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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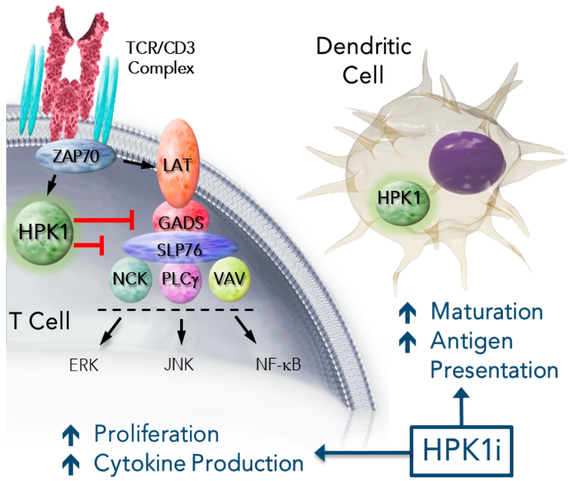
BACKGROUND

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.



METHODS

A structure-based drug design approach was used to identify potent and selective inhibitors of HPK1. Biochemical assays, as well as primary human and mouse immune cell-based activation assays, were utilized for multiple iterations of structure-activity relationship (SAR) studies. *In vivo* efficacy, target engagement and pharmacodynamic data were generated using murine syngeneic tumor models.

FIGURE 1. Potency, Selectivity, and Drug-like Properties of HPK1 Inhibitor NMBS-1

			NMBS-1
Biochemical HPK1 IC_{50} (nM)			< 1
Cellular HPK1 IC_{50} (nM)			31
MAP4K Kinase Family (Fold Selectivity over HPK1)	GCK (MAP4K2)		> 100
	GLK (MAP4K3)		> 100
	HGK (MAP4K4)		> 100
	KHS (MAP4K5)		> 80
	MINK (MAP4K6)		> 400
Select T Cell Kinase Panel (Fold Selectivity over HPK1)	TNIK		> 400
	c-SRC		> 400
	FYN		> 400
	LCK		> 400
	SYK		> 400
Mouse *In Vivo* Exposure Parameters	15mpk, BID	C_{max} (μM)	12
		$AUC_{(μM.hr)}$	71
	75mpk, QD	C_{max} (μM)	69
		$AUC_{(μM.hr)}$	270

FIGURE 2. HPK1 Inhibition Enhances IL-2 Production From Stimulated Human T Cells

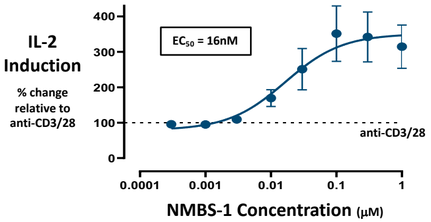


FIGURE 3. HPK1 Inhibition Alleviates PGE2- Mediated Immunosuppression of Human T Cell Activation

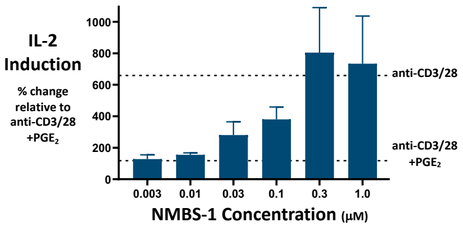
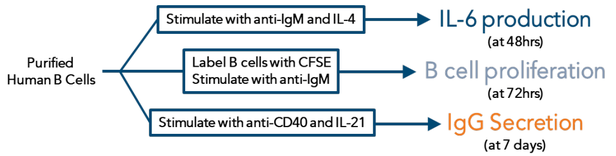


FIGURE 4. HPK1 Inhibition Enhances IL-6 Production, Proliferation, and IgG Secretion from Human B Cells



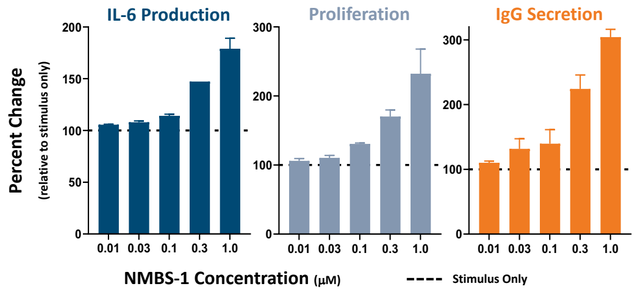


FIGURE 5. NMBS-1 Inhibits TCR-Stimulated pSLP-76 in Splenic T Cells and Increases Serum IL-2 Levels in CT-26 Syngeneic Mice

Splenic T cells were purified from tumor-bearing animals that received anti-CD3 antibody. pSlp76 in cell lysates was analyzed by ELISA.

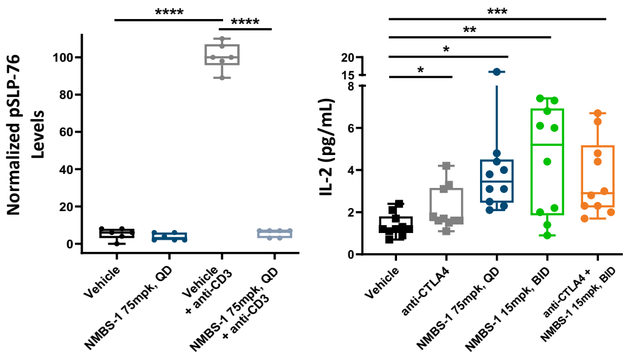
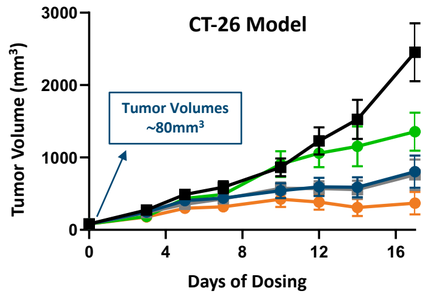
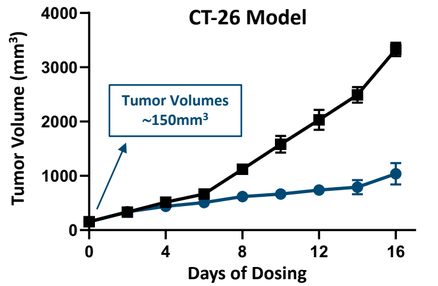


FIGURE 6. NMBS-1 Induces Robust Tumor Growth Inhibition as a Single Agent and in Combination with anti-CTLA4



Treatment Group	P_{value} (t-test)	*CRs
Vehicle		
anti-CTLA4	0.003	2
NMBS-1 75mpk, QD	0.002	2
NMBS-1 15mpk, BID	0.038	
anti-CTLA4 + NMBS-1 15mpk, BID	0.0002	4

*CRs, Complete Responses; No measurable tumor



Treatment Group	P_{value} (t-test)	*CRs
Vehicle		
NMBS-1 75mpk, QD	< 0.0001	

*CRs, Complete Responses; No measurable tumor

CONCLUSION AND FUTURE STEPS

An oral, potent, highly selective small molecule HPK1 inhibitor demonstrates *in vivo* tumor growth inhibition as both monotherapy and in combination with anti-CTLA4 in the CT-26 model. Effects shown are consistent with *in vivo* activity demonstrated in published genetic knock-out / kinase dead knock-in studies[1,2]. Further evaluation of this selective inhibitor in other *in vivo* models will continue to elucidate the value of HPK1 inhibition as a novel immunomodulatory approach for anti-tumor immunity.

ACKNOWLEDGEMENTS

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REFERENCES

1. Hernandez et al., 2018, Cell Reports 25, 80–94.
2. Liu et al., 2019, PLOS One 14 (3), 1-18.

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