than traditional T cells in the host. We explored the characteristics of CpG-expanded T cells and found that T cells generated from a CpG culture incur a unique proteomic and cell surface signature phenotype. Of all the cell types present in the starting culture (CD4+ T cells, NK cells, B cells, DCs, macrophages), B cells were the only cell type critical to achieve a more potent T cell therapy with CpG. In a direct comparison of CpG class A (targeting DCs) and CpG class B (targeting B cells), only the B cell-activating CpG improved cell therapy. Finally, we found that B cells alone could improve purified CD8+ T cells for ACT when the co-culture was activated with CpG, indicating that B cells become potent APCs in this context.

Conclusions Collectively, our findings indicate a novel way to use TLR agonists to improve ACT and reveal a critical role for B cells in the expansion of potent anti-tumor CD8+ T cells. Translating these findings to ACT therapies could provide dramatic improvements in patients with late stage malignancies.

Acknowledgements Proteomic analysis was performed at the Mass Spectrometry Facility, a University Shared Research Resource at the Medical University of South Carolina, using instrumentation acquired through the NIH shared instrumentation grant program (S10 OD010731-Orbitrap Elite Mass Spectrometer or Orbitrap Fusion Lumos ETD/UVD MS (S10 OD025126).

Trial Registration NA

Ethics Approval All animal procedures were approved by the Institutional Animal Care & Use Committee of the Medical University of South Carolina, protocol number 0488.

Consent NA

REFERENCES
1. NA

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161 DEVELOPMENT OF A CD8 CO-RECEPTOR INDEPENDENT T CELL RECEPTOR SPECIFIC FOR TUMOR-ASSOCIATED ANTIGEN MAGE-A4 FOR NEXT GENERATION T CELL-BASED IMMUNOTHERAPY

Background The cancer-testis antigen MAGE-A4 is an attractive target for T cell-based immunotherapy, especially for indications with unmet clinical need like non-small-cell lung carcinoma or triple-negative breast cancer. Overcoming high tumor burden using adoptive transfer of T cells modified to express a transgenic T cell receptor (TCR) demands optimal recognition of the corresponding target on tumor cells by the TCR-modified T cells (TCR-Ts). Here we describe the isolation and pre-clinical characterization of high avidity TCR-Ts expressing a human leucocyte antigen (HLA)-A*02:01-restricted MAGE-A4-specific TCR that is fully functional in T cells irrespective of CD4 or CD8 co-receptor expression.

Methods An unbiased CD137-based sorting approach was first used to identify an immunogenic MAGE-A4-derived candidate epitope that was properly processed and presented on HLA-A2 molecules encoded by the HLA-A*02:01 allele. To isolate high avidity T cells via subsequent multimer sorting, an in vitro priming approach using HLA-A2-negative donors (allogenic-HLA-restricted priming approach) was conducted to bypass central tolerance to this self-antigen. Pre-clinical parameters of safety and activity were assessed in a comprehensive set of in vitro and in vivo studies of the lead TCR candidate derived from a selected T cell clone.

Results A TCR recognizing the MAGE-A4-derived decapeptide GVYDREHTV was isolated from primed T cells of a non-tolerant HLA-A2-negative donor. The respective TCR-T cell product bbT485, expressing the lead TCR in T cells from healthy donors, was demonstrated pre-clinically to have a favorable safety profile and superior in vivo potency compared to TCR-Ts made using a TCR derived from an HLA-A2-positive donor bearing a tolerated T cell repertoire to self-antigens. The natural high avidity allogenic (allo)-derived TCR was found to be CD8 co-receptor-independent, allowing effector functions to be elicited in transgenic CD4+ T helper cells. These CD4+ TCR-T cells not only supported an anti-tumor response by direct killing of MAGE-A4-positive tumor cells, but also upregulated hallmarks associated with helper function, such as CD154 expression and release of key cytokines upon tumor-specific stimulation.

Conclusions The extensive pre-clinical assessment of safety and in vivo potency of this non-mutated high avidity, CD8 co-receptor-independent, MAGE-A4-specific HLA-A2 restricted TCR provide the basis for its use in clinical TCR-T immunotherapy studies. The ability of this co-receptor-independent TCR to activate all transduced T cells (irrespective of CD4 or CD8 expression) could potentially provide enhanced cellular responses in the clinical setting through the induction of functionally diverse T cell subsets that goes beyond what is currently tested in the clinic.

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163 NICE: NEOANTIGEN-CYTOKINE-CHEMOKINE MULTIFUNCTIONAL ENGAGER FOR NK CELL IMMUNOTHERAPY OF SOLID TUMORS
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Background The effectiveness of natural killer cell-based immunotherapy against solid tumors is limited by the lack of specific antigens and the immunosuppressive tumor microenvironment. To improve the clinical efficacy and specificity of NK cell therapy, we are designing, developing, and characterizing a new generation of multi-specific killer engagers, which consists of a neoantigen-targeting moiety, together with cytokine and chemokine-producing domains.

Methods Targeting a neoantigen-an antigen formed specifically in response to tumor genome mutations-enables substantially enhanced tumor specificity to be achieved. We evaluated the responsiveness of NK cells to Wilms Tumor 1 (WT1) antigen in GBM by synthesizing an antibody that is able to recognize the WT1/HLA complex. Incorporation of cytokine (namely IL-2, IL-15, and IL-21)-essential for the maturation, persistence, and expansion of NK cells in vivo-favors the proliferation and survival of NK cells in the tumor microenvironment, thereby leading to more sustained anti-tumor responses. Additionally, our data have indicated that the chemokine CXCL10 plays an important role in the infiltration of immune cells into GBM, yet the chemokine itself is expressed at low levels in GBM. Incorporation of a CXCL10-producing element into our
construct further supports NK cell recruitment and may stimulate the recruitment of other immune cells. NK activation through the tri-specific engager is achieved through NKP46-mediated signaling. We are investigating the ability of the tri-functional engager to support and enhance NK cell-mediated cytotoxicity against GBM in vitro and in patient-derived GBM xenografts in vivo.

Results We hypothesize that taking advantage of our multi-functional engager, NK cells will exhibit, at once, superior persistence, infiltration and antitumor activity, simultaneously addressing three of the main limitations to the use of NK cells in immunotherapy of GBM and other solid tumors.

Conclusions N/A

Acknowledgements N/A

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**Abstracts**

**164 AGENT-797, A NOVEL ALLOGENIC AND ‘OFF-THE SHELF’ iNKT CELL THERAPY PROMOTES EFFECTIVE TUMOR KILLING**

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**Background** Harnessing both the innate and adaptive immune system could increase the efficiency of current cancer immunotherapies and promote durable anti-tumor immunity. Invariant natural killer T (iNKT) cells are innate-like lymphocytes that bridge innate and adaptive immune responses and promote anti-cancer immunity. iNKT cells are activated and respond rapidly via multiple signals such as recognition of lipid antigens through the invariant T cell receptor (TCR), pro-inflammatory cytokines or recognition of stress ligands. Here we describe, AgenT-797, a novel, allogeneic and ‘off-the-shelf’ iNKT cell therapy, designed to promote effective anti-cancer immunity against a wide range of malignancies.

**Methods** iNKT cells isolated from healthy donors were expanded by stimulation of the invariant TCR with alpha-Galactosylceramide (αGalCer) and cytokines using the AgenTus manufacturing protocol. The phenotype and functional activity of the expanded unmodified iNKT cells, AgenT-797, were characterized by flow cytometry. The cytotoxic potential of AgenT-797 was assessed in tumor co-culture assays against CD1d-expressing cancer cell lines. To further direct anti-tumor responses, iNKT cells were engineered to express Chimeric Antigen Receptors (CARs), and the cytotoxic potential assessed against antigen-expressing cancer cells.

**Results** iNKT cells were rapidly expanded up to 2 × 1010 cells in 30 days, with over 99% purity. Expanded, unmodified iNKT cells, AgenT-797, were found to secrete both Th1 (IFNγ, TNFa, GM-CSF) and Th2 (IL4, IL13) type cytokines. After rapid expansion, AgenT-797, retained their inherent cytotoxic capacity against CD1d-expressing tumor cell lines. Further, killing of tumor target cells, in vitro, was mediated through their endogenous invariant TCR or engineered CAR receptor.

**Conclusions** AgenT-797 is an ‘off-the-shelf’ and allogenic cell therapy with effective cancer killing properties. Strategies to engineer iNKT cells using CAR technology further enhance the tumor killing potential of iNKT therapy.

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**165 ACTIVATING ANTIGEN CARRIERS GENERATED WITH MICROFLUIDICS CELL SQUEEZING DRIVE EFFECTIVE ANTI-TUMOR RESPONSES**

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**Background** Activation of T cell responses is essential for effective tumor clearance, however generating targeted, effective antigen presentation to stimulate T cell response remains challenging. We can harness the natural process of red blood cell (RBC) clearance from the body to activate the antigen-specific immune responses. Using the Cell Squeeze® microfluidics platform, we generate activating antigen carriers (AACs) from RBCs to drive antigen presentation and T cell activation in human and murine models.

**Methods** We loaded proteins or synthetic long peptide antigens together with adjuvants into murine or human RBCs with Cell Squeeze® (SQZ’ing) to generate AACs and investigated the effects of SQZ’ing on the RBC membrane. Following intravenous AAC injection into mice, we measured AAC clearance kinetics and characterized the site and cell type of AAC uptake. We investigated the regulation of activation markers on phagocytes that engulf AACs, clearance of endogenous RBCs, and the effect of boosting with AACs on endogenous T cell responses. To determine the ability of AACs to control subcutaneously implanted tumors, we measured tumor growth rates in mice therapeutically treated and boosted with AACs. Finally, we observed in vitro uptake of human AACs loaded with adjuvant and resultant maturation of monocyte-derived dendritic cells (MOs) to qualify adjuvant delivery. Peptide antigen delivery to human AACs was measured with flow cytometry and fluorescence microscopy.

**Results** We demonstrated that SQZ’ing effectively loads AACs without reducing CD47 expression. When administered into a mouse, AACs were cleared from circulation within one hour and were engulfed by professional phagocytes in both the spleen and liver. In vivo, AACs upregulated activation markers on macrophages and DCs, and administration of AACs does not affect clearance or half-life of endogenous RBCs. Therapeutic AAC administration to mice strongly impedes tumor growth and extends survival; the anti-tumor responses correlate with >10x increase in antigen-specific CD8+ tumor-infiltrating lymphocytes compared to untreated mice. Boosting enhances endogenous T cell responses and boosting at early time points in the tumor model enhances low dose vaccinations. In an in vitro human system, we demonstrated that human AACs can be loaded with peptide antigen and adjuvant such that upon engulfment, AACs stimulated MOs maturation.

**Conclusions** In summary, these results indicate that AACs loaded with antigen and adjuvant can effectively drive antigen presentation and prime a potent anti-tumor response in mice. These data support the further study of SQZ AACs as an immunotherapy for cancer treatment.

**Ethics Approval** All methods were performed in accordance with the relevant guidelines and regulations. Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at SQZ Biotechnologies, using the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Office of Laboratory Animal Welfare. All activities were also