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**From the Selected Works of Shayna Zelcer**

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**IMMU-13. Dual CTLA4/ PD-1 blockade improves survival for replication-repair deficient high-grade gliomas failing single agent PD-1 inhibition: An IRRDC study**

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aim to characterize the tumour immune microenvironment (TIME) of intracranial syngeneic mouse models of diffuse hemispheric glioma, H3G34 (DHG-H3G34) and diffuse midline glioma, H3K27 (DMG-H3K27). We also demonstrate how an oncolytic reovirus (Reolysin) can “heat-up” the TIME of our syngeneic models. Orthotopic immunocompetent mouse models of DHG-H3G34 (C57BL/6, NRASG12V + shp53 + shATR1X +/- H3.3G34R) and DMG-H3K27 (Nestin-Tv-a/p53<sup>tm</sup>, RCAS-ACVR1R206H + RCAS-H3.1K27M) were profiled using single-cell RNA-sequencing (scRNA-seq) (10x genomics), a 22-colour custom flow cytometry immune panel and spatial transcriptomics. Differential marker expression was validated with immunohistochemistry and immunofluorescence in tissue sections. Syngeneic mouse tumours treated systemically with Reolysin were also profiled to evaluate the effects of the oncolytic virus on the TIME. Cell type predictions in scRNA-seq using singleR, ssGSEA and expression of individual marker genes suggested that the predominant immune cell types within hemispheric tumours were monocytes (11-21%) and macrophages (10-19%) with much smaller proportions of CD4+ and CD8+ T-cells (4-10%). By contrast, much smaller proportions of monocytes (2%) and macrophages (3%) were observed in the H3.1K27M pontine model. Flow cytometry, immunohistochemistry and immunofluorescence validated scRNA-seq immune profiles and characterised signalling of the PD-1/PD-L1 checkpoint pathway. Spatial transcriptomics allowed immune cell populations to be positioned within tumour sections and showed significant co-localization of CD4+ and CD8+ lymphocytes at tumour margins. Treatment of syngeneic mouse tumours with Reolysin resulted in reduced tumour volumes and altered the TIME, in particular increasing cytotoxic T-cell tumour infiltration. Our results highlight immunological heterogeneity within molecular subgroups of PDHGG and demonstrate ability of a systemically delivered oncolytic virus, Reolysin, to “heat-up” the TIME, contributing to a more immune actionable profile. Future work will help to identify optimal combinations for the next generation of immunotherapies in PDHGG.

**IMMU-13. DUAL CTLA4/ PD-1 BLOCKADE IMPROVES SURVIVAL FOR REPLICATION-REPAIR DEFICIENT HIGH-GRADE GLIOMAS FAILING SINGLE AGENT PD-1 INHIBITION: AN IRRC STUDY**  
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**BACKGROUND:** High-grade gliomas (HGG) with replication-repair deficiency (RRD) harbour high mutation burden (TMB) and are rapidly fatal following chemo-radiation approaches. Although hypermutation results in objective responses and prolonged survival in >30% of patients undergoing PD1-blockade, salvage following failure of PD1-inhibition remains a challenge. **METHODS:** We performed a real-world study of Ipilimumab (anti-CTLA4) in combination with Nivolumab/Pembrolizumab for patients failing single-agent PD1-inhibition. **RESULTS:** Among 68 consortium patients with relapsed HGG treated with single-agent PD1-inhibitors, progression was observed in 43 (63%). Ipilimumab was added to 20/43 (46.5%),

14 (32.5%) received best supportive care (BSC), and 9 (21%) received miscellaneous therapies. For patients receiving CTLA4/PD1-inhibition, median age at progression was 12.3-years (IQR: 9; 15.6). Time from anti-PD1 initiation to progression was 8-months (IQR: 3.8; 18.5). Germline predisposition was observed in all patients (CMMRD: 70%, Lynch: 25%, polymerase-proofreading deficiency: 5%). All HGG were hypermutant (median TMB: 182 mutations/Mb; IQR: 15.6; 369.4). Centralized radiology review revealed objective responses in 3/20 (15%, all ultra-hypermutant: 320, 496, 834 mutations/Mb), stable disease in 5 (25%), and 12 (60%) eventually progressed (iRANO). Following failure of PD1-blockade, estimated progression-free and overall survival at 18-months for patients receiving CTLA4/PD1-inhibition were 11% and 25%, respectively. Importantly, survival was superior to patients receiving BSC (median OS <1-month versus 12-months on CTLA4/PD1-inhibition; p<0.001). All patients receiving BSC died within 3.5-months, while 4/8 survivors were alive for >1-year on the anti-CTLA4/PD1 combination (range:1-48 months). The combinational immunotherapy resulted in significant autoimmune toxicity in 11/20 (55%), warranting immunosuppressive therapy in all, and treatment abandonment in 6 patients. **CONCLUSION:** Combined CTLA4/PD1-blockade after failure of single-agent PD1-inhibition revealed objective responses and prolonged survival in an otherwise rapidly-fatal disease. This needs to be assessed in the context of significant autoimmunity, supporting the need for the current prospective trial (NCT04500548), and novel strategies to limit treatment-related toxicity.

**IMMU-14. SYNNOTCH CHIMERIC ANTIGEN RECEPTOR (CAR) T-CELLS AS A POTENTIAL TREATMENT FOR DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)/DIFFUSE MIDLINE GLIOMA (DMG)**  
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**BACKGROUND:** The development of effective chimeric antigen receptor (CAR) T-cell therapies for malignant pediatric brain tumors remains a challenge due to multiple barriers, including antigenic heterogeneity, on-target off-tumor toxicities, and T cell exhaustion. We have adopted a novel synthetic Notch “synNotch” receptor system and developed innovative T-cell circuits that recognize tumor cells based on the “prime-and-kill” strategy. **METHODS:** We created a novel synNotch-CAR circuit in which the brain/glioma-specific antigen Brevican (BCAN) primes the T cells to induce expression of a CAR that recognizes interleukin-13 receptor  $\alpha 2$  (IL-13R $\alpha 2$ ) and ephrin type A receptor (EphA2), thereby eradicating glioma cells expressing either antigen. Immunocompromised mice bearing the SF8628 DIPG cell line in the frontal lobe or brain stem received a single intravenous (IV) infusion of synNotch CAR T-cells ( $2.5 \times 10^6$  each of CD4+ and CD8+ T cells) on day 6 following the tumor inoculation. Mice were monitored for toxicity and tumor growth. **RESULTS:** Following this synNotch CAR T-cell dose, although tumors in the brainstem did not regress, 3 of the 5 mice with frontal lobe tumors demonstrated complete and sustained remission. Our histological analyses revealed primed CAR T-cells both within and surrounding the tumor in both settings. By flow cytometry, we confirmed the CAR T-cells in the CNS were primed and mostly did not express an exhaustive phenotype. In the spleen, we also found the CAR T-cells in a more naïve and central memory state. **CONCLUSIONS:** Our work so far has demonstrated that synNotch-CAR T-cells are able to traffic to the tumor microenvironment even in the brainstem, are primed to express the CAR, and most do not express an exhaustive phenotype. Future work will include CAR T-cell dose optimization, continued assessments of the tumor microenvironment, and investigating for antigen escape.

**IMMU-15. THE IMMUNOLOGIC CONTEXT OF PEDIATRIC CENTRAL NERVOUS SYSTEM MALIGNANCIES AND IDENTIFICATION OF PAN-HISTOLOGY IMMUNOMODULATORY TARGETS**  
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Central nervous system tumors account for the most childhood cancer mortality. Immunotherapies have made major contributions to treat adult cancers, but application of immunotherapy for childhood brain tumors has been limited, in part due to the unique CNS microenvironment and mechanisms of immune escape in this context. To investigate the immunologic context, we query the transcriptomic profile of ~700 primary brain tumors released by the Children's Brain Tumor Network. An immune subtype