HPK1 Inhibition Enhances T Cell Activation and Relieves the Immunosuppressive Effects of Extracellular Substances Found in the Tumor Microenvironment

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INTRODUCTION

- Effective anti-tumor immune responses rely on sufficient T cell activation, which is actively suppressed by extracellular substances found in the tumor microenvironment (TME), including extracellular adenosine, prostaglandin E2 (PGE_2), and transforming growth factor beta (TGF-β).
- Hematopoietic progenitor kinase 1 (HPK1), a hematopoietic cell-specific Ste20-related serine/threonine kinase, is a negative regulator of TCR signaling, with the potential to enhance T cell activity while bypassing these immunosuppressive factors.
- Herein, we demonstrate that HPK1 inhibition enhances antigen-specific T cell response and can be combined with adenosine receptor blockade to overcome individual and combined suppressive factors found in the TME to restore T cell activation.

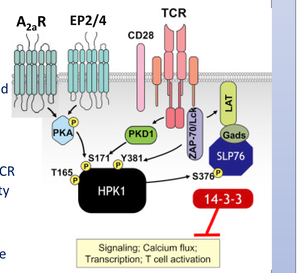

Figure 1. HPK1 functions as a negative regulator of TCR signaling. Schematic from Wu, P. *et al.* Structure 2019 Jan 2;27(1):125-133.

METHODS

- **T cell experiments:** $CD8^+$ T cells were isolated from human blood and activated using anti-CD3/CD28 stimulation. Supernatants were assayed for IL-2, IFN-γ and TNF-α using cytokine bead array (CBA). Activation markers and SLP-76 phosphorylation (S376) were assayed by flow cytometry. OT-1 splenocytes were isolated from OT-1 mice and activated using ovalbumin specific residues (257-264) of varying affinity. Supernatants were assayed for cytokine secretion using CBA.
- **CRISPR knockout:** $HPK1^{KO}$ $CD8^+$ T cells and Jurkat cells were generated using Amaxa nucleofection of cells with Cas9 and gene-specific sgRNA sequences. Cells were recovered for at least 7 days prior to use.
- **Compounds:** Arcus HPK1 inhibitors and reference compound (You et al. *J Immunother. Cancer*. 2021) were used. Dual adenosine ($A_{2a}R/A_{2b}R$) receptor antagonist etrumadenant (etruma) was used to block the effects of synthetic adenosine receptor agonist NECA.

RESULTS

HPK1 is the Most Abundant MAP4K Protein in $CD8^+$ T cell subsets

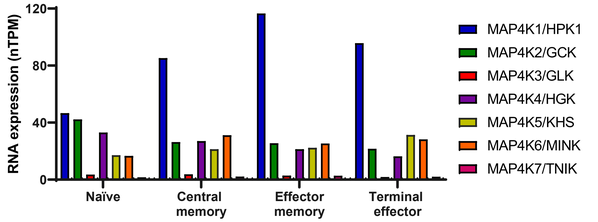

Figure 2. RNA expression data from Human Protein Atlas shows that MAP4K1 (HPK1) is the most abundantly expressed member of the MAP4K family in $CD8^+$ T cell subsets.

Genetic Deletion of HPK1 Amplifies T cell Activation and Restores Cytokine Secretion Suppressed by Inhibitory Signals

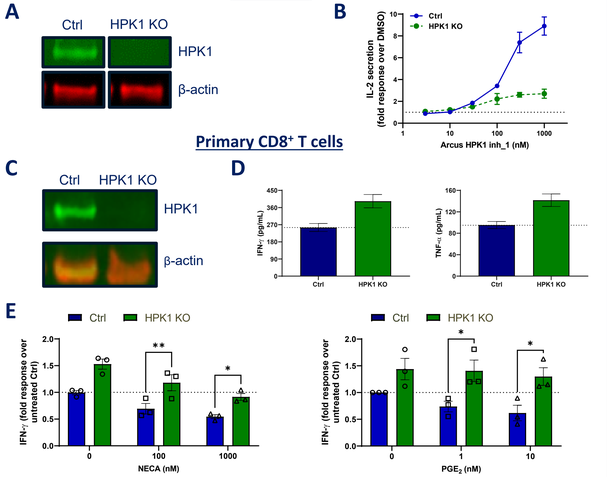

Figure 3. (A) CRISPR knockout of HPK1 in Jurkat cells was confirmed by Western blot. **(B)** Demonstration of on-target activity of Arcus HPK1 inh_1 in increasing IL-2 secretion in Ctrl vs $HPK1^{KO}$ Jurkat cells. **(C)** CRISPR knockout of HPK1 in primary human $CD8^+$ T cells was confirmed by Western blot. **(D)** CD3/28/2 activated $HPK1^{KO}$ $CD8^+$ T cells display increased IFN-γ (left panel) and TNF-α (right panel) secretion. **(E)** $HPK1^{KO}$ $CD8^+$ T cells display increased IFN-γ secretion in presence of individual inhibitory signals NECA (left panel) and PGE_2 (right panel). *=p<0.05,**=p<0.01.

Pharmacological Inhibition of HPK1 Increases Early T cell Activation and Cytokine Secretion

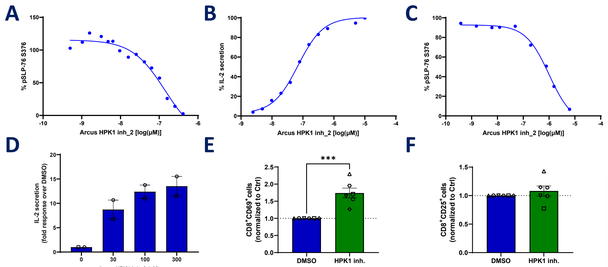

Figure 4. In activated Jurkat cells, Arcus HPK1 inh_2 **(A)** decreases phosphorylation of SLP-76 (S376) and **(B)** increases IL-2 secretion. Arcus HPK1 inh_2 decreases CD3/28 activation-induced phosphorylation of SLP-76 (S376) in healthy human whole blood **(C)**. In activated $CD8^+$ T cells, Arcus HPK1 inhibitor increases IL-2 secretion **(D)**. HPK1 inhibition increases $CD8^+CD69^+$ cells in $CD8^+$ T cells after 24 hr activation **(E)**, but it does not affect $CD8^+CD25^+$ cells from matched donors after 72 hr activation **(F)**. ***=p<0.001.

Concentration-dependent Inhibition of HPK1 Kinase Activity Correlates with Increased Cytokine Secretion in Human Whole Blood

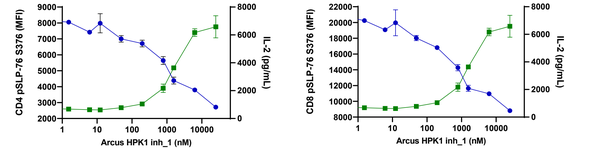

Figure 5. Arcus HPK1 inh_1 decreases CD3/28 activation-induced phosphorylation of SLP-76 (S376) in **(A)** $CD4^+$ and **(B)** $CD8^+$ T cells in healthy human whole blood in a concentration dependent manner. Inhibition of pSLP-76 S376 (blue line) correlates with an increase in IL-2 secretion (green line) in a matched human whole blood donor.

HPK1 Inhibition Relieves the Effect of Multiple Immunosuppressive Signals Found in the TME

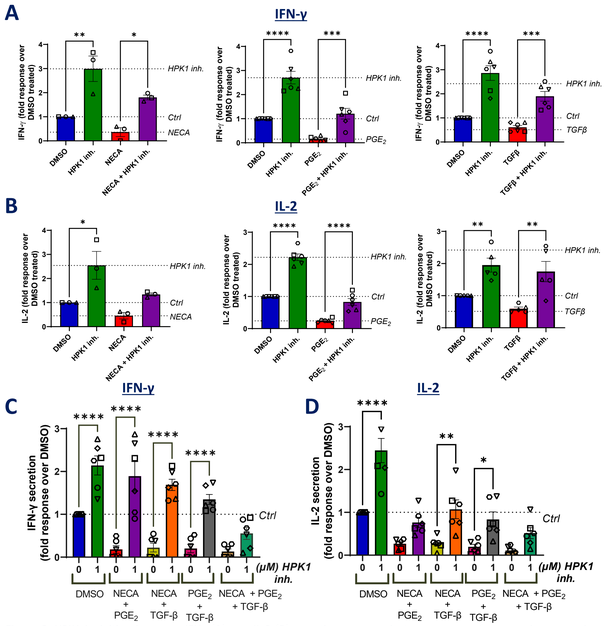

Figure 6. HPK1 inhibition increases IFNγ **(A)** and IL-2 **(B)** secretion alone or in the presence of suppressive adenosine (NECA, 5 μM), prostaglandin (PGE_2, 10 nM) and TGFβ (TGFβ , 3 ng/mL) signaling in human $CD8^+$ T cells. HPK1 inhibition significantly increases **(C)** IFN-γ and **(D)** IL-2 secretion with the majority of combined suppressive signals. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

HPK1 Inhibition Combined with Adenosine Receptor Blockade Relieves the Effect of Multiple Inhibitory Signals Found in the TME

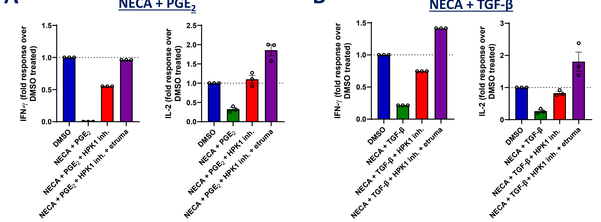

Figure 7. HPK1 inhibition in combination with adenosine receptor blockade (etrumadenant) fully restores T cell activity under NECA + PGE_2 **(A)** and NECA + TGF-β **(B)** mediated suppression.

HPK1 Inhibition Enhances Cytokine Secretion in OT-1 Splenocytes

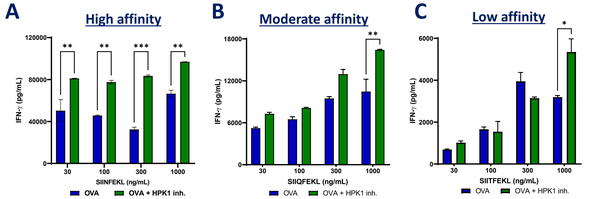

Figure 8. HPK1 inhibition increases IFN-γ secretion in high **(A)**, moderate **(B)** and low **(C)** affinity ovalbumin peptide activated OT-1 splenocytes. *=p<0.05, **=p<0.01, ***=p<0.001..

CONCLUSIONS

- HPK1 (MAP4K1) is the most abundantly expressed member of the MAP4K family in $CD8^+$ T cell subsets. Genetic deletion of HPK1 in Jurkat cells and human $CD8^+$ T cells increase cytokine secretion alone or in presence of individual suppressive signals.
- Inhibiting HPK1 kinase activity using Arcus HPK1 inhibitor reproduces the phenotype of genetic deletion in Jurkat cells and $CD8^+$ T cells, decreasing SLP-76 (S376) phosphorylation and increasing IL-2 secretion.
- Arcus HPK1 inhibitor decreases phosphorylation of SLP-76 (S376) in human whole blood in a concentration-dependent manner and concurrently increases IL-2 secretion.
- HPK1 inhibition increases cytokine secretion in the presence of single or combination of suppressive signals - adenosine (NECA), PGE_2 and TGFβ in human $CD8^+$ T cells. This effect is further enhanced by combining HPK1 inhibition with antagonism of adenosine A_2R receptors using etrumadenant.
- HPK1 inhibition increases cytokine secretion in OT-1 splenocytes with the most enhanced effect observed in high affinity antigen activated splenocytes.
- **These data highlight HPK1 as an attractive target for immunotherapy as inhibiting HPK1 can boost T cell responses and amplify anti-tumor responses.**