DIPG-11. DIPGS SHOW ROBUST MHC CLASS I EXPRESSION BUT LOW LYMPHOID AND HIGH MYELOID INFILTRATION PARTLY REVERSIBLE BY DNA METHYLTRANSFERASE INHIBITION

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Diffuse intrinsic pontine gliomas (DIPGs) are the most aggressive tumors of the central nervous system in children. Median survival of patients is less than one year post-diagnosis. Radiotherapy remains the only standard treatment but is rarely curative. Immunotherapy is an emerging and promising treatment strategy for children with DIPG. However, general immunotherapy has not lived up to its promise to treat many cancers, including DIPGs, in part due to incomplete understanding of the barriers posed by the tumor microenvironment. We therefore evaluated the immune cell infiltration in a syngeneic mouse model of DIPG and in DIPG tumors collected from patients. We evaluated the expression profiles of T lymphocytes and myeloid cell markers in a cohort of 28 DIPG tumors compared to matched normal tissue specimens. Our data indicate that the expression of MHC I components in DIPG tumors is similar to that of matched normal tissue. Moreover, the well-known immune checkpoint, PD-L1, was not overexpressed in DIPG tumors. Using immunohistochemistry, we demonstrated only rare infiltration of lymphocytes but high enrichment of myeloid cells in tumor tissue. We found similar results in a syngeneic mouse model of DIPG. Collectively, our results indicate that DIPG tumors harbor rare lymphocytes and are enriched in myeloid cells. Recent studies have demonstrated that DNA methyltransferase inhibitors prime some tumors for more effective checkpoint blockade by activating expression of human endogenous retroviruses and sparking a T-cell mediated immune response through a process called "viral mimicry." We tested the effect of decitabine in a syngeneic mouse model of DIPG. Decitabine reduced the tumor growth kinetics compared to vehicle but was unable to induce the recruitment of lymphoid cells in DIPG tumors. Importantly, we observed a noticeable reduction in the myeloid component of the DIPG microenvironment suggesting a possible role of myeloid cells in tumor growth and progression.

DIPG-12. INDUCED MITOTIC ABNORMALITIES ASSOCIATED WITH BMI-1 MODULATION SENSITIZE DIFFUSE INTRINSIC PONTINE GLIOMA CELLS TO IONIZING RADIATION

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Diffuse intrinsic pontine glioma (DIPG) remains an incurable childhood brain cancer with a median overall survival of less than 12 months, affecting 200-300 children annually in the United States. Hence, there is an unmet need for the development of novel and effective targeted therapies. BMI-1 is a subunit of the multimeric protein complex Polycomb repressor complex 1 (PRC1) implicated in self-renewal of normal and cancer cells, and in DNA damage signaling. We have previously identified BMI-1 as a potential therapeutic target in DIPG and have shown that BMI-1 is highly expressed in DIPG tumors regardless of H3K27 mutational status. Treatment of DIPG cells with PTC596, a small molecule initially identified as a BMI-1 modulator, and ionizing radiation (IR) impairs the kinetics of DNA damage response. *in vivo*, treatment with PTC596 alone delayed tumor growth kinetics and induced in-tumor apoptosis. However, we observed tumor regrowth once PTC596 treatment is completed or discontinued. In the present study, we evaluated the use of PTC596 in combination with IR. Our *in vivo* results indicate that PTC596 sensitizes DIPG cells to IR inducing a prolonged cell growth arrest 14 days post-treatment compared to IR or PTC596 alone. The effectiveness of this combination is currently evaluated in murine orthotopic DIPG models and the results will be presented. PTC596 is being tested in newly diagnosed children with DIPG and high-grade gliomas (NCT03605550). Data collected from this study will support the development of a novel therapy including PTC596 in combination with radiotherapy to treat children with DIPG.

DIPG-13. IMMUNE PROFILING BY RNA-SEQ DECONVOLUTION AND SINGLE-CELL SEQUENCING REVEAL MYELOID CELL ENRICHMENT IN DIPG TUMOR MICROENVIRONMENT

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Diffuse intrinsic pontine glioma (DIPG) remains an incurable disease with median overall survival <12 months despite decades of clinical trials investigating multimodal therapies. Immunotherapy represents a promising treatment paradigm which has been successfully used in other cancers. An adequate understanding of the tumor microenvironment and immunologic profile is essential to identify potential immunotherapeutic targets to inform immunotherapy design. Previous studies have shown that most DIPG tumors harbor low mutational burden compared to adult cancers and are characterized by a non-inflammatory microenvironment, limiting the development of immunotherapies in this disease. Our team's prior work similarly demonstrated that most DIPG tumors have an immunologically "cold" microenvironment, but a subset of tumors harbors a more inflammatory gene expression profile and/or higher mutational burden, with trends toward improved survival and favorable radiographic response to radiation. Here, we applied a deconvolution analysis using CIBERSORTx on bulk RNA-seq data from 28 DIPG patients' tumors paired with matched normal tissue specimens, to profile the immune microenvironment of DIPG and evaluate immune-related gene expression to determine percentages of different types of immune cells. Our results indicate that DIPGs have very limited lymphocyte infiltration. However, the infiltration of macrophages "M2-like" type cells and CD4 memory resting T cells were significantly higher in tumors compared to normal tissue samples. Similar results were found using single-cell RNA sequencing performed on biopsy and autopsy tissue, with less than 5% of total cells identified as immune cells. MHC I components were widely expressed in DIPGs with no significant difference between tumor and normal tissue. Expression of *CD11B* and *CD68* were higher in tumor compared to normal tissue, suggesting enrichment of myeloid cells. Overall, deconvolution analysis of bulk RNA-seq can be used to profile DIPG tumors' immune microenvironment to aid in the thoughtful design of effective immunotherapeutic strategies for this disease.

DIPG-14. NEOGENIN KNOCKOUT STOPS THE INVASION AND DISSEMINATION OF DIFFUSE INTRINSIC PONTINE GLIOMAS *IN VIVO*.

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INTRODUCTION: Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating high-grade glioma localized in the brainstem that occurs almost exclusively in children. The significant mortality rates of DIPG stem largely from its capacity to invade adjacent normal brain. We have previously published studies identifying the axon guidance factor, neogenin as a key driver regulating brain tumor cell invasion in medulloblastoma, glioblastoma and DIPG in vitro. Here we expand this work with an in vivo model to validate neogenin as promising inducer of DIPG invasion. METHODS: CRISPR-Cas9 system was used to knock-out the neogenin gene in DIPG cells. After validation of the knockout (KO) *in vitro* by RTqPCR, Western blot, and immunofluroresence, the cells were orthotopically implanted in the pons of nude mice (n=8/group) with a stereotactic frame. Tumor growth and dissemination were monitored using the In Vivo Imaging System (IVIS). Then, brains and spinal cords were collected, and H&E staining was performed. RESULTS: *In vitro* studies confirmed the KO of neogenin in DIPG cells. *In vivo* studies revealed that the neogenin total KO group had no progression of the tumor without any dissemination after 4-6 weeks, in marked contrast to the WT group, which exhibited steady growth and widespread dissemination into the spine. Immunohistochemistry confirmed the IVIS results. CONCLUSION: Expanding on previous work, this data suggests that neogenin is a major contributor of DIPG cell invasion and dissemination. The blockade of neogenin in DIPG cells demonstrates the potential utility of neogenin as potential therapeutic target, capable of reducing tumor growth and stopping tumor cell dissemination.

DIPG-15. MAJOR TUMOR REGRESSIONS IN H3K27M-MUTATED DIFFUSE MIDLINE GLIOMA (DMG) FOLLOWING SEQUENTIAL INTRAVENOUS (IV) AND INTRACEREBROVENTRICULAR (ICV) DELIVERY OF GD2-CAR T-CELLS

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BACKGROUND: H3K27M-mutated DMGs express high levels of the disialoganglioside GD2 and GD2-CAR T-cells (GD2-CART) regress DMG in

preclinical models. METHODS: NCT04196413 is a 3 + 3 Phase I dose escalation trial testing GD2-CART in patients with biopsy-proved H3K27M DMG, with dose-limiting toxicities (DLT) considered independently for DIPG and spinal DMG (sDMG). Arm A tested escalating doses of IV GD2-CART (DL1=1e6 GD2-CART/kg; DL2=3e6 GD2-CART/kg) following lymphodepletion (LD). After the DLT period, patients with clinical and/or radiographic benefit were eligible for subsequent ICV GD2-CART infusions (10-30e6 GD2-CART) administered via Ommaya without LD. RESULTS: Twelve subjects were treated after standard radiotherapy, 7 of whom began treatment at the time of progression [n=4 DL1 (3 DIPG/1 sDMG); n=8 DL2 (6 DIPG/2 sDMG)]. No DLTs were observed on DL1. Three subjects experienced DLT on DL2 (2 DIPG/1 sDMG) due to grade-4 cytokine release syndrome (CRS). On both dose levels, all subjects exhibited transient symptoms related to on-tumor inflammation, termed Tumor Inflammation-Associated Neurotoxicity (TIAN); no DLT due to TIAN has occurred. Ten subjects experienced radiographic and/or clinical benefit after IV infusion and received subsequent ICV infusions (median=4 ICV infusions/pt, range=1-7). ICV infusions were not associated with high-grade CRS. Four patients continue to receive ICV infusions on study and have experienced continued clinical and radiographic benefit, currently 7-11 months following enrollment. Two patients (one sDMG, one DIPG) have experienced near-complete (>95%) tumor volume reduction. CONCLUSIONS: IV treatment of DIPG and sDMG with GD2-CART is safe at a dose of 1e6/kg, but associated with frequent high-grade CRS at 3e6/kg. ICV GD2-CART has been well tolerated and has mediated impressive sustained clinical benefit in some patients with DIPG/sDMG. Given these findings, we are launching a new arm to assess safety and activity and to define the recommended phase 2 dose for ICV delivery of GD2-CART without LD.

DIPG-16. EVALUATION OF MYELOID COMPONENT OF DIPG MICROENVIRONMENT

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Diffuse intrinsic pontine glioma (DIPG) is an aggressive and incurable disease of the central nervous system in children with median overall survival of less than one year. In recent years, several immunotherapy strategies have emerged as an option to treat DIPG. However, the low mutational burden and rare infiltration of T lymphocytes, render these tumors immunologically "cold" and therefore pose challenges for general immunotherapy. The myeloid component was implicated in the immunosuppression in other solid tumors. Previous data have shown that DIPG tumors are enriched in macrophages, but their role in tumor growth and progression have not been elucidated. Specifically, it remains unclear whether the myeloid cells are recruited to the tumor microenvironment from the peripheral circulation. Here, we examined the recruitment of myeloid cell populations to the tumor microenvironment and further delineated their role in tumor progression in a syngeneic mouse model of DIPG. We showed that this DIPG mouse model displays an immune microenvironment similar to that of patients' DIPGs. DIPG tumors harbored rare tumor infiltrating lymphocytes and are enriched in myeloid cells. To further characterize the phenotype and functions of these myeloid populations, we evaluated the changes in proportions of myeloid cell subsets using flow cytometry (CD11b, Ly6c, Ly6G, MHCII, F4/80, CD206, Arg1) in the bone marrow, peripheral blood, and in the tumor microenvironment during tumor progression. Also, we investigated the role of these myeloid cells in angiogenesis and immune suppression by performing histological and expression analyses of endothelial markers and chemokines (CD31, CD34, KDR, IL-10, IL-13, IL-4, CCL2, CCL5). Furthermore, decitabine (DNA methyltransferase inhibitor) treated tumors showed a decrease in myeloid population associated with a reduction in tumor growth, suggesting an important role of myeloid populations in tumor growth and progression.

DIPG-17. CD155 REGULATES CELL GROWTH AND IMMUNE EVASION IN DIFFUSE INTRINSIC PONTINE GLIOMA

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There is an unmet need for more effective treatment strategies for diffuse intrinsic pontine glioma (DIPG), a devastating brain tumour arising in children and young adults. While immunotherapy is emerging as a powerful approach to treatment of other cancers, clinical trials with immune checkpoint inhibitors have failed to show a survival benefit for DIPG patients. In this study, we analysed the expression of immune checkpoint molecules on the surface of human and murine DIPG cells by flow cytometry and identified CD155 and B7-H3 as the most highly expressed checkpoint molecules, with minimal expression of PD-L1, PD-L2, Galectin-9, CEACAM-1, CD86, CD252 and CD137. These findings were confirmed in primary patient samples from pediatric brain tumours, including high-grade gliomas, medulloblastomas and ependymomas. To test whether CD155 inhibition increases susceptibility to CD8+ T cell killing *in vitro*, we cultured DIPG cells expressing ovalbumin (OVA) with CD8+ T cells from OT-I mice, which express T cell receptors specific for OVA. Addition of CD155 blocking antibodies to these cultures increased expression of T cell activation markers (CD25, CD44 and CD69) as well as T cell-mediated tumour killing, supporting the notion that CD155 can function as an immune checkpoint in DIPG. In addition to its effects on T cells, CD155 also exerted direct effects on tumour cells: treatment with anti-CD155 antibodies led to impaired cell viability, and shRNA-mediated knockdown of CD155 resulted in reduced cell proliferation *in vitro*. Strikingly, knockdown of CD155 also led to reduced growth of DIPG cells *in vivo*, and mice transplanted with the CD155-deficient cells had a clear survival benefit compared to mice transplanted with wild type cells. These studies demonstrate that CD155 functions as an immune checkpoint and as a regulator of tumor growth in DIPG, and suggest that targeting CD155 could be a valuable therapeutic strategy for this devastating disease.

DIPG-18. EVALUATING DRUG DISTRIBUTION IN CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) TREATED WITH CONVECTION-ENHANCED DRUG DELIVERY

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BACKGROUND: There is currently no method for evaluating drug distribution and tumour coverage using the convection-enhanced drug delivery (CED) technique in diffuse midline glioma of the pons (previous DIPG). **AIMS**: To determine an imaging protocol that can be used to assess the distribution of infusate in children with DIPG treated with CED of carboplatin and sodium valproate. METHODS: 12 children with DIPG received between 4–18ml of infusate, through 2 pairs of catheters to encompass tumour volume on 2 days. Volumetric T2W and Diffusion Weighted Imaging (DWI) MRI sequences were performed before and after the first cycle of CED therapy and Apparent Diffusion Coefficient (ADC) maps were calculated. The tumour volume pre and post CED was automatically segmented (ITKSnap) on T2W and ADC on the basis of signal intensity. The ADC maps pre and post infusion were registered and subtracted (FSL) to visualize the infusate distribution. RESULTS: ADC and T2W demonstrated a significant (<0.001) change in mean tumour volume post-infusion (mean ADC volume pre: 19.8ml, post 24.4ml; mean T2W volume pre 19.4ml, post 23.4ml). A significant correlation ($p<0.001$) was observed for the difference in tumour volume and the actual infused volume (ADC, r=0.76, T2W, r=0.70). There was a significant increase ($p<0.001$) in mean ADC and mean T2W signal intensity ratio post-infusion, no significant correlation with infusion volume. Finally, pixel-by-pixel subtraction of the ADC maps pre and post infusion visually demonstrated high signal intensity, presumed infusate coverage of the tumour. CONCLUSIONS: Our study provides the preliminary evidence that measurement of change in tumour ADC and T2W MR sequences, has a potential value for quantifying the distribution of infusate delivered by the intermittent CED, which will facilitate the use of CED in future clinical trials.

DIPG-19. FOXR2 IS AN ONCOGENIC DRIVER ACROSS PEDIATRIC AND ADULT CANCERS

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