classification from The Cancer Genome Atlas project reveals that 81% of high-grade tumors across molecular subtypes are characterized by an immunosuppressive phenotype (C4) while an inflammatory phenotype (C3) is more common in low-grade lesions (p<0.001). Adjusting for histologic grade and extent of resection, C4 associates with worse overall (OS) and progression-free survival (PFS) in this cohort (HR 3.1, p=0.008 and 1.6, p=0.03 respectively). Deconvolution of the transcriptome reveals that relative to C3, C4 tumors have decreased T-cell signature, OR 0.4 (0.2–0.8), and increased macrophage and tumor-proliferation signature (OR 2.0, 1.3–3.3, and 3.1, 2.3–4.2, respectively). In contrast to C3 tumors, T-cell signature in C4 tumors adversely impacts survival and correlates with multiple immunosuppressive genes and cytokines. Among them, the immune checkpoint *CD276* has the highest associated impact on survival in C4 tumors (HR of log increase is 1.9, p<0.001). Additionally, high-grade lesions have suppressed expression of antigen-presenting genes. *EZH2* is implicated in downregulating antigen presentation and is found to be significantly upregulated in all high-grade lesions in this cohort. Treatment with the EZH1/2 inhibitor valemetostat resulted in upregulation of antigen-presenting genes and tissue differentiation pathways across three murine syngeneic models, one modeling diffuse midline glioma and two embryonal models. Future in-vivo studies with genetic and chemical modification of immunomodulatory genes of interest aim to identify immunotherapeutic targets with potential for broad applicability in pediatric neuro-oncology.

IMMU-16. NEXT-GENERATION CAR T-CELLS TARGETING IL13RA2 AND SECRETING IL-15 ACHIEVE DURABLE TUMOUR CLEARANCE IN PRE-CLINICAL MODEL OF DIFFUSE MIDLINE GLIOMA

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T-cells engineered with a chimeric antigen receptor (CAR) to acquire tumour specificity provide a possible new treatment approach for childhood brain tumours. Durable clinical remissions have been achieved with CD19-directed CAR T-cells in refractory B-cell malignancies including control of leptomeningeal disease and parenchymal deposits. Early clinical data of CAR-T cells in adult glioblastoma and diffuse midline glioma (DMG) further support the rationale for development of CAR-T cell therapy for paediatric high-grade gliomas (pHGG) and other high-risk childhood brain tumours. IL13RA2 provides a suitable CAR target. It is expressed in the majority of pHGG including DMGs while expression is absent on normal (paediatric) brain tissue. Early results with CAR T-cell therapy for adult HGG has shown that IL13RA2 can be safely targeted, and potent anti-tumour activity is possible. However, responses are variable and ultimately transient. This is likely due to the immunosuppressive environment encountered by CAR T-cells at tumour sites which hampers persistent tumour-directed immunity. Here we develop a next generation CAR approach engineering T-cells with both a CAR and a cytokine signal which supports persistent anti-tumour immunity. We have generated novel IL13RA2-specific antibodies and used these to construct CARs. Using a functional screening approach assessing target-specific cytolytic function, T-cell proliferation and cytokine release, we selected the optimal IL13RA2-CAR design. Then, we generated a next generation CAR construct co-expressing inflammatory cytokine IL-15 with the IL13RA2-CAR. In a PDX model of DMG, T-cells transduced with IL13RA2-CAR^{IL-15}, in contrast to IL13RA2-CAR alone, induced long-term tumour clearance. Further in vitro testing of IL13RA2-CAR^{IL-15} showed enhanced proliferation and resistance to immune-suppressive secreted factors such as TGF-β. In conclusion, co-expression of IL-15 with IL13RA2-CAR enhances CAR T-cell proliferation and in vivo persistence and achieves durable tumour clearance. IL13RA2-CAR^{IL-15} is being translated into a phase I clinical trial for patients with DMG.

IMMU-17. COMPREHENSIVE IMMUNOLOGICAL GENE EXPRESSION PROFILING OF PEDIATRIC BRAIN TUMORS

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Immunotherapy, predominantly through immune checkpoint inhibition (ICI), has had incredible success in treating some metastatic cancers, however, outside of rare cases of mismatch repair deficient (MMRD) gliomas, brain tumors have not had consistent responses to ICI. This can be attributed to a variety of factors including a low tumor mutation burden, lack of T cell infiltrates, and the CNS immune privilege. There are numerous strategies to target the tumor immune microenvironment (TIME) beyond ICI, include CAR-T cells, tumor vaccines, and myeloid cell modulation. The investigation of these depends critically on detailed characterization of the cell populations and interactions in the CNS TIME. We developed a 103 gene NanoString immune-oncology gene expression panel that includes markers reflecting selected cell types, therapeutic targets, and cellular pathways, as well as the 18-gene Tumor Inflammation Signature, a well validate biomarker for ICI response. We have used this to characterize over 500 brain tumors, including a diverse set of 227 pediatric low-grade gliomas (LGG), 86 MMRD gliomas, 47 diffuse intrinsic pontine gliomas (DIPG), 26 ependymomas, 36 medulloblastomas, 70 adult gliomas, and 35 non-tumor brain samples. Our results demonstrate a broad range of immunologic states, including within groups of tumors with the same genetic driver alteration. In pediatric LGG with BRAF V600E, there was clear histologic correlation with immune status, as glioneuronal tumors had substantial upregulation of T cell markers and regulatory genes, while diffuse astrocytomas had a near normal immune profile. In DIPG there was strong upregulation of macrophage markers, contradicting prior reports that have characterized these tumors as immunologically neutral. In a set of MMRD gliomas treated with ICI we identified several differentially expressed genes correlating with therapeutic response, including CCL4, CXCL9, and HGPD. In sum, this provides a characterization of diverse immune activation states across pediatric gliomas and other brain tumors.

IMMU-18. TARGETING ANTIGEN PRESENTING CELLS TO IMPROVE VIROTHERAPY EFFICACY IN DIFFUSE MIDLINE GLIOMAS

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With a 2-year survival less than 20%, Diffuse Intrinsic Pontine Glioma (DIPG) is the principal cause of pediatric death. Despite recent advances in the current treatments, the outcome for children with DIPGs remains dismal. Since the approval of T-VEC for melanoma by the FDA, oncolytic adenoviruses have emerged as a promising therapeutic strategy for brain tumors. Thus, our group launched the first world clinical trial phase I with the oncolytic adenovirus Delta-24-RGD (DNX-2401 in the clinic) for newly diagnosed DIPG (NCT03178032), which has shown safety and feasibility. Despite DNX-2401 increases the recruitment of T cells into the tumor, they usually become inactive due to the non-responsive tumor microenvironment evidencing the urgent need to improve this strategy focusing on the generation of effective long-term immune responses. Therefore, we decided to combine the Delta-24-RGD with the targeting of the costimulatory molecule CD40 in immunocompetent mice bearing orthotopic DIPG. The activation of the CD40 receptor, which is expressed by antigen presenting cells (APC) such as microglia, macrophages, and dendritic cells, is known to increase antigen presentation and enable T-cell priming and activation. Here, we observed that in addition to Delta-24-RGD anti-tumor effects, the stimulation of CD40 (using an agonistic antibody) on the tumor APCs results in a remodeling of the tumor immune compartment towards a proinflammatory scenario and a more efficient T-cell infiltration. Of importance, the combination therapy extends survival of treated mice as compared to single treatments or non-treated mice. In addition, we observe a complete regression of tumors in more than 40% of treated mice and the development of long-term anti-tumor immunity. We believe that these results provide a translational breakthrough in the treatment of these lethal tumors and open the door for a future innovative clinical trial.

IMMU-19. OUTCOMES OF PEDIATRIC PATIENTS WITH HIGH-RISK CNS TUMORS TREATED WITH MULTI-TUMOR ASSOCIATED ANTIGEN SPECIFIC T CELL (TAA-T) THERAPY: THE REMIND TRIAL

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BACKGROUND: The ReMIND trial hypothesizes that autologous T-cells specific for three tumor-associated antigens (TAA) -WT1, PRAME, and survivin-will be safe and elicit anti-tumor immunity in pediatric patients with CNS cancer. METHODS: Patients (n=25) received autologous TAA-T for newly-diagnosed DIPG (Stratum A, up to 4x107/m2) or recurrent CNS malignancies (Stratum B, up to 8x107/m2) in this dose-escalation study (NCT03652545) and