A Highly Selective and Potent HPK1 Inhibitor Enhances Immune Cell Activation and Induces Robust Tumor Growth Inhibition in a Syngeneic Tumor Model

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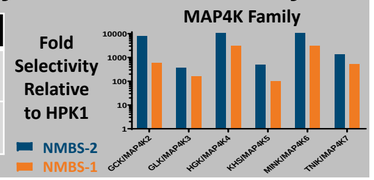
BACKGROUND

HPK1, a member of the MAP4K family of protein serine/threonine kinases, is involved in regulating signal transduction cascades in cells of hematopoietic origin. Recent data from HPK1 knockout animals and kinase-inactive knock-in animals underscores the role of HPK1 in negatively regulating lymphocyte activation. This negative-feedback role of HPK1 downstream of lymphocyte activation and function, combined with its restricted expression in cells of hematopoietic origin, make it a compelling drug target for enhancing anti-tumor immunity.

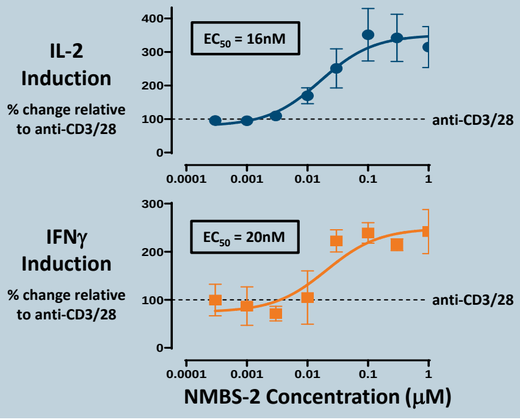
Potency & Selectivity

Assay	NMBS-1	NMBS-2
HPK1 Biochemical IC_{50}	< 1nM	< 1nM
pSLP-76 Jurkat Cell IC_{50}	35nM	42nM

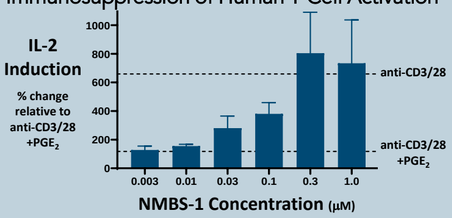


T Cells

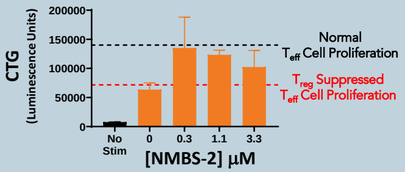
HPK1 Inhibition Enhances IL-2 and IFNγ Production From Stimulated Human T Cells



HPK1 Inhibition Alleviates PGE_2-Mediated Immunosuppression of Human T Cell Activation

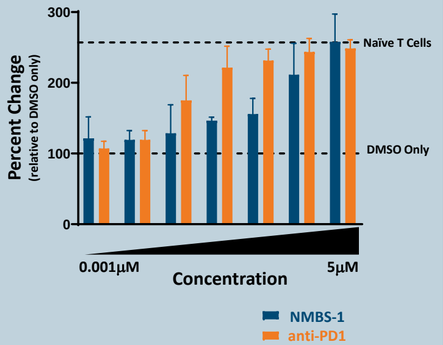


HPK1 Inhibition Reverses the Suppressive Activity of Mouse T Regulatory Cells



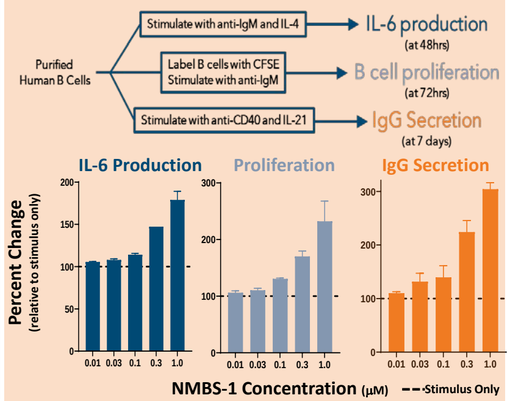
HPK1 Inhibition Restores the Proliferative Capacity of Exhausted Human T Cells

In vitro exhausted human T Cells (iterative stim/rest cycles) were tested in an MLR +/- NMBS-1 or +/- anti-PD1 and assayed for proliferation



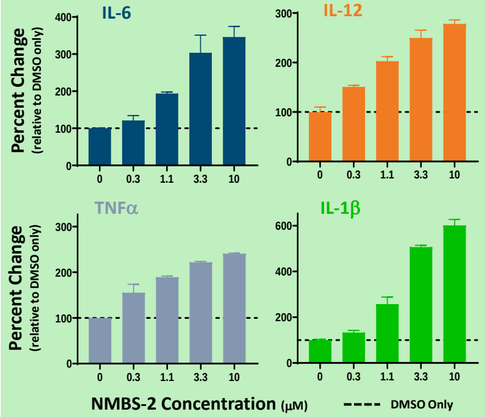
B Cells

HPK1 Inhibition Enhances IL-6 Production, Proliferation, and IgG Secretion from Human B Cells

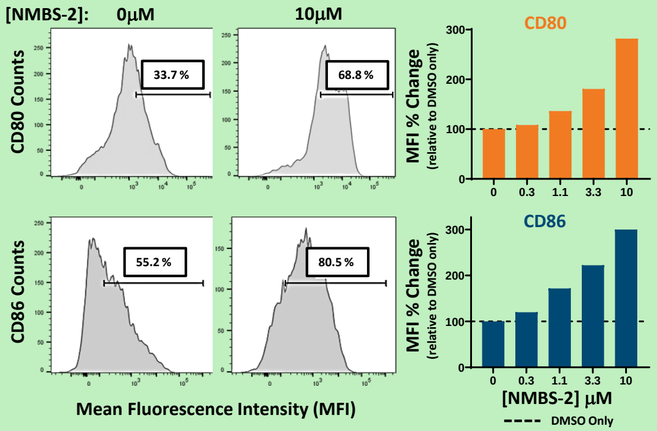


Dendritic Cells

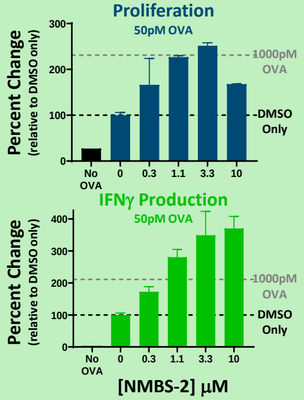
HPK1 Inhibition Enhances Cytokine Production From LPS-stimulated Mouse Bone Marrow DCs



HPK1 Inhibition Increases the Number of CD80/86+ Cells and Enhances the Expression of CD80/86 on Mouse Bone Marrow DCs

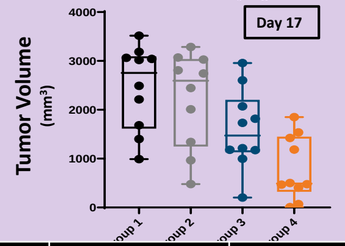


HPK1 Inhibition Enhances Ag-Specific OTI $CD8^+$ T Cell Activation in Mouse MLR



Syngeneic Studies

HPK1 Inhibition Induces Robust Tumor Growth Inhibition as a Single Agent and in Combination with anti-PD1 in the CT-26 Syngeneic Model



Group	Treatment	P_{value} (t-test)	*CRs
2	anti-PD1 10mpk, i.p., BIW	0.56	
3	NMBS-2 75mpk/day, p.o.	0.03	
4	anti-PD1 10mpk, i.p., BIW NMBS-2 75mpk/day, p.o.	0.0001	2

CONCLUSIONS AND FUTURE STEPS

We show here that potent, highly selective small molecule HPK1 inhibitors mediate activation of several immune cell types, including T, B and dendritic cells. Immune activation upon HPK1 inhibition is observed even in immune-suppressed environments (PGE_2, Treg, and T cell exhaustion). This enhancement of immune responses *ex vivo* translates into effective tumor growth inhibition *in vivo*, as a single agent and in combination with anti-PD1 in syngeneic models. Further evaluation of these selective inhibitors in additional immune cell-based assays will continue to build upon our mechanistic understanding of HPK1 inhibition as a novel immunomodulatory approach for anti-tumor immunity.

ACKNOWLEDGEMENTS

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