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

David Ciccone[1], Vad Lazari[2], Ian Linney[2], Michael Briggs[2], Samantha Carreiro[1], Ben Whittaker[2], Stuart Ward[2], Grant Wishart[2], Eric Feyfant[3], Jeremy Greenwood[3], Abba Leffler[3], Alexandre Cote[3], Steven Albanese[3], Ian Waddell[2], Chris Hill[2], Christine Loh[1], Peter Tummino[1], Joshua McElwee[1], Alan Collis[1], and Neelu Kaila[1]

[1]Nimbus Therapeutics, Cambridge, MA, USA; [2]Charles River Laboratories, Chesterford Research Park, United Kingdom; [3]Schrödinger, New York, NY, USA

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. NMBS-2 is a Highly Potent and Exquisitely Selective HPK1 Inhibitor

Assay	NMBS-2
HPK1 Caliper IC_{50} @ 1mM ATP	< 1 nM
pSLP76 Jurkat Cell IC_{50}	42 nM

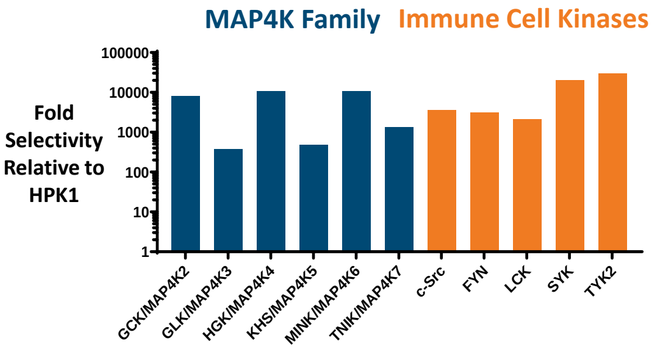


FIGURE 2. HPK1 Inhibition Enhances IL-2 and IFNγ Production From Stimulated Human T Cells

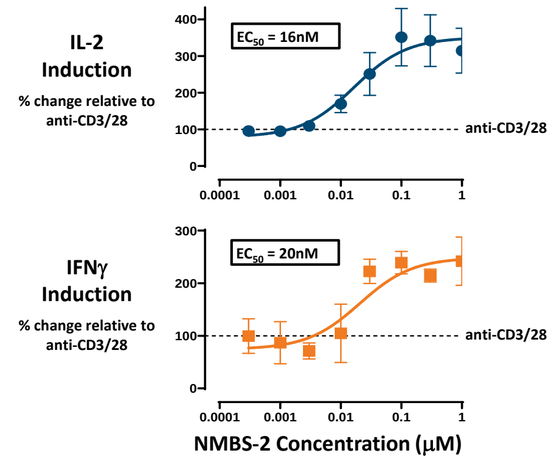


FIGURE 3. NMBS-2 *In Vivo* Dosing ≤30mpk Results in Complete HPK1 Target Engagement

Splenic T cells were purified from NMBS-2 treated animals 2hrs post anti-CD3 administration. pSlp76 in cell lysates was analyzed by ELISA.

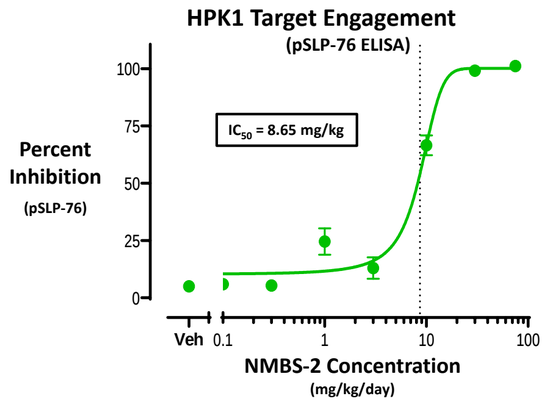
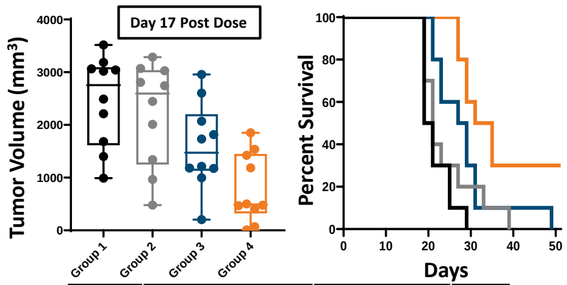


FIGURE 4. NMBS-2 Induces Robust Tumor Growth Inhibition as a Single Agent and in Combination with anti-PD1 in the CT-26 Syngeneic Model



Group	Treatment	#P_{value} (t-test)	*CRs
1	Vehicle, p.o.		
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

#P_{value}, Vehicle Comparison *CRs, Complete Responses; No Measurable Tumor

FIGURE 5. Increased Serum Cytokines and Circulating Antibodies (Pharmacodynamic Responses) in NMBS-2 Treated CT-26 Animals

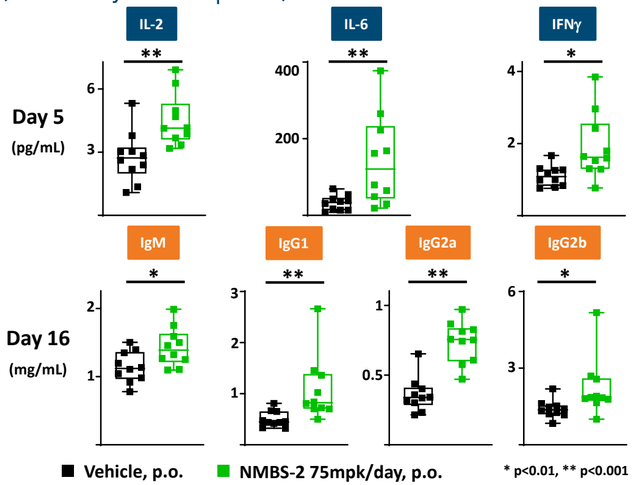
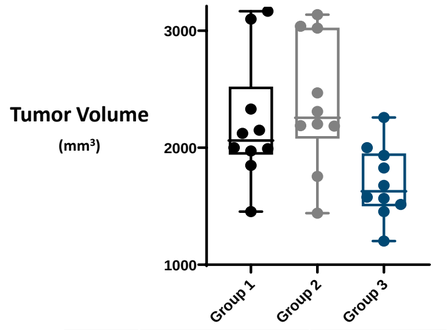


FIGURE 6. NMBS-2 Induces Robust Tumor Growth Inhibition as a Single Agent in the B16-F10 Syngeneic Model



Group	Treatment	P_{value} (t-test)
1	Vehicle, p.o.	
2	anti-PD1 10mpk, i.p., BIW	0.52
3	NMBS-2 150mpk/day, p.o.	0.02

#P_{value}, Vehicle Comparison

CONCLUSIONS AND FUTURE STEPS

An oral, potent, highly selective small molecule HPK1 inhibitor demonstrates *in vivo* tumor growth inhibition as a single agent and in combination with anti-PD1 in syngeneic models. Observed pharmacodynamic responses in syngeneic models, including increased serum cytokines and circulating plasma antibody levels, are consistent with comprehensive immune cell activation. Further evaluation of this selective inhibitor in additional immune cell-based assays will continue to build upon the mechanistic understanding of HPK1 inhibition as a novel immunomodulatory approach for anti-tumor immunity.

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130 Prospect Street, Suite 301, Cambridge, MA 02139 | Office Tel. +1 (857) 999-2009 nimbustx.com