**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 (PGE2), and transforming growth factor beta (TGF-β).
- HPK1, a serine/threonine kinase, is a negative regulator of TCR signaling, with the potential to enhance T cell activity by 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.

**METHODS**

- **T cell experiments**: CD8 T-cells were isolated from human blood and activated using anti-CD3/CD28 stimulation. Supernatants were assayed for IFN-γ, IL-2, and TNF-α using cytokine bead array (CBA). Activation markers and SLP-76 phosphorylation (S376) were assayed by flow cytometry.
- **Arcus HPK1 inhibitors**: Arcus HPK1 inhibitors and reference compound (You et al. 2019) were used, and their effects on IL-2 secretion alone or in presence of individual suppressive signals were measured.
- **HPK1 CRISPR knockout**: Cas9, gene-specific sgRNA sequences, and DMSO were used to knock down HPK1 expression in primary human CD8+ T cells.

**RESULTS**

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

- HPK1 inhibition increases cytokine secretion in the presence of single or combination of TGF-β, IFN-γ, and adenosine receptor blockade (You et al. 2019), activates OT-1 splenocytes, and boosts T cell responses and amplifies anti-tumor responses.

**CONCLUSIONS**

- HPK1 (MAP4K6) 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 cell subsets, decreasing PGE2 (20ng/mL) phosphorylation and increasing IL-2 secretion.
- Arcus HPK1 inhibitor decreases phosphorylation of SLP-76 (20 ng/mL) 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), and TGF-β in human CD8+ T cells. This effect is further enhanced by combining HPK1 inhibition with antagonism of adenosine A2A receptors.
- HPK1 inhibition increases cytokine secretion in OT-1 splenocytes with the most enhanced effect on high affinity antigen receptor engagement, which may be a hot target for the evolved T cells in the TME.

**ACKNOWLEDGMENTS**

- The authors thank the ARCUS Biosciences team for support.

**Supporting Information**

- **Figure 1**: HPK1 inhibition enhances T cell activation and relieves the immunosuppressive effects of extracellular substances found in the tumor microenvironment.
- **Figure 2**: HPK1 inhibition combined with adenosine receptor blockade relieves the effect of multiple immunosuppressive signals found in the TME.
- **Figure 3**: HPK1 inhibition enhances cytokine secretion in primary human CD8+ T cells, resulting in a more robust T cell response.

**REFERENCES**

- Arcus Biosciences, Inc.; 3292 Point Eden Way, Hayward, CA 94545 (USA).