Development of Small-Molecule HPK1 Inhibitors to Unleash Tumor-Specific T Cell Responses

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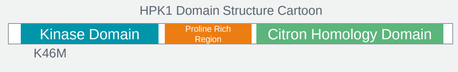
Abstract

Hematopoietic progenitor kinase 1 (HPK1) is an intracellular protein kinase that negatively regulates T cell signaling and proliferation. Upon T cell receptor (TCR) activation, active HPK1 phosphorylates the adaptor protein SLP76 in the TCR complex, recruiting the negative regulator 14-3-3 and targeting components of the TCR signaling complex for degradation. HPK1 thus limits the TCR signaling important for mounting an effective immune response against tumor cells. We are employing structure-guided drug design to develop potent small-molecule inhibitors of HPK1. Our compounds potently inhibit HPK1 in biochemical assays, reduce levels of phosphorylated SLP76 and concomitantly increase IL-2 production by Jurkat T cells. Importantly, our HPK1 inhibitors enhance cytokine production by human and mouse primary T cells above that observed with TCR activation alone. Treatment of mice with orally available HPK1 inhibitors results in increased activation of antigen-specific CD8$^+$ T cells in vivo and decreased tumor growth as single agent and in combination with clinically relevant checkpoint inhibitor antibodies. Our work confirms the importance of HPK1 for T cell function and supports HPK1 as a promising next-generation immuno-oncology target.

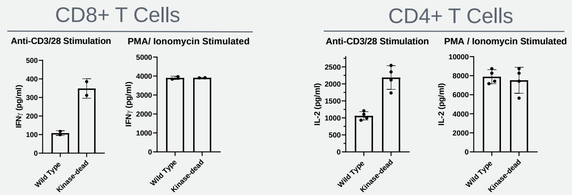
Genetic Validation of HPK1

Mice with a kinase inactivating mutation (K46M) in HPK1 were generated on a C57BL/6J background



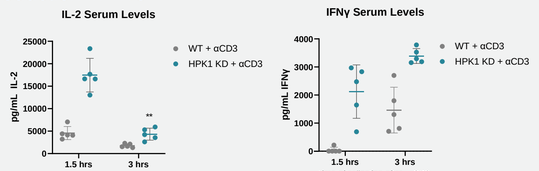
Targeted mutation of catalytic lysine to methionine selectively abrogates kinase activity. K46M = Kinase Dead

Loss of HPK1 kinase activity enhances TCR driven cytokine production in vitro



Mouse T cells were isolated using negative selection and stimulated as indicated for 24hrs. Cytokine levels were determined using AlphaLISA.

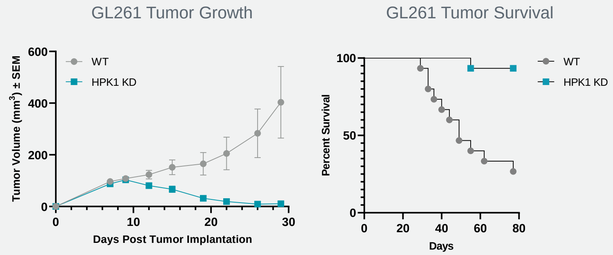
Loss of HPK1 kinase activity enhances TCR driven cytokine production in vivo

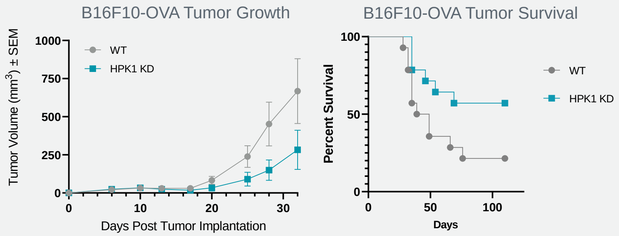


Wild type (WT) or Kinase dead (KD) mice were administered Anti-CD3ε (145-2C11) antibody I.V. Serum cytokines were measured by ELISA at the indicated timepoint. N=5 animals per group.

HPK1 K46M Mice Have Enhanced Tumor Control

Loss of HPK1 kinase activity enhances tumor rejection



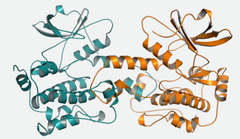


Wild type (WT) or K46M Kinase dead (KD) mice were implanted with the indicated syngeneic tumor lines and monitored for tumor growth and time to an endpoint tumor volume of 1,500 mm^3. A minimum of 14 animals per group were used.

Small Molecule HPK1 Kinase Inhibitor Development

Development of potent and selective, orally bioavailable small molecule inhibitors of HPK1 kinase activity

High resolution X-Ray structures of HPK1 Kinase domain enable RAPT structure-based drug design program



1.7 Ångstrom resolution structure of representative HPK1 kinase domain displaying domain swapped dimer architecture. Monomers individually colored

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

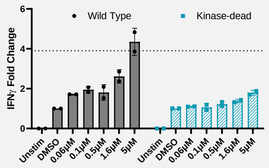
Kinome Selectivity:

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.

HPK1 Inhibition Enhances Effector Cytokine Production in Primary Immune Cells

HPK1-054 increases effector cytokine levels in mouse primary T cells and in an HPK1 kinase-dependent manner

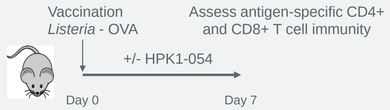


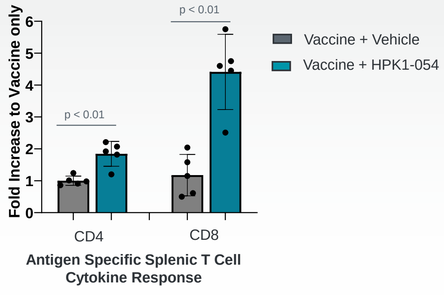
KD/WT DMSO Ratio
(Maximum fold change from inhibiting HPK1)

Purified mouse CD8+ T cells from WT and KD mice were stimulated with anti-CD3/28 beads for 24 hrs with either DMSO or an increasing concentration of HPK1-054. Interferon gamma levels were measured using AlphaLISA and normalized to the DMSO only controls. KD/WT DMSO ratio represents the maximal effect from HPK1 kinase inhibition. Viability was unaffected in any samples (data not shown).

HPK1 Inhibition Enhances Antigen-Specific T Cell Responses in Vivo

Inhibition of HPK1 in a vaccination model results in enhanced antigen-specific responses in both CD4+ and CD8+ T cells





Mice were vaccinated with 1x10^3 CFU of attenuated *Listeria* expressing ovalbumin. HPK1-054 was dosed at 100 mg/kg BID. Splenic cells were isolated at day 7. Cells were restimulated with either LLO or OVA peptides for CD4 and CD8 T cells, respectively. Responses were measured by intracellular cytokine staining for interferon gamma. N=5 mice per group. Responses are normalized to vaccine only.

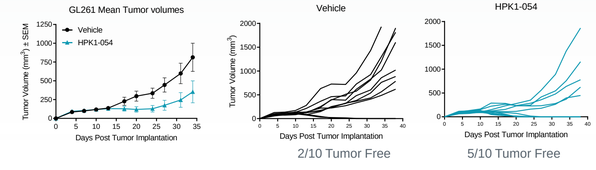
HPK1-054 In Vivo Target Engagement

HPK1-054 blocks S376 phosphorylation of SLP76 in vivo

Mice were dosed orally with HPK1-054 at the indicated doses. Anti-CD3ε (145-2C11) antibody was administered IV. Splenic T cells were stained for phosphorylated S376 SLP-76. N=4 animals per group.

HPK1 Inhibition Demonstrates Single Agent Antitumor Activity

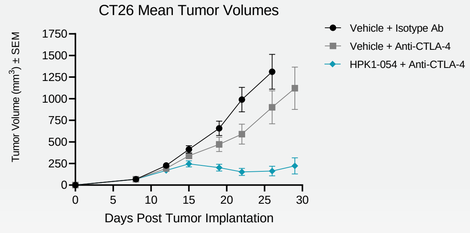
HPK1-054 inhibits tumor growth as monotherapy in GL261 tumors

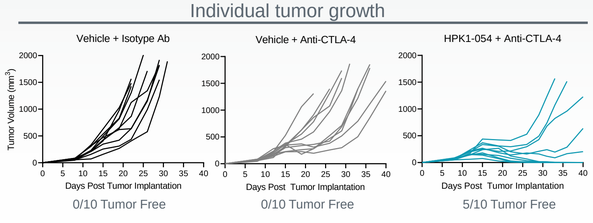


Mice were randomized at ~80 mm^3 tumor volume on day 10. HPK1-054 was dosed at 150 mg/kg BID starting at day 10. At day 38 animals with no measurable tumor were considered tumor free. N=10 animals per group.

HPK1 Inhibition Enhances T Cell Checkpoint Inhibitor Antibody-Mediated Antitumor Activity

HPK1-054 inhibits tumor growth in combination with anti-CTLA-4 antibody





Mice were randomized at ~100 mm^3 tumor volume on day 8. HPK1-054 was dosed at 150 mg/kg BID starting at day 8.100μg of αCTLA-4 (9D9) or isotype control antibody was dosed IP on day 8, 12 and 16. At day 40 animals with no measurable tumor were considered tumor free. N=10 animals per group.

Conclusions

Here we show that in vivo genetic loss of HPK1 kinase activity increases T cell cytokine levels in multiple settings. HPK1 kinase dead mice have delayed tumor growth and enhanced survival in multiple syngeneic tumor models. These results demonstrate that inhibitors of HPK1 kinase activity have the potential to enhance the immune response to tumors. We have developed selective, oral small molecule inhibitors of HPK1 that robustly block HPK1 activity in vivo and enhance T cell cytokine production. Monotherapy dosing of our small molecule HPK1 inhibitor in GL261 tumors leads to delayed tumor growth and complete tumor regressions. Combination of our HPK1 inhibitor and a checkpoint inhibitor antibody in the CT26 tumor model leads to delayed tumor growth and complete tumor regressions. Our data demonstrates that HPK1 is a promising novel druggable target with the potential to enhance antitumor immunity.

Disclosures

All authors are current or former employees of RAPT Therapeutics, Inc., and hold stock and/or intellectual property in the company.

Acknowledgments

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