

the murine GBM cell line GL261 into CD45+ immune cells accompanying morphological changes to less adhering cells by a combination of four factors (PU.1, IRF8, BATHF3, ID2). Of these induced CD45 positive cells, a significant fraction also expresses high levels of the myeloid marker CD11b and antigen presenting molecules MHCII and MHCI by flow cytometry, suggesting these induced CD45+ cells are myeloid lineage APCs (iAPC). These iAPC exhibit phagocytic property, tested by incubating pHrodo Red bioparticles conjugated with Zymosan, an antigen found on the surface of fungi. More importantly, these induced iAPC appear to have lost their proliferative capacity characteristic of the parental GBM cells. Total live cell numbers were significantly reduced in 4F-induced culture compared to the EV control. In conclusion, we successfully transdifferentiated mouse GBM cells into APC-like cells based on *NETZEN* prediction and our work can potentially provide a novel therapeutic approach for developing an in-situ APC vaccine immunotherapy for GBM, and for other cancers.

IMMU-38. PRECLINICAL VALIDATION AND MANUFACTURE OF IL-8 RECEPTOR-MODIFIED CD70 CAR T CELLS FOR CLINICAL TRIAL

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BACKGROUND: Chimeric antigen receptor (CAR) modified T cell therapy is a promising treatment strategy for cancer therapy. We developed IL-8 receptor-linked CD70CAR (8R-70CAR), aiming to enhance T-cell intratumoral trafficking and persistence, guided by IL-8-secreting tumors. The preclinical results show that 8R-70CAR T cells induce complete tumor regression and long-lasting antitumor immunity, which necessitate further clinical development of this technology. **OBJECTIVE:** To assess the toxicity of 8R-70CAR T cells, evaluate *in vitro* tumor recognition and expansion of the 8R-70CAR T cells, and develop clinically compliant SOPs for the CAR T cell manufacture. **METHODS:** The m8R-70CAR (mouse), h8R-70CAR (human), and control CAR were constructed. **Toxicology:** C57BL/6J mice were intravenously injected with or without 2×10^6 m8R-70CAR T cells after total body irradiation (5Gy). The body weight, clinical signs, hematology, serum chemistry, serum cytokines, gross pathology, and histopathology of 12 vital organs were evaluated. **Tumor recognition:** T cells derived from GBM patients were transduced with h8R-70CAR and then co-cultured with CD70+ glioblastoma. INF-g production was measured. **Ex vivo expansion:** The G-Rex system was utilized for CAR T cell production, and the expansion of h8R-70CAR T cells was assessed by cell count. **RESULTS:** No CAR T cell-related toxicity was observed in mouse study. In addition to a robust antitumor reactivity, the h8R-70CAR T cells present an auto-stimulative property that elicits greater *ex vivo* expansion compared to other CAR T cells tested. An approximately 1200-fold expansion was achieved during the 2 weeks CAR T cell production without additional stimulation, which allows production of the CAR T cells on a clinical trial scale using < 100mL of whole blood. **CONCLUSION:** The 8R-70CAR T cells are effective, auto-stimulative, and safe. These properties are fundamental for successful clinical applications in cancer treatment. A phase I trial using the 8R-70CAR T cells in newly diagnosed GBM will be initiated soon.

IMMU-39. TIM-3 APTAMER IN COMBINATION WITH RADIOTHERAPY RESULTS IN ENHANCED SURVIVAL IN DIPG MODELS

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Pediatric diffuse midline gliomas-H3-K27M-mutant are aggressive brain tumours that arise during childhood. Despite new advances in genomic knowledge and the significant number of clinical trial testing new targeted therapies, patient outcome is still insufficient. Cancer immunotherapy is opening new therapeutic options representing a hope for this orphan disease. Aptamers are single-stranded nucleic acid ligands design to achieve a remarkable affinity and specificity to their targets, comparable to antibodies. TIM-3, is a potential immune checkpoint target, typically involved in T-cell exhaustion. Recent studies showed that TIM-3 is also expressed in tumour and glial cells and it plays an important role in brain tumour responses mediated by myeloid cells. In this work, we examined the anti-tumour effect of an aptamer against TIM-3 alone or in combination with radiotherapy. Of importance, we tested TIM-3 aptamer in a murine glioma and DIPG model, where we not observed any toxicity. TIM-3 administration increased overall survival but was unable to control the disease. Of importance, TIM-3 combination with radiotherapy improved the overall survival of treated mice when compared with single treatments leading to 50% of long-term survivors. TIM-3 aptamer administration increase T-infiltration in the tumour

site compared to non-treated or library control. Mechanistic studies performed on day 16 showed an increase in CD8 effector cells, a decrease in T-regulators Foxp3+ cells and an increase in IFN-gamma expression suggesting the triggering of an antitumor-immune response. Rechallenge experiments demonstrated immune memory in the long-term responders that led to reject tumour re-implantation, confirming that TIM-3 aptamer treatment in combination with RT elicits specific antitumor immunity in mouse glioma models. These results suggest that immuno-therapies approaches in combination with radiotherapy would be worth exploring in the treatment of deadly DMG-H3K27-Mutant tumours.

IMMU-40. IMPROVING OUTCOMES IN OLDER ADULTS WITH GLIOBLASTOMA BY REVERSING AGE-RELATED CHANGES OF THE CENTRAL NERVOUS SYSTEM

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De novo glioblastoma (GBM) represents $\geq 90\%$ of all GBM diagnoses and has a median age of onset at 65 years old. Because of its preponderance during advanced age, we investigated the mortality rate of older adult C57BL/6 (WT) mice with CT-2A glioma. Strikingly, we discovered a significant decrease of overall survival among animal subjects depleted for both CD4+ and CD8+ T cells as compared to the group treated with IgG control antibodies. This negative effect of lymphopenia only applied to older adults. We next questioned which of the major T cell subsets contribute to this effect and determined that the depletion of CD4+ T cells significantly decreased overall survival as compared to IgG control or CD8+ T cell-depleting antibodies (n=12/group; $p < 0.01$). We previously found increased immunosuppressive IDO levels in the mouse and human brain during advanced age. Although 78–86 week old WT mice have no survival benefit after triple combination immunotherapy, age-matched IDOKO mice have an impressive increase in median and overall survival – despite both groups being treated with a pharmacologic IDO enzyme inhibitor. Since IDO is potentially induced by proinflammatory cytokines, we hypothesized that a potential mechanism for the increased IDO during advanced age is associated with an accumulation of senescent cells in the brain with a proinflammatory secretory phenotype. To investigate this, 80 week old mice with intracranial GL261 were treated with (i) vehicle with IgG antibodies, (ii) the senolytic drugs dasatanib and quercetin, (iii) whole brain radiation, anti-PD-1 mAb, and IDO enzyme inhibitor, or (iv) the 5 agent combination. Only animal subjects treated with the 5 agent cocktail showed a significant increase in long-term survival in a subset of mice (n=12–15/group; $p < 0.01$). These results suggest that optimization of senescent cell eradication treatment may be particularly beneficial to older patients with GBM treated with immunotherapy.

IMMU-41. HIGH-THROUGHPUT RETRIEVAL OF THERAPEUTIC T CELL RECEPTORS FROM GLIOMA

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Gliomas are tumors with low mutational burden with the majority of them being resistant to checkpoint inhibition due to few immunogenic antigens. Multicenter vaccine trials targeting personalized neoantigens in gliomas demonstrated feasibility and illustrated the challenges of retrieving neopeptide-specific T cells based on the prediction of immunogenic neopeptides. Here we took an entirely different T cell-centric approach and established a single cell sequencing-based high-throughput T cell receptor (TCR) retrieval platform, exploiting the therapeutic potential of spontaneous intratumoral T cell clonotypes for the development of adoptive cell therapy. We conducted direct *ex vivo* TCR single cell sequencing from freshly sorted human glioma-infiltrating T cell samples. High fidelity PCR was established to clone TCRs from single cell libraries directly into episomal expression vectors further optimized for T cell therapy. In parallel to standard therapy, patient-derived xenografts were developed and characterized. Tumor-reactivity of retrieved TCRs was demonstrated against patient-derived cell lines. Collectively, we provide a novel sequencing-based platform for high-throughput identification and validation of endogenous glioma-targeting TCRs and demonstrate their therapeutic applicability.

IMMU-42. DUAL ACTIVATION OF THE CGAS-STING PATHWAY AND AIM2-INDUCED PYROPTOSIS BY TUMOR-TREATING FIELDS PRODUCES ANTI-TUMOR IMMUNITY IN GLIOBLASTOMA

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OBJECTIVES: Tumor Treating Fields (TTFields) was approved in combination with adjuvant temozolomide chemotherapy for newly diagnosed