protocols used to expand T cells often exhibit suboptimal tumor control. Failure in these therapies has been attributed to premature differentiation and impaired metabolism of the infused T cells. Previous work done in our lab showed that reduced PI3Kδ signaling improved ACT. Because PI3Kγ and PI3Kδ have critical regulatory roles in T cell differentiation and function, we tested whether inhibiting PI3Kγ could recapitulate or synergize PI3Kδ blockade.

Methods To test this, we primed melanoma specific CD8+ pmel-1 T cells, which are specific to the glycoprotein 100 epitope, in the presence of PI3Kγ (IPI-459), PI3Kδ (CAL101 or TGR-1202) or PI3Kγ/δ (IPI-145) inhibitors following antigen stimulation with hgp100, and then infused them into 5Gy total body irradiated B16F10 tumor bearing mice. We characterized the phenotype of the transferred product by flow cytometry and then assessed their tumor control by measuring the tumor area every other day with clippers. For metabolic assays we utilized the 2-NBDG glucose uptake dye and the real time energy flux analysis by seahorse.

Results Sole inhibition of PI3Kδ or PI3Kγ in vitro promoted greater tumor immunity and survival compared to dual inhibition. To understand how PI3Kδ or PI3Kγ blockade improved T cell therapy, we assessed their phenotype. CAL101 treatment produced more CD62LhiCD44lo T cells compared to IPI-459, while TGR-1202 enriched mostly CD62LhiCD44hi T cells. Because decreased T cell differentiation is associated with mitochondrial metabolism, we focused on CAL101 treated T cells to study their metabolism. We found that CAL101 decreased glucose uptake and increased mitochondrial respiration in vitro, indicating augmented mitochondrial function.

Conclusions These findings indicate that blocking PI3Kδ is sufficient to mediate lasting tumor immunity of adoptively transferred T cells by preventing premature differentiation and improving mitochondrial fitness. Our data suggest that addition of CAL101 to ACT expansion protocols could greatly improve T cell therapies for solid tumors by preventing T cell differentiation and improving mitochondrial function.

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159 ADOPTIVELY TRANSFERRED CD8+ T CELLS THAT TARGET NEOANTIGEN PERSIST AND REGRESS MELANOMAS TO A GREATER EXTENT THAN THOSE THAT TARGET SELF/TUMOR-ANTIGEN

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Background In patients treated with immunotherapy, mechanisms underlying why some respond to or fail treatment are not fully understood. Higher tumor mutational burden is often correlated with better responses, because the immune system reacts more strongly against mutated antigens (versus self antigens) due to a higher affinity interaction with the T cell receptor. In adoptive T cell transfer therapy (ACT), engraftment and persistence of the T cells are critical to prolonged antitumor responses. It remains unclear whether the affinity of the interaction between tumor antigen and TCR alone impacts the engraftment and persistence of tumor-specific T cells post ACT.

Methods To simulate this clinical scenario in mice, we used two different melanoma models: 1) B16F10 expressing a low affinity peptide (mgp100 = i.e. tumor/self-antigen), or 2) B16F10 expressing a higher affinity peptide (hgp100), which represents a neoantigen-expressing tumor.[1] Pmel-1 CD8+ T cells expressing a TCR that recognizes gp100 were adoptively transferred into mice bearing B16F10 melanoma.

Results We posited that the function and persistence of adoptively transferred pmel-1 T cells would be increased in mice with neoantigen- compared to self- antigen expressing tumors. Indeed, we found that pmel-1 were less exhausted as well as engrafted and persisted far better in mice bearing tumors expressing neoantigens. Moreover, these large subcutaneous hot tumors shrank post ACT treatment and the animals survived long-term. Beneficial outcome was correlated with the appearance of vitiligo. Importantly, these cured mice were protected when rechallenged with a secondary tumor even after an intravenous rechallenge, implicating this ACT treatment mediates durable memory responses.

Conclusions Herein, we underscore how tumor antigen affinity can drastically change T cell fate. Future work will concentrate in exploring in depth the correlation of less differentiated cytotoxic T cells treating neo/self-antigen expressing melanomas mimicking a clinical setting.

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160 TLR9-ACTIVATED B CELLS IMPRINT ADOPTIVELY TRANSFERRED CD8+ T CELLS WITH POTENT TUMOR IMMUNITY AND PERSISTENCE IN VIVO

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Background Compared to traditional therapies for advanced malignancies, adoptive T cell transfer (ACT) therapy has increased the number of patients who achieve complete regressions; however, only about 20% of patients achieve lasting progression-free survival. Thus, more potent cell therapies for cancer are urgently needed. Preconditioning patients with chemo- or radiotherapy prior to cell transfer provides several benefits to the transferred T cells. One of these benefits is activation of the host immune system Toll-like receptors (TLRs) via microbes leaked from the injured gut. Direct administration of TLR agonists has been used in numerous preclinical and clinical trial settings, but has shown toxicity and limited success in promoting tumor immunity in patients. We hypothesized that TLR agonists could be used in an alternative way – in the ex vivo propagation of potent T cells for ACT.

Methods To test our hypothesis, we employed the pmel-1 ACT model, where all CD8+ T cells express a transgenic TCR which recognizes the gp100 epitope expressed by melanoma. To determine if TLR agonists could improve cell therapy, we activated CD8+ T cells in the presence of APCs and the TLR9 agonist, CpG, and transferred T cells to B16F10 melanoma-bearing mice.

Results Pmel-1 CD8+ T cell products expanded with CpG elicited potent anti-melanoma immunity in vivo and improved survival compared to traditionally expanded T cell therapy. CpG-derived T cells engrafted robustly and persisted longer

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.

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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

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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

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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 (allogeneic-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 GVYDGREHTV 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 tolerized T cell repertoire to self-antigens. The natural high avidity allogeneic (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