407 MITOCHONDRIAL TRANSCRIPTION IS REQUIRED FOR THE ENHANCED ANTI-TUMOR ACTIVITY OF ADOPTIVELY TRANSFERRED STEM-LIKE MEMORY T CELLS

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Background Durable responses have been observed with adoptive T cell therapy in chemotherapy and immunotherapy refractory patients. However, current T cell products do not always lead to therapeutic responses. T cell intrinsic factors that lead to failure in immunotherapy have been attributed to T cell exhaustion and poor mitochondrial quality. T cell mediated immunity can be impaired by overt PI3Kδ signaling through altering transcriptional rewiring and metabolism. Recently, chronic PI3K signaling is associated with loss of mitochondrial transcription and mitochondrial DNA. We hypothesized that PI3Kδ inhibition would generate stem-like memory T cells (T_{scm}) that provide protection against melanoma by sustaining stemness and enhancing mitochondrial fitness.

Methods To test this, we primed melanoma specific $CD8^+$ pmel-1 T cells in the presence of increasing concentrations of Idelalisib, a PI3Kδ specific inhibitor, and infused them into B16F10 tumor bearing, following non-myeloablative total body irradiation. We modeled both high and low immunogenic tumors using two different tumors lines of B16F10 melanoma (expressing low affinity mouse gp100) and a modified B16F10 line (expressing high affinity human gp100 antigen). *In vitro* we tested T cell stemness by flow cytometry and RNA sequencing. We assessed mitochondrial qualities such as mass, membrane potential, reactive oxygen species, and respiratory capacity. Mitochondrial transcription was measured via mitochondrial mRNA relative quantification and protein levels of electron transport proteins. We further tested the effect of ablating mitochondrial transcription by CRISPR knockout using guides against a known regulator of mitochondrial transcription.

Results We found that PI3Kδ inhibited T cells provide potent antitumor activity in both poor and highly immunogenic tumor models of melanoma. The adoptively transferred T cells transcriptionally resemble intratumoral T_{scm} cells. Transcriptionally they showed elevated *Tcf7*, and *Lef1*, and suppressed *Havcr2*, and *Prdm1*. Metrics of improved mitochondrial quality were elevated in a dose dependent manner. Mitochondrially encoded electron transport chain gene expression was selectively enhanced at the RNA and protein level. Ablation of mitochondrial transcription vastly impaired the antitumor activity of stem-like memory T cells generated with PI3Kδ inhibition.

Conclusions These findings indicate that blocking PI3Kδ in T cells mediates lasting tumor immunity of adoptively transferred T cells by preserving stemness features and improving mitochondrial fitness. We discovered that enhanced mitochondrial transcription is required for T_{scm} anti-tumor immunity. These findings suggest that modulating mitochondrial transcription is a potential target to bolster the activity of tumor specific T cells.

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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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