HPK1 INHIBITION RELIEVES SUPPRESSION DOWNSTREAM OF TCR ACTIVATION TO DRIVE ENHANCED CYTOKINE PRODUCTION AND ANTIGEN-SPECIFIC KILLING, AN EFFECT THAT IS FURTHER ENHANCED BY IMMUNE CHECKPOINT BLOCKADE

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Background T cell activation is critical in the initiation and potentiation of anti-tumor immune responses. Hematopoietic Progenitor Kinase 1 (HPK1) is a member of the MAP4K family whose activity restrains T cell activation through phosphorylation of SLP-76 (pSLP-76) at Serine 376, leading to disassembly of the TCR complex. Mouse genetic deletion and kinase dead mutants of HPK1 have been shown to enhance T cell activity and combine with immune checkpoint inhibition. Therefore, we sought to demonstrate that inhibitors of HPK1 activity can increase T cell activation, drive antigen-specific cancer cell killing, and combine with immune checkpoint blockade to amplify anti-tumor T cell responses. Herein, we describe the characterization of novel inhibitors of HPK1 and assess their effects on T cell activation in combination with PD-1 blockade.

Methods Measurement of pSLP-76 using a flow cytometry-based assay was employed to assess HPK1 activity in human and mouse whole blood. Jurkat cells, human CD8⁺ T cells and PBMCs were activated using anti-CD3/CD28 stimulation, CEF peptide pools, or Staphylococcal enterotoxin A (superantigen), in the presence of HPK1 inhibitors; levels of IL-2, IFN-γ and TNF-α were assayed in supernatants by cytokine bead array. Mouse OT-1 splenocytes were stimulated with SIINFEKL peptide and co-cultured with E.G7-OVA cells to assess cytokine production and target cell killing.

Results HPK1 inhibitors exhibited a potent, concentration-dependent reduction in pSLP-76, with a concurrent increase in IL-2 secretion in both Jurkat and human CD8⁺ T cells. Human T cells also demonstrated increased IFN-γ and TNF-α secretion as well as a greater percentage of activated CD69⁺ cells with HPK1 inhibition. These increases in T cell activation were mirrored in antigen-specific OT-1 T cell assays, in which HPK1 inhibition enhanced IFN-γ production in response to OVA peptide and greater killing of E.G7-OVA cancer cells. Similarly, antigen-recall assays demonstrated that inhibition of HPK1 increased IFN-γ production from human PBMC stimulated with CEF peptide and combined with PD-1 blockade to further enhance cytokine production. Consistent with these results, HPK1 inhibition and PD-1 blockade increased cytokine secretion in superantigen-stimulated human PBMC individually and further enhanced cytokine production in combination. Taken together, these data demonstrate that the combined activity of HPK1 inhibition with checkpoint therapy may yield greater anti-tumor T cell activity.

Conclusions These data demonstrate that pharmacological inhibition of HPK1 amplifies antigen-specific T cell activation alone or in combination with immune checkpoint blockade and provide a strong mechanistic rationale for targeting HPK1 to amplify anti-tumor immune responses.

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