD1- targeting drugs with the goal of identifying a combination with optimal synergy and blood brain barrier (BBB) penetration suitable for clinical translation. We were surprised to find that traditional catalytic LSD1 inhibitors had minimal effect in isolation and did not seem to synergize with HDAC inhibitors, while a recently described CoREST/LSD1 degrader named UM171 phenocopied the effects seen in our CRISPR screen. Degraders are a class of compounds that recruit an E3 ubiquitin ligase to a protein-of-interest and cause target ubiquitination and proteasomal degradation. Given our unexpected finding, we hypothesized that UM171 induces synergy with HDAC inhibitors through elimination of a scaffolding function of LSD1. To prove this, we knocked out LSD1 using CRISPR/Cas9 and subsequently treated with a panel of HDAC inhibitors, which showed a signification sensitization of DIPG cells to HDAC inhibitors compared to standard controls. We also confirmed that UM171 interacts with the CoREST complex (members include LSD1, RCOR1, HDAC1/2) by performing streptavidin bead pull down with a newly synthesized biotin-conjugated UM171 probe. In summary, our results show that targeting LSD1 for degradation in combination with HDAC inhibition is a synergistic strategy in DIPG worthy of further translational study.

DIPG-27. BEHAVIORAL DISTURBANCES AS UNDERESTIMATED PRESENTING SYMPTOMS IN CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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PURPOSE: to describe how often behavioral and emotional changes occur at diagnosis in children with DIPG, or precede it. METHODS: the anamnesis, clinical history, psychological evaluation, and onset symptoms of all cases of DIPG diagnosed at Gaslini Institute between January 2010 and December 2020 were reviewed. RESULTS: 20 DIPGs were diagnosed, 7 males, with a median age of 7,6 years (range 2,4-16,2). All patients presented typical neurological symptoms: 16 had cranial nerves palsy, 12 ataxia, 8 dysarthria, 5 dysphagia, 5 hemiparesis, 5 headache, and 2 obstructive hydrocephalus. Behavioral disorders were found in 14 cases, with several manifestations and in various association: irritability and aggressive behavior in 6, ideomotor slowdown and apathy in 5, emotional dysregulation in 4, mood deflection in 3, sleep disturbances (*i.e.* nightmares, insomnia, and somniloquy) in 3, marked behavioral changes, school phobia and separation anxiety in 2, depersonalization crisis and phobia of waterdrops in the eyes in 2 patients each. In 6 cases behavioral disturbances were the presenting symptom, appearing one to twelve months earlier than the classic neurological deficits. In all patients, behavioral symptoms improved during Radiotherapy. CONCLUSIONS: behavioral disturbances, although well-known and described in the literature, are not commonly reported among the onset symptoms of DIPG, thus being probably underestimated. Their pathogenesis can be explained by neurophysiology: the brainstem contains reciprocal cerebro-ponto-cerebellar connections whose disruption compromises their modulatory function on affective and cognitive behavior. Furthermore, the reticular formation contains aggregates of neurons regulating several complex functions including the state of alertness (*e.g.* sleep and wakefulness), the perception of pain, and cognitive functions (*e.g.* attention, mood, and memory). A careful anamnestic and medical history together with a detailed psychological assessment should be always performed in all DIPGs at diagnosis, in order to bring out those underlying behavioral disorders which could benefit from early neuropsycological support.

DIPG-28. INDOLENT *H3 K27M*-MUTANT DIFFUSE MIDLINE GLIOMA

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INTRODUCTION: *H3 K27M*-mutant diffuse midline glioma (DMG) is an aggressive central nervous system tumor that is universally fatal with a median survival of 8-12 months after diagnosis. Here we present a patient who was incidentally found to have a lesion within the right thalamus on brain magnetic resonance imaging (MRI). Twelve years later, she was found to have a brain mass within the same area with pathology consistent with an *H3 K27M*-mutant DMG. CASE DESCRIPTION: A 14-year-old female presented with new onset left-sided numbness and weakness, blurry vision and a right-sided temporal headache. Her past medical history is significant for severe persistent asthma and anoxic brain injury secondary to a cardiopulmonary arrest at 2-years-of-age due to an asthma exacerbation. She has had multiple MRIs of her brain since her initial insult which initially showed the presence of a T2/FLAIR hyper-intense lesion within the right medial thalamus in addition to chronic central nervous system changes. The right thalamic lesion was stable in size between images obtained at 2-years and 4-years of age. Her current physical exam is significant for decreased strength to her left upper and lower extremities. Brain MRI with and without contrast is obtained with partial effacement of the third ventricle due to mass effect from an enlarging mass from the right thalamus. Biopsy of the lesion demonstrated an *H3 K27M*-mutant DMG, WHO grade IV. Our patient went on to receive palliative radiation therapy with 59.4 Gy over 33 fractions. DISCUSSION: This case illustrates an unusual presentation of an *H3 K27M*-mutant DMG with an indolent course, diagnosed twelve years after the initial MRI finding. This proposes the possibility of multiple factors playing a role in the oncogenesis of these aggressive tumors and that further research is warranted.

DIPG-29. HIGHLY MULTIPLEXED DIGITAL SPATIAL PROFILING OF THE TUMOR IMMUNE ENVIRONMENT OF PEDIATRIC AND ADULT *H3 K27M*-MUTANT DIFFUSE MIDLINE GLIOMAS

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BACKGROUND: *H3 K27M*-mutant diffuse midline gliomas (DMGs) are highly aggressive malignancies of the central nervous system that affect both pediatric and adult populations. The immune layout and genetic changes within the tumor microenvironment associated with these high-grade malignancies are thought to play an integral role in the phenotypic differences in tumor presentation and clinical course between both populations. Comparative immune landscape between pediatric and adult DMGs is not known. METHODS: The NanoString GeoMx™ Digital Spatial Profiler platform was used to determine the immune marker and genetic layout in a cohort of both pediatric and adult *H3 K27M*-mutant DMG tissue samples. Three fluorescently labeled antibodies targeting immune cells (CD45), epithelial cells (PanCK), tumor cells (*H3 K27M*) and a nucleic acid stain (SYTO-13) were used to identify and separate out the various components within the tumor tissues from selected regions of interest. The resultant information was then pooled into libraries that were run through the Illumina sequencing system to assess gene expression and proteomics for both cohorts of samples.