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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