Adoptively transferred CD8+ T cells that TLR9-activated B cells imprint adoptively transferred CD8+ T cells with potent tumor immunity and persistence in vivo

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), 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 PI3Kδ 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 PI3Kγδ (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 last 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.

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 B16F10 expressing a higher affinity peptide (hgp100), which represents a neoantigen-expressing tumor. 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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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. Pmel-1 CD8+ T cells expressing a TCR that recognizes gp100 were adoptively transferred into mice bearing B16F10 melanoma.