POTENT INHIBITION OF PI3Kδ PROMOTES ENHANCED T CELL STEMNESS AND MITOCHONDRIAL FITNESS

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Background Durable responses have been observed with adoptive T cell therapy (ACT) in chemotherapy and immunotherapy refractory patients. However, current T cell products do not always lead to therapeutic responses. Therapy failure has been attributed to terminally-differentiated T cell profiles, diminished host engraftment and poor mitochondrial metabolism. T cell activation and a switch towards glycolysis is regulated by signals downstream of the PI3K signaling pathway. We hypothesized that by inhibiting the T cell specific PI3Kδ subunit, that we would prevent overt T cell differentiation, enhance mitochondrial metabolism and improve anti-tumor immunity of adoptively transferred T cells.

Methods To test this, we primed melanoma-specific CD8+ pmel-1 T cells in the presence of increasing concentrations of CAL-101, a PI3Kδ specific inhibitor, and infused them into B16F10 tumor-bearing mice, following non-myeloablative total body irradiation. In vitro we tested how PI3Kδ inhibition altered T cell stemness features by flow cytometry and RNA sequencing. Further, we tested the ability of PI3Kδ inhibited T cells to survive against antigen rechallenge, and assessed how mitochondrial phenotypes changed using dyes indicating mass, membrane potential and reactive oxygen species. Moreover, we tested how real time mitochondrial respiration was changed with PI3Kδ inhibition via Seahorse.

Results Potent inhibition of PI3Kδ in vitro enhanced tumor immunity and survival in a dose dependent manner. High doses of CAL-101 enriched T cells with phenotypic and transcriptional signatures of stemness. We found that CAL-101 decreased glucose uptake and increased mitochondrial mass and membrane potential while reducing mitochondrial reactive oxygen species. Potent inhibition of PI3Kδ enhanced mitochondrial respiration, however gene expression of fatty acid oxidation was not enriched, suggesting an alternative pathway involved in the enhanced spare respiratory capacity of CAL-101 treated T cells.

Conclusions These findings indicate that blocking PI3Kδ is sufficient to mediate lasting tumor immunity of adoptively transferred T cells by preserving stemness features and improving mitochondrial fitness. Our data suggest that addition of CAL-101 to ACT expansion protocols could greatly improve responses to solid tumors by rewiring T cell stemness and promoting mitochondrial fitness.

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Ethics Approval All animal procedures performed at the Medical University of South Carolina or Emory University were approved by each university's Institutional Animal Care & Use Committee, protocol number 0488 or 201900225, respectively

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