965 DEFICIENCY OF METABOLIC REGULATOR PKM2 ACTIVATES THE PENTOSE PHOSPHATE PATHWAY TO GENERATE TCF1+ PROGENITOR CD8 T CELLS TO IMPROVE EFFICACY OF PD-1 CHECKPOINT BLOCKADE

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Background TCF1^{high} progenitor CD8+ T cells have been shown to mediate the efficacy of PD-1 checkpoint blockade.[1-3] However, the mechanisms that govern generation of TCF1^{high} cells are poorly understood.

Methods We sequenced bulk RNA from tumor-infiltrating lymphocytes to identify differentially expressed genes based on tumor progression. We utilized *in vitro* co-cultures of tumor-specific T cells tumor cells to examine differentiation, effector function, and metabolism of T cells with different genetic and pharmacologic manipulations by flow cytometry, metabolic flux analyses, and metabolomic profiling. We performed *in vivo* adoptive transfers of control and genetically manipulated tumor-specific T cells into tumor-bearing mice from both a non-small cell lung cancer and a melanoma model to examine effects of genetic manipulation on differentiation and effector function, as well as determine tumor burden and overall mouse survival both in the treatment-naïve and anti-PD-1 treated contexts.

Results RNA Sequencing demonstrated a metabolically active response in tumor-infiltrating CD8+ T cells isolated from large and late-stage tumors. Using a genetic screen targeting glycolytic enzymes up-regulated in tumor-infiltrating CD8+ T cells, we demonstrate that PKM2 deficiency (PKM2[KO]) enriched for TCF1^{high} progenitor cells (figure 1). Antigen-specific PKM2[KO] CD8+ T cells from draining lymph nodes and tumors exhibited a central memory-like phenotype (figure 2) with reduced effector cytokine production, increased CD44/CD62L expression, and increased TCF1 and Eomes in non-small cell lung cancer and melanoma. Adoptive transfer of PKM2[KO] CD8+ T cells in combination with PD-1 blockade impaired tumor growth and improved survival (figure 3). PKM2[KO] CD8+ T cells showed reduced glycolytic flux and accumulation of glycolytic intermediates with concomitant increases in pentose phosphate pathway (PPP) metabolites. Importantly, small molecule agonism of PPP was sufficient to skew activated CD8+ T cells towards the TCF1^{high} population (figure 4).

Conclusions Here we show that targeting glycolytic flux by deletion of pyruvate kinase muscle 2 (PKM2) results in elevated pentose phosphate pathway activity, leading to generation of an altered differentiation state responsive to PD-1 blockade. Our study demonstrates a novel metabolic reprogramming that contributes to a memory-like T cell state amenable to checkpoint blockade.

REFERENCES
1. Escobar G, Mangani D & Anderson AC. T cell factor 1: A master regulator of the T cell response in disease. *Sci Immunol* 2020;**5.**
2. Siddiqui I, *et al*. I Intratumoral Tcf1 + PD-1 + CD8 + T Cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity* 2019;**50**:195–211.e110.
3. Kurtulus S, *et al*. Checkpoint blockade immunotherapy induces dynamic changes in PD-1-CD8+ Tumor-Infiltrating T Cells. *Immunity* 2019;**50**:181–194.e186.
4. Luckey CJ, *et al*. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. *Proc Natl Acad Sci USA* Feb 2006;**28**:103(9):3304–9.

Ethics Approval All animal work was performed in accordance with an animal protocol approved by the institutional Animal Care and Use Committee at Weill Cornell Medical College (Protocol number 0806-762A).
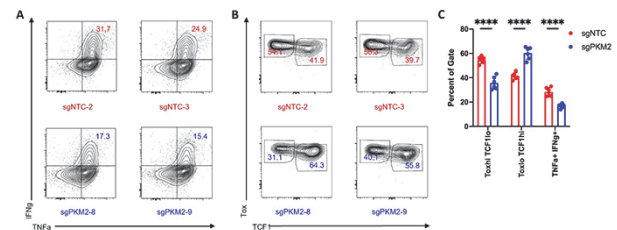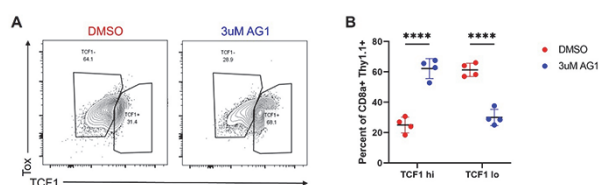

Abstract 965 Figure 1 Flow cytometry analysis of OT-I+ Thy1.1+ T cells co-cultured with HKP1-ova-GFP tumor cells. Activated T cells were electroporated with guides targeting PKM2 (sgPKM2) or non-targeting controls (sgNTC), and co-cultured with tumor cells for 4 days. a, Representative contour plots for IFN? and TNFa staining in T cells electroporated with two non-targeting control guides (red, sgNTC-2 and sgNTC-3) and two guides targeting PKM2 (blue, sgPKM2-8 and sgPKM2-9) after 4 days of co-culture. b, Representative contour plots for Tox and TCF1 staining in T cells electroporated with two non-targeting control guides (red, sgNTC-2 and sgNTC-3) and two guides targeting PKM2 (blue, sgPKM2-8 and sgPKM2-9) after 4 days of co-culture. c, Quantification of populations of T cells electroporated with sgNTC (red) and sgPKM2 (blue) after 4 days of co-culture. **** p<0.0001
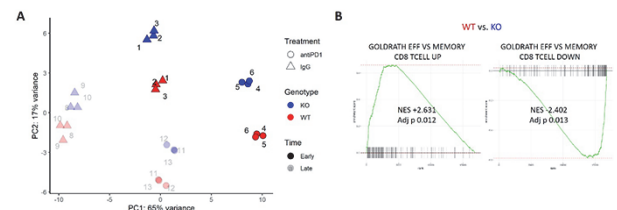
Abstract 965 Figure 2 Transcriptomic analysis of adoptively-transferred T cells. Adoptive co-transfers of activated OT-I+ Thy1.1+ PKM2WT (NTC-2, red) or PKM2KO (PKM2-8, blue) CD8+ T cells distinguished by Thy1.1 zygosity were performed into lymphodepleted C57Bl/6 mice 7 days after implantation of HKP1-ova-GFP tumors. 3 doses of either IgG control or anti-PD-1 were administered on days 10, 14, and 17 after orthotopic implantation. T cells were subsequently sorted back from tumors based on Thy1.1, phenotyped by flow cytometry, and underwent bulk RNA sequencing. a, Principal component analysis of bulk RNA sequencing data with mouse batch effects removed. b, Gene set enrichment analyses from Luckey et al[4] examining signatures for effector and memory cells in PKM2WT compared with PKM2KO.
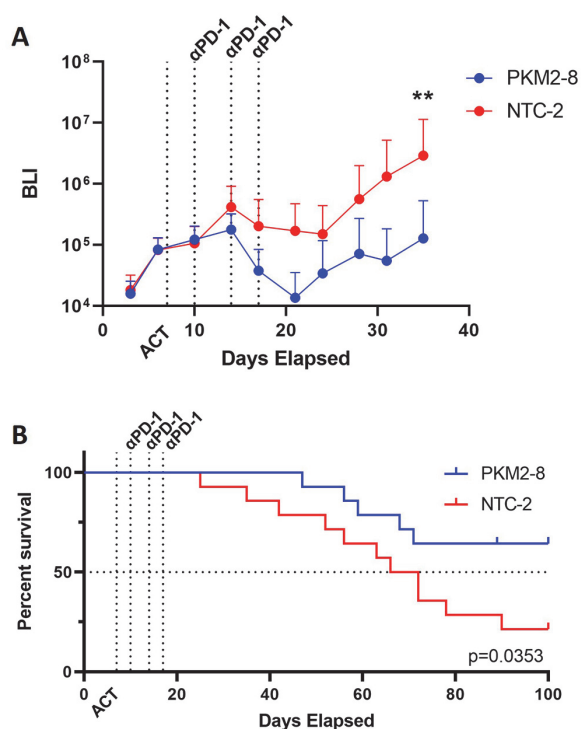
Abstract 965 Figure 3 Activated OT-I+ Thy1.1+ PKM2WT (NTC-2, red) or PKM2KO (PKM2-8, blue) CD8+ T cells were adoptively transferred into lymphodepleted C57Bl/6 mice 7 days after orthotopic implantation of HKP1-ova-GFP tumors. 3 doses of anti-PD-1 were administered on days 10, 14, and 17 after tumor implantation. Bioluminescence imaging to measure tumor burden (a) and overall mouse survival monitoring (b) were performed. ** p<0.01

Abstract 965 Figure 4 Flow cytometry analysis of activated OT-I+ Thy1.1+ T cells treated with either DMSO control (red) or 3µM glucose-6-phosphate dehydrogenase agonist AG1 (blue) and co-cultured with HKP1-ova-GFP tumor cells. T cells were activated for 24 hours, treated with DMSO or AG1 for another 24 hours, then co-cultured with HKP1-ova-GFP tumor cells at a 5:1 effector:target ratio for 4 days with continuing DMSO or AG1 treatment. a, Representative contour plots for Tox and TCF1 staining in T cells treated with DMSO (left) or AG1 (right), with gates for populations with differential TCF1 expression. b, Quantification of populations from (a) as percent of cultured CD8+ Thy1.1+ T cells in DMSO (red) and AG1 (blue) treated co-cultures. **** p<0.0001

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0965