HPK1 inhibits CD8+ T cell effector gene expression following T cell activation

RAPT THERAPEUTICS

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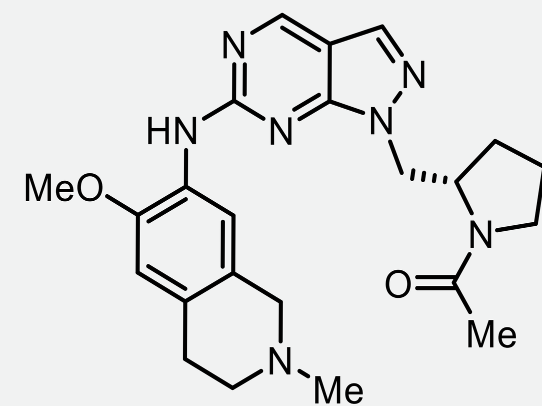
Abstract

Hematopoietic progenitor kinase 1 (HPK1) has been shown to act as a negative regulator of T cell receptor signaling and of subsequent effector T cell function. Inhibiting HPK1 to enhance T cell activity has emerged as a promising strategy for cancer immunotherapy. Upon T cell receptor engagement, the kinase domain of HPK1 interacts with and targets components of the T cell receptor (TCR) signaling pathway for degradation, including SLP76. However, the molecular events connecting HPK1 kinase activity to observed T cell functions downstream of proximal TCR signaling are not well understood. Through transcriptional profiling of HPK1-kinase-dead (HPK1-KD) versus wild-type CD8+ T cells following an acute, attenuated *Listeria monocytogenes* infection, we observed increased expression of many genes associated with T cell activation and effector function, including changes in expression of several key transcription factors and their targets. Upon activation, HPK1-KD T cells, as well as T cells treated with our small-molecule HPK1 inhibitor, produce higher levels of a wide range of effector cytokines in vitro and in vivo. Treatment of mice with the HPK1 inhibitor also inhibited tumor growth in several syngeneic tumor models. These data expand our understanding of the role of HPK1 in inhibiting CD8+ T cell effector programs, and, importantly, the impact of HPK1 inhibitors on T cell function.

HPK1-054 is a potent, selective, orally bioavailable inhibitor of HPK1 kinase activity



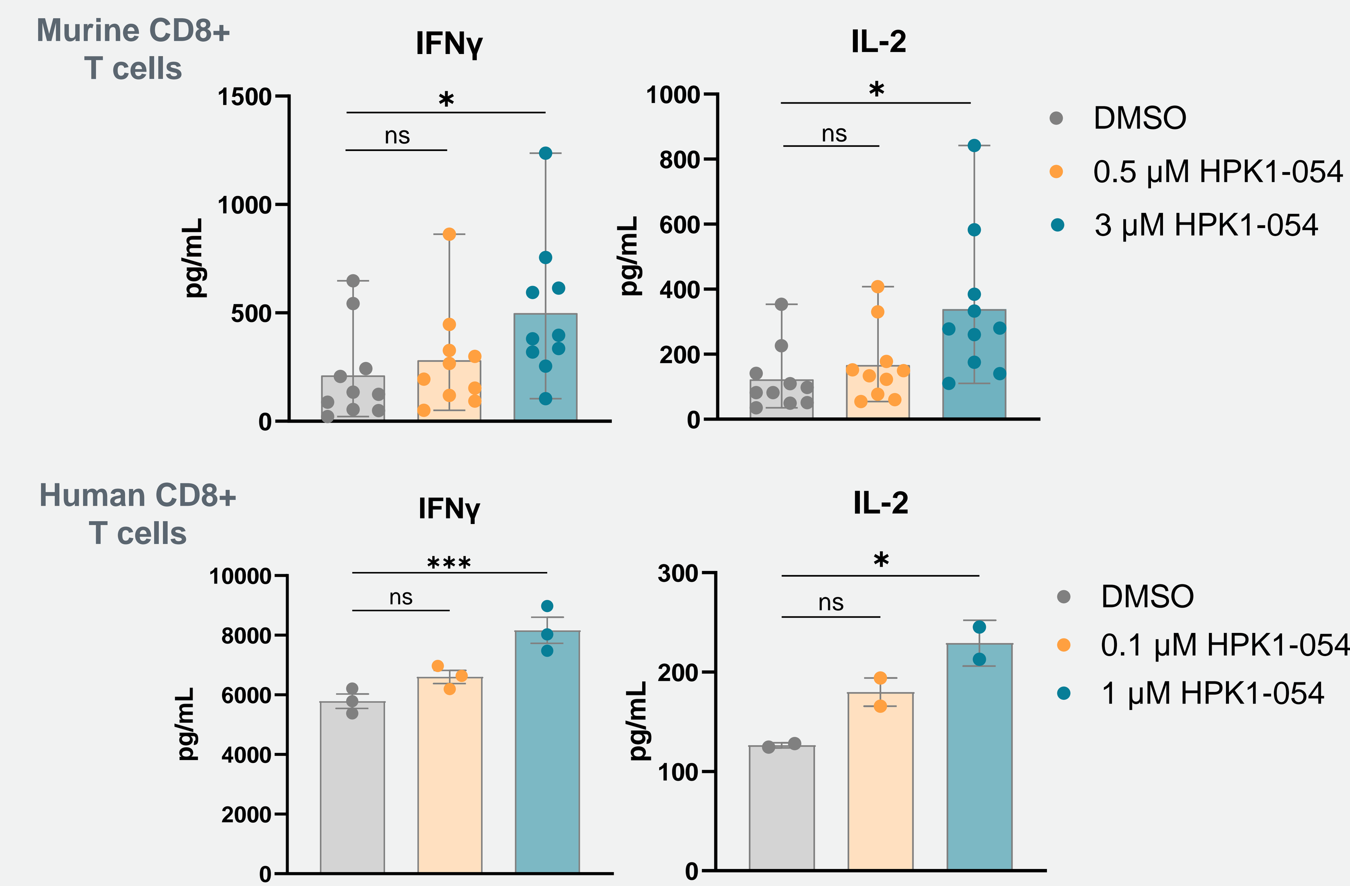
HPK1-054 In Vitro Profile:

HPK1 Biochemical IC_{50}:	2 nM
MAP4K family selectivity (biochemical):	> 10-fold
LCK selectivity (biochemical):	> 100-fold
Jurkat IL-2 EC_{50}:	2.8 µM
Jurkat pSLP-76 IC_{50}:	2.3 µM

Compound Concentration	# of kinases bound (< 35% of control) including HPK1
100 nM	3

Kinome selectivity was determined against 468 human kinases using the KINOMEscan platform in a competition binding assay.

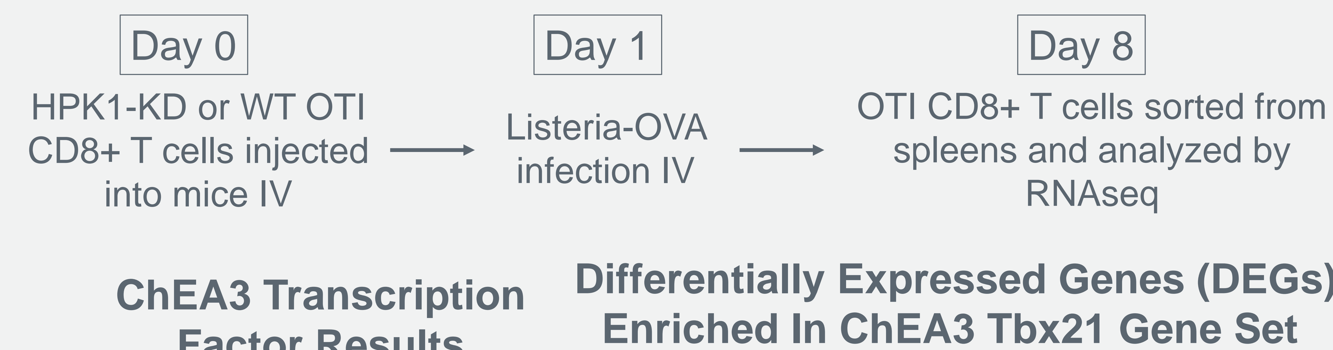
Antigen-specific CD8+ T cells secrete elevated levels of cytokines following exposure to HPK1-054

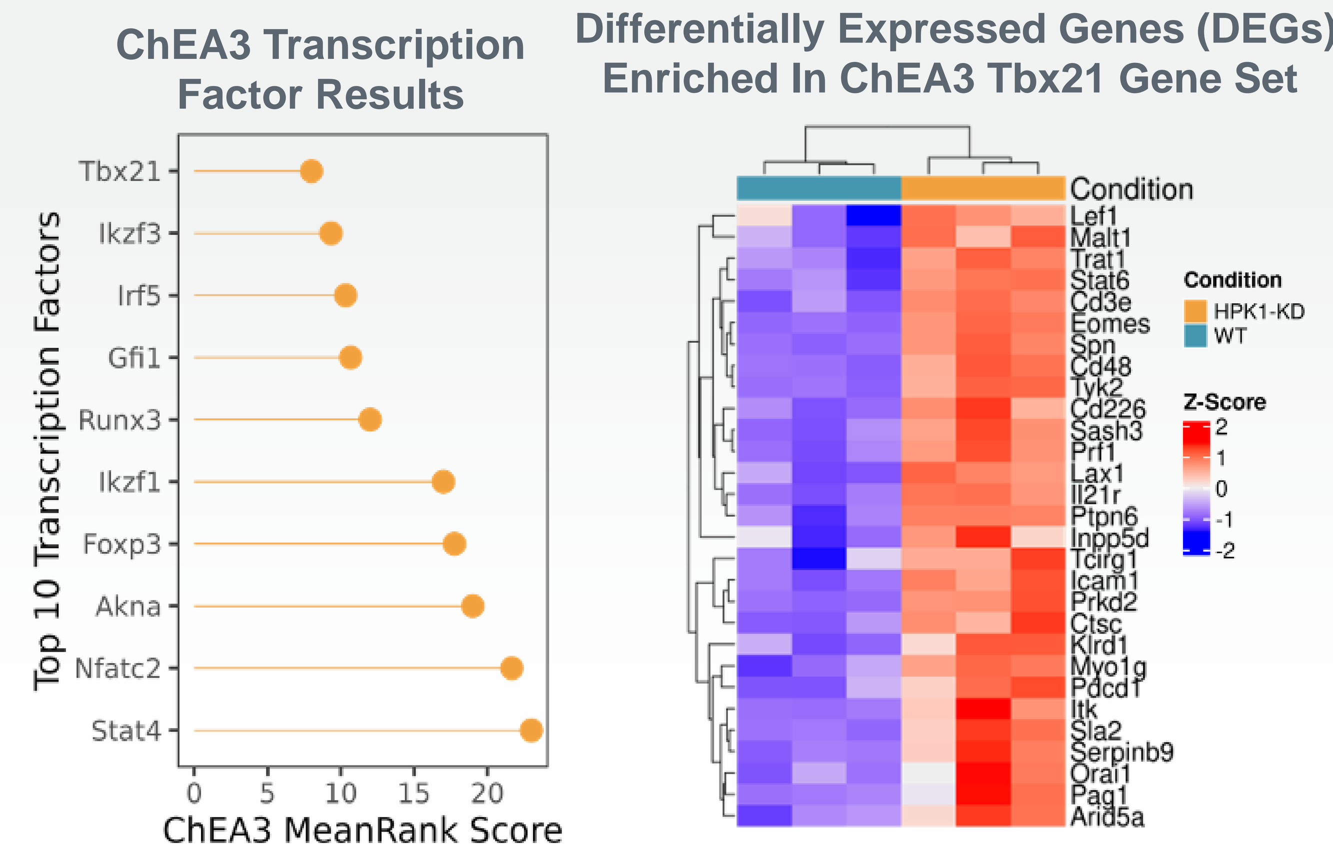


Murine T cells: Naïve CD8+ T cells were purified from OTI mice and co-cultured with RMA-S cells and OVA peptide (SIINFEKL, 0.1uM) in the presence of DMSO or HPK1-054 for 24 hrs. Cytokine levels in supernatants measured by Luminex. Human T cells: T2 cells were co-cultured with anti-MART-1 CD8+ T cells alone or in the presence of HLA-A*02:01 restricted analog MART-1 26-35 decamer peptide (ELAGIGILTV) for 20 hrs. IFNγ in supernatants measured by AlphaLISA and IL-2 by Luminex. Human CD8+ T cell data shown is representative of results from 3 unique donors. Significance determined by one-way ANOVA. ns, not significant, *p<0.05, **p<0.01, ***p<0.001

RNAseq analysis of HPK1-KD CD8+ T cells following *L. monocytogenes-OVA* vaccination shows changes in expression of targets of several key transcription factors

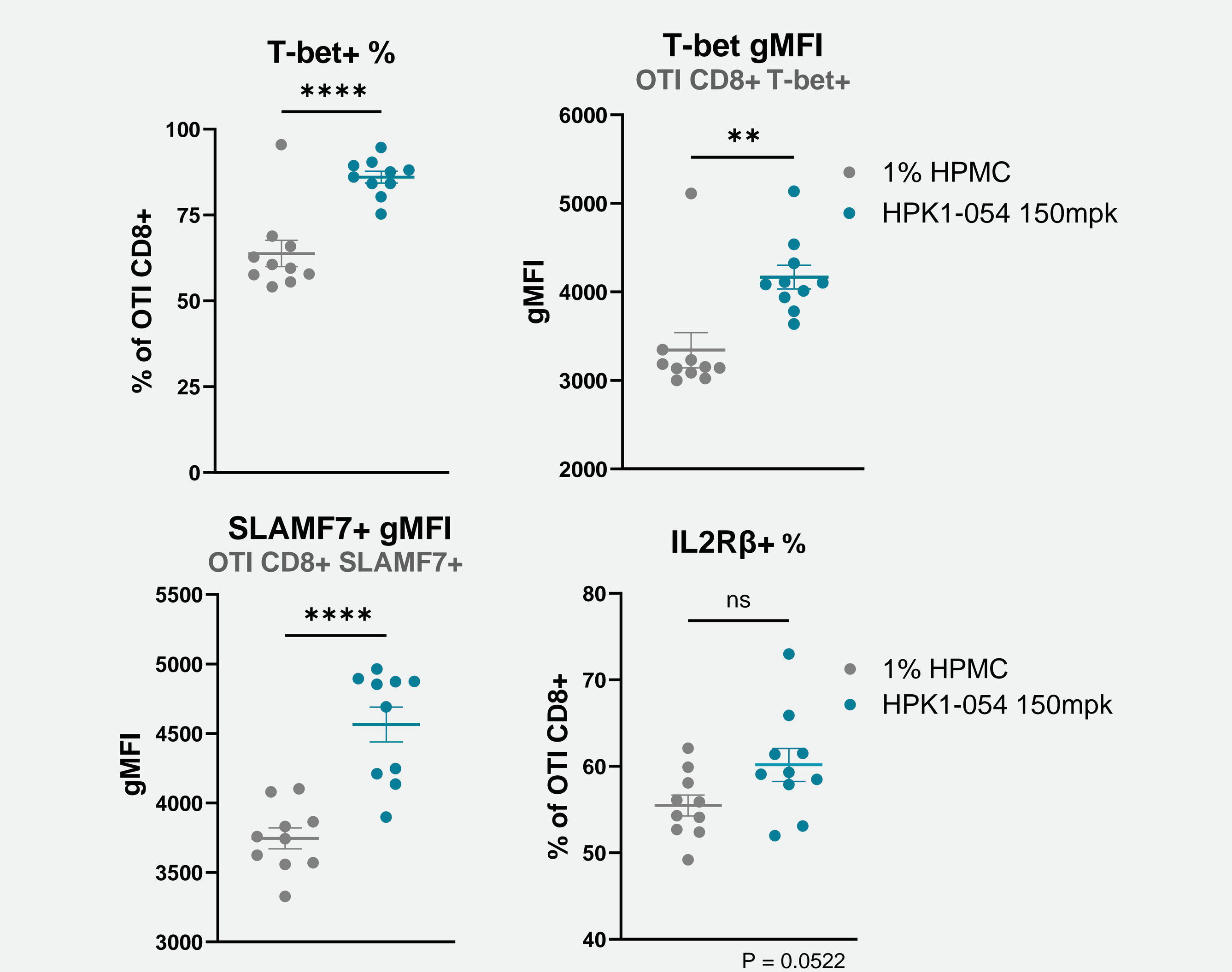
Mice with a kinase-inactivating mutation (K46M) in *Map4k1 (Hpk1)* were generated on a C57BL/6J background and crossed with OTI mice to generate OTI-HPK1-KD CD8+ T cells





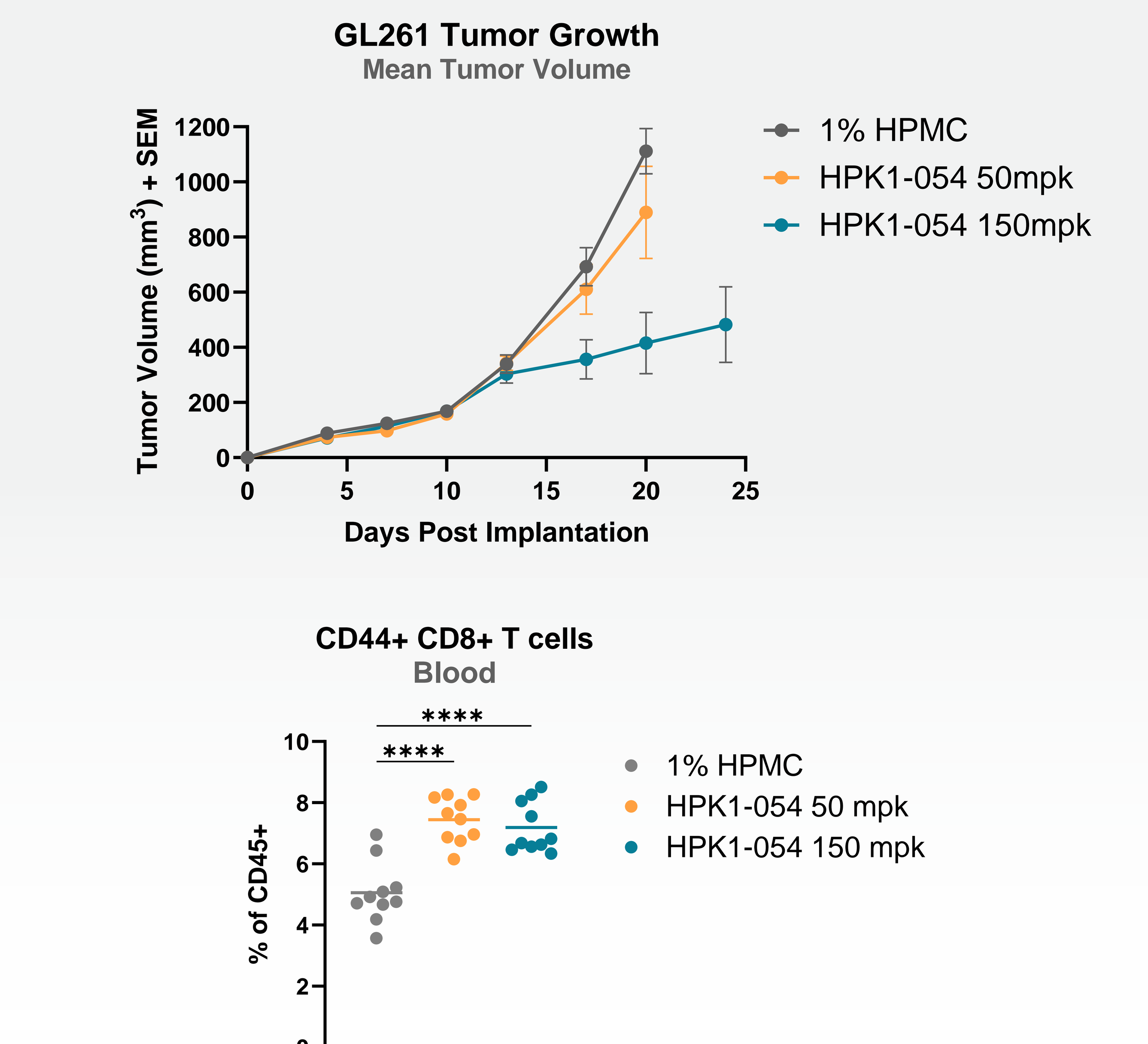
B6.SJL-Ptprca Pepcb/BoyJ mice were injected with OTI T cells on Day 0 followed by challenge with *Listeria monocytogenes* expressing ovalbumin (Lm-OVA) on Day 1. On Day 8, spleens were collected, and OTI T cells were purified by FACS. RNAseq analysis was performed using Illumina TruSeq stranded library preparation and NovaSeq (PE100) sequencing. ChEA3 is a transcription factor enrichment analysis tool that ranks transcription factors associated with user-submitted gene sets.

HPK1-054 treatment results in increased T-bet and SLAMF7 in CD8+ T cells stimulated by *L. monocytogenes-OVA*



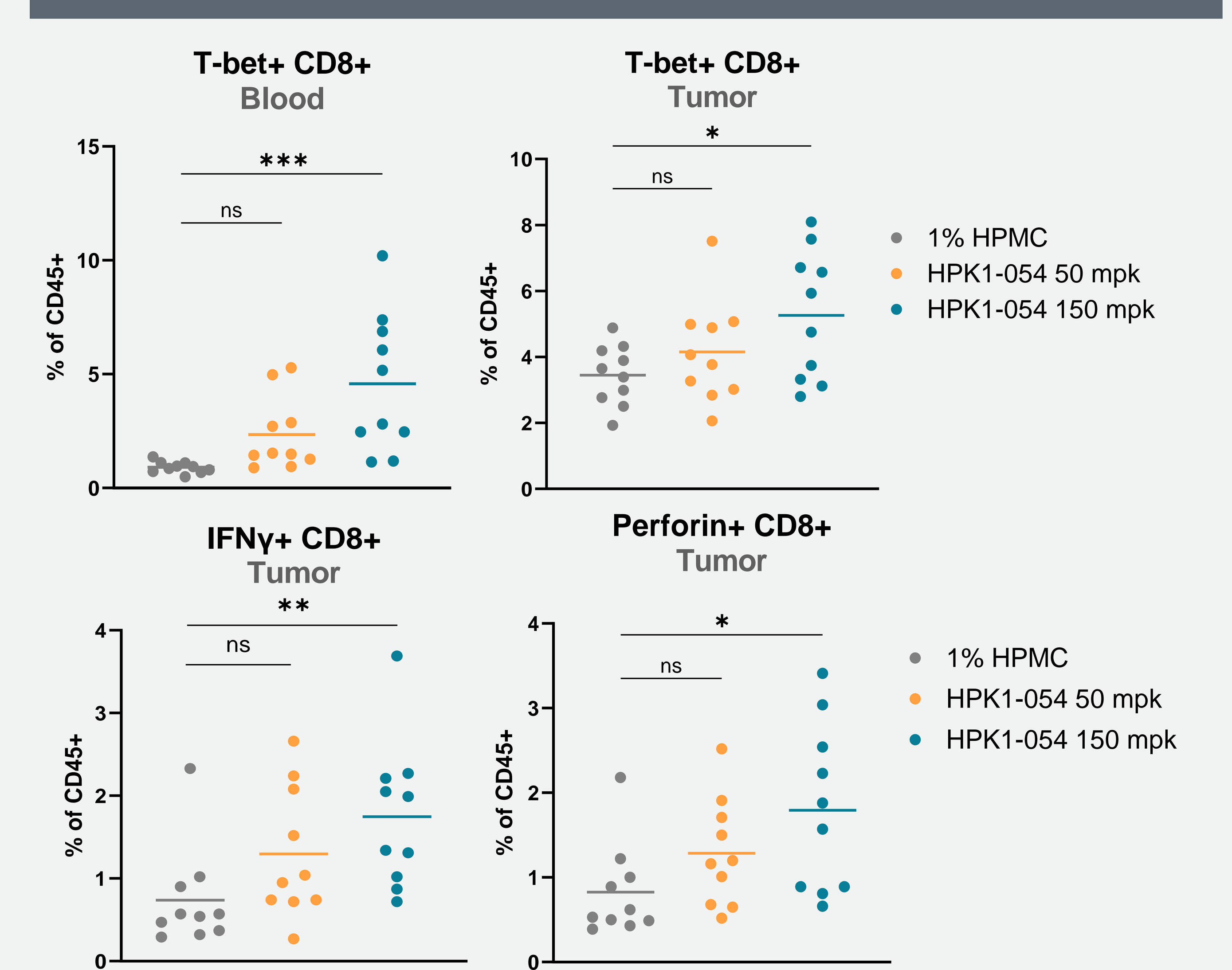
B6.SJL-Ptprca Pepcb/BoyJ mice were injected with OTI T cells on Day 0 followed by challenge with Lm-OVA on Day 1. Mice were dosed orally BID with HPK1-054 starting on Day 1. On Day 8, spleens were collected and processed into single cell suspensions and stained with antibodies to the indicated proteins before flow cytometry analysis on Day 8. Significance determined by two-tailed unpaired t tests. ns, not significant, *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001

Treatment of GL261 tumor-bearing mice with HPK1-054 results in greater tumor control and increased CD8+ T cell activation



C57BL6/J mice were implanted intradermally with GL261 tumor cells on Day 0 and monitored for tumor growth up to an endpoint tumor volume of 1500mm³. Mice were randomized and dosed orally BID with HPK1-054 starting on Day 10. CD8 and CD44+ T cells in the blood were measured by flow cytometry at Day 14 post implantation.

HPK1-054 treatment increases expression of T-bet and several of its targets in CD8+ T cells in mice bearing GL261 tumors



C57BL6/J mice were implanted with GL261 tumor cells intradermally on Day 0. Mice were randomized and dosed orally BID with HPK1-054 starting on Day 10. Tumors and blood were collected on Day 14. T-bet+ CD8+ T cells were measured by flow cytometry. IFNγ+ and Perforin+ CD8+ T cells were measured by flow cytometry following 6 hrs of brefeldin A and monensin treatment ex vivo in cell media.

Conclusions

- HPK1-054 is a potent and selective small-molecule HPK1 inhibitor.
- HPK1-054-treated antigen-specific CD8+ T cells secrete more IFNγ and IL-2 in vitro following stimulation via the TCR.
- WT vs HPK1-KD CD8+ T cells challenged with Lm-OVA were analyzed by RNAseq. ChEA3 analysis revealed that significantly upregulated genes in HPK1-KD T cells vs WT were enriched in targets of the transcription factor T-bet, among others.
- Increased expression of T-bet was observed in peripheral CD8+ T cells following Lm-OVA challenge and in tumor-bearing mice.
- We observed increased frequency of CD8+ T cells expressing T-bet targets IFNγ and Perforin in the TME.
- HPK1-054 treatment in GL261 tumor-bearing mice results in inhibition of tumor growth and increased CD44+ CD8+ T cells in the blood.
- These data support HPK1 kinase inhibition as a means of increasing T cell function in tumors and increase our understanding of molecular events downstream of HPK1 inhibition.

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