template copy number from RNA data owing to B cell subtype specific variation in the expression of the B cell receptor. Hypothetically, however, RNA input based monitoring could be advantageous both owing to reduced input requirements and superior ability to detect B call malignancies of plasmablast and plasma cell origin, where the BCR is robustly expressed. Here we compared the ability of RNA and DNA based IGH chain sequencing to detect Burkitt's Lymphoma cell lines and Chronic Lymphocytic Leukemia samples at a frequency of 10^{-6} from peripheral blood.

Materials and Methods Here we present performance for rare clone detection utilizing the Ion Oncomine™ BCR IGH-SR assay and the Ion Oncomine™ BCR IGH-LR assay. These assays use multiplex primers targeting all known IGH germline variable genes in the framework 1 (FR1) or framework 3 (FR3) regions of the B cell receptor using either DNA or RNA as input. To evaluate detection sensitivity of the IGH-SR assay we utilized DNA or RNA from Burkitt's lymphoma cell lines as well as clinical chronic lymphocytic leukemia (CLL) samples controllably added to a background of peripheral blood leukocytes (PBL) by mass ratio to create specimens with a known target B cell frequency. Automated downsampling analysis was used to confirm libraries were sequenced to saturation. Library preparation and analysis was performed in replicate to quantify sensitivity of detection.

Results For each cell line, we prepared and sequenced (1) 30 libraries derived from amplification of 2ug gDNA spiked with 2pg cell line gDNA and (2) 10 libraries derived from amplification of 100ng RNA spiked with 0.1pg cell line total RNA. The Burkitt's lymphoma cell line and CLL samples were detected in 10/30 and 8/30 libraries respectively, consistent with the performance of orthologous DNA-based sequencing approaches. For RNA libraries, the Burkitt's lymphoma and CLL samples were detected in each library (10/10 and 10/10, respectively).

Conclusions Here we demonstrate the ability to detect B cell clones down to 10^{-6} from gDNA and RNA inputs utilizing the Ion Oncomine™ BCR IGH-SR assay. Feasibility for rare clone detection is shown in gDNA or RNA enabling B cell minimal residual disease research, and high sensitivity characterization the B cell role in response to checkpoint blockade within the tumor microenvironment. Importantly, we find that RNA based IGH sequencing may significantly reduce input requirements for rare clone detection, potentially enabling routine detection of clones at 10^{-6} frequency from a single library.

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P05.02 AN INTEGRATED VIRTUAL TISSUE PLATFORM FOR INCORPORATING EXERCISE ONCOLOGY INTO IMMUNOTHERAPY

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We introduce a novel in silico platform for simulating solid tumor growth and anti-tumor immune response. We present the model, test the sensitivity and robustness of its parameters, and calibrate it with pre-clinical and clinical data from exercise oncology experiments which offer a natural biological backdrop for modulation of anti-tumor immune response. We then perform two virtual experiments with the model that demonstrate its usefulness in guiding pre-clinical and clinical studies on immunotherapy. The first virtual experiment probes the intricate dynamics in the tumor microenvironment between the tumor and the infiltrating immune cells. Such dynamics is difficult to probe during a pre-clinical study as it requires significant of redundancy in lab animals and is time and labor intensive. The result is a time series of spatiotemporal observational 'windows' into the tumor microenvironment that can serve as a platform to test several mechanistic hypotheses on the role and dynamics of different immune cells in ant-tumor immune response. The second virtual experiment shows how dosage and frequency of immunotherapy drugs can be optimized based on the aerobic fitness of the patient, so that possible adverse side effects of the treatment can be minimized.

Disclosure Information A. Hagar: None. **J. Aponte Serrano:** None.

P06 Cell Therapy in Solid Tumors

P06.01 BISPECIFIC ANTIBODY-DRIVEN SYNTHETIC AGONISTIC RECEPTOR – TRANSDUCED T CELLS MEDIATE SPECIFIC AND CONDITIONAL THERAPY IN MELANOMA CANCER MODELS

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Background Immunotherapeutic approaches, including immune checkpoint blockade and adoptive T cell therapy (ACT) in the form of tumor-infiltrating lymphocytes (TILs), have had marked success in the treatment of melanoma. Despite these successes, many patients are refractory to treatment or relapse with therapy-resistant disease. To overcome these limitations, we propose a controlled ACT approach, where T cells are armed with synthetic agonistic receptors (SARs) that are conditionally activated only in the presence of a target melanoma-associated antigen, and a cross-linking bispecific antibody (BiAb) specific for both (SAR) T cell and tumour cell.

Materials and Methods A SAR composed of an extracellular EGFRvIII, trans- membrane CD28, and intracellular CD28 and CD3z domains was fused via overlap- extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. We validated our approach in two murine as well as two human cancer models expressing our melanoma-associated target antigens TYRP (murine) and MCSP (human). We confirmed conditional and specific stimulation and proliferation of our T cells, as well as their tumour-antigen-directed cytotoxicity, *in vitro* and *in vivo*.

Results Crosslinking TYRP-EGFRvIII (murine) and MCSP-EGFRvIII (human) BiAb, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation

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of SAR-T cells and directed tumour cell lysis with specificity towards two TYRP-expressing murine melanoma and two MCSP-expressing human melanoma cancer models. *In vivo*, anti-tumoural activity was mediated by the co-administration of SAR-T cells and BiAb, in an A375 melanoma xenograft model. Further, overexpression of IDO (a key immunosuppressive enzyme implicated in the suppression of T cell function in the tumor microenvironment) in a melanoma model did not influence the killing kinetics of SAR T cells.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of synthetic agonistic receptor transduced T cells, and targeted tumour cell lysis. The modularity of our platform is key for a targeting approach in a tumor entity with a high mutational load such as melanoma and is fundamental in our drive towards personalised immunotherapies. Further, the SAR approach has demonstrated resistance to IDO-mediated inhibition in the context of melanoma, an interesting axis that requires further investigation.

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P06.02 ENHANCING CAR T CELL PERSISTENCE AND MEMORY THROUGH MODULATING MITOCHONDRIAL FUNCTION

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Background CAR T cell therapy for solid tumours has achieved limited success compared to its application to B cell malignancies. One reason for this failure is the low differentiation rate to memory subsets and low persistence of CAR T cells due to activation-induced cell death (AICD) in lymphoid tissue and the tumour microenvironment. In this study, we have expressed the MCL1 gene within CAR T cells to overcome losses by AICD in adoptively transferred T cells. The MCL1 gene expresses two isoforms; the long isoform localises to the outer membrane of mitochondria and inhibits the CD95 signalling death pathway, while the short isoform localises to the inner membrane of mitochondria to enhance mitochondrial oxidation, phosphorylation and fusion. In addition, we have also utilized a microRNA (miR) 429 to promote memory T cell formation through the suppression of genes such as T-cell-restricted intracellular antigen-1 (TIA-1), T cell activation inhibitor, mitochondrial (TCAIM) and mitochondrial fission factor (MFF).

Materials and Methods Overexpression of MCL1 was confirmed at both mRNA and protein level by real time RT-PCR (qPCR) and western blot. Similarly, overexpression of miR-429 was measured by qPCR and specific binding of miR-429 to the 3′ UTR of target genes was confirmed by luciferase reporter assay. Mitochondrial depolarization and cell viability were assessed by TMRE mitochondrial membrane potential assay (flow-cytometry) and resazurin assay. The effect of MCL1 or miR429 overexpression on HER2-CAR T cells was determined by flow cytometry. Soluble leucine-zipper CD95L (https://www.addgene.org/104349/) was expressed and purified from Expi293 cells.

Results Overexpression of MCL1 in both Jurkat T cells and primary human T cells protected cells against mitochondria depolarization as well as the loss of cell viability in response to CD95L-triggering. Expression of miR429 downregulated TIA1, TCAIM and MFF. A HER2-CAR construct with either MCL1 or miR429 in a lentiviral system was successfully designed and transduced into primary T cells. Mitochondria in transduced T demonstrated enlarged and fusion morphology - a classic feature of memory T cells.

Conclusions Overexpressing MCL1 or miR429 significantly improves mitochondrial function in T cells. This approach will be used to increase persistence of adoptively transferred CAR T cells.

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P06.03 C-C CHEMOKINE RECEPTOR 8 TUMOR-DIRECTED RECRUITMENT ENABLES CAR T CELLS TO REJECT SOLID TUMORS

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Background CAR T cell therapy is remarkably successful in patients with hematological malignancies, in some cases inducing durable remissions. However, it remains ineffective in solid tumors, in part due to poor T cell infiltration into the tumor mass. Determinants of successful T cell infiltration to the tumor site remain to be defined. In contrast, tumors actively attract T regulatory (T_{reg}) cells for immune suppression through the C-C chemokine receptor 8 (CCR8) - CCL1 axis. As this axis is functional across cancer entities, we postulated that CCR8 could also be used to target tumor-ablating T cells to the tumor site.

Material and methods Murine and human CCR8 have been cloned in a retroviral expression vector. CCR8 can be expressed in murine and human T cells upon transduction. A chimeric antigen receptor (CAR) targeting the murine epithelial cell adhesion molecule (EpCAM) was used for syngeneic pancreatic tumor models and a CAR targeting human mesothelin was used for a xenograft pancreatic tumor model.Mechanistically, we use flow cytometry and multi-photon intra-vital microscopy to interrogate infiltration of CCR8-transduced CAR T cells.

Results Here we show that genetically engineering CAR T cells to express CCR8 improves their migration into solid tumors and allows rejection of tumors that are otherwise resistant to CAR T cell therapy. We demonstrate the capacity of these enhanced CAR T cells to stunt solid tumor growth and improve survival in both murine syngeneic and human xenograft tumor models.

Conclusion Our results demonstrate the viability of using CCR8 to confer T_{reg}cell trafficking-properties in CAR T cells to enable their effectiveness in solid tumors. This receptor may be combined with other promising strategies to improve the efficacy of cellular approaches.

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