Background: The signaling of key metabolic pathways in T cells within the tumor microenvironment (TME), strongly impacts their antitumor response. Cancer cells compete with T cells for essential nutrients such as glucose and amino acids; in addition, cancer cells produce T-cell inhibitory metabolites such as lactate and adenosine. Therefore, metabolic reprogramming of T cells to confer adaptability to the TME is a promising therapeutic strategy for advanced cancers.

Methods: To identify detrimental metabolic genes for T-cell tumor infiltration, we performed an in vivo shRNA screen with a pooled metabolome library (~300 genes) in Pmel CD8 T cells adoptively transferred into B16 tumor-bearing mice. We identified genes whose disruption in T cells resulted in increased infiltration in B16 melanoma.

Results: We found that disruption of the PDHB gene enhances T-cell tumor infiltration. Pdhb encodes for the E1 Subunit Beta of the Pyruvate Dehydrogenase (PDHB), an enzyme linking glycolysis and the tricarboxylic acid cycle. We validated this result using CRISPR/Cas9 RNP transfection (>90% knockout efficacy) to knockout (KO) Pdhb in Pmel CD8 T cells. Our in vivo studies showed that PDHB KO improves T-cell infiltration and tumor rejection, vs non-targeting control T cells. PDHB KO cells produced higher IFNg levels and displayed improved tumoricidal capacity. The functional metabolomic analyses (seahorse assays) indicated that PDHB KO cells have an impaired mitochondrial function (reduced respiratory and FAO capacities) while having an increased glycolytic activity. The transcriptome and pathway analyses indicated that PDHB disruption, strongly induced lipid biosynthesis. Analyzing the most upregulated metabolites in the PDHB KO cells revealed that inosine, a product of adenosine metabolism, reported to serve as an alternative carbon source for T cells, was within the top 5 upregulated metabolites. The integrated transcriptome, metabolomics and pathway analyses indicated that PDHB KO cells display a metabolic reprogramming with upregulation of unsaturated fatty acid biosynthesis, glycolysis/gluconeogenesis, pyruvate metabolism, chemokine signaling and purine metabolism. Remarkably, the transcriptome analysis suggests that the PDHB KO T cells have an expression pattern of Tscm/Tcm cells, which might be associated with their capacity to survive within tumors.

Conclusions: In tumors characterized by glucose deprivation, the inosine upregulation by PDHB KO T cells might support T-cell function. Overall, our data suggests that PDHB disruption in T cells could contribute improving adoptive cell therapy. Thus, our study indicates that CRISPR-mediated metabolic reprogramming of T cells could be a powerful approach for generating T cells able to overcome tumor-driven metabolic restrictions.

Ethics Approval: Animal protocol was approved by the University of South Florida, IACUC IS00008501.