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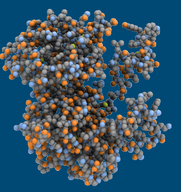


Hematopoietic Progenitor Kinase 1 (HPK1) Inhibition Enhances Antibody Secretion, Pro-inflammatory Cytokine Production and Proliferation of Primary Human B Cells

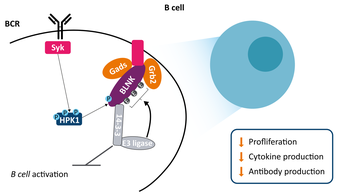
David Ciccone[a], Fu-Shan Kuo, Scott Boiko, Scott Daigle, Samantha Carreiro, Alexander Benzell, Neelu Kaila, Gene Yau, Esha A. Gangolli, Denise Levasseur, Bhaskar Srivastava, Frank G. Basile and Christine Loh
Nimbus Therapeutics, Boston, MA, USA

nimbus THERAPEUTICS

BACKGROUND

- Hematopoietic progenitor kinase 1 (HPK1), a member of the MAP4K family of protein serine/threonine kinases, is involved in negatively regulating signal transduction cascades in cells of hematopoietic origin (Liu et al, 2019, Hernandez et al, 2018).
  - Upon B-cell receptor (BCR) engagement, HPK1 is rapidly phosphorylated and activated by the BCR-associated kinase Syk (Wang et al, 2012).
  - Activated HPK1 phosphorylates Thr 152 of the B-cell linker protein (BLNK), an adapter protein that potentiates B-cell signaling by stabilizing a signaling 'hub' at localized phospholipid rafts (Wang et al, 2012).
  - Phosphorylation of BLNK leads to 14–3–3 recruitment, ubiquitylation, and proteasomal degradation of activated BLNK, thus inhibiting BCR signaling and B-cell activation (**Figure 1**) (Wang et al, 2012).
  - In vivo studies in HPK1 kinase-dead mice showed that HPK1 activity was pivotal for suppression of a wide range of immune cells including CD4+, CD8+, dendritic cells, natural killer cells, and regulatory T cells (Liu et al, 2019).
  - Taken together, the above information suggests that HPK1 inhibition should enhance B-cell activation

Figure 1. B-Cell Activation is Negatively Regulated by HPK1

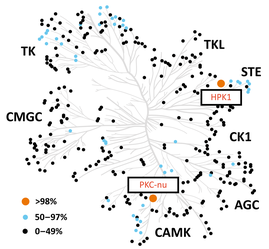


- NDI-101150 is a potent and highly selective HPK1 small molecule inhibitor
  - In vitro and in vivo studies of NDI-101150 showed dose-dependent activation of a wide range of immune cells and significant tumor growth inhibition in murine syngeneic mouse models, respectively (Ciccone et al, 2023)
  - Preliminary NDI-101150 clinical data show antitumor activity and good tolerability in patients with advanced solid tumors (Sommerhalder et al, 2023)
- We assessed the effects of NDI-101150 on antitumor B-cell activation both in vitro and in Balb/c animals following keyhole limpet hemocyanin (KLH) immunization, as well as in the EMT-6 syngeneic murine model

METHODS/RESULTS

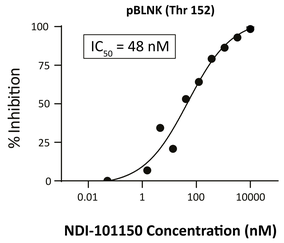
Figure 2. NDI-101150 is a Potent and Exquisitely Selective HPK1 Inhibitor

A structure-based drug design approach was used to generate potent and selective inhibitors of HPK1. Biochemical and biophysical assays, as well as primary human and mouse immune cell-based activation assays, were utilized for multiple rounds of structure-activity relationship studies. Ultimately, NDI-101150 was identified as a highly potent HPK1 inhibitor that shows high selectivity against immune cell-specific kinases and kinases in the MAP4K family



NDI-101150	
Potency	
HPK1 biochemical IC_{50}	0.7 nM
HPK1 cellular IC_{50}	41 nM
FOLD selectivity against MAPK4 family	
GLK (@ 1 mM ATP)	377
KHS (@ 1 mM ATP)	489
TNIK (@ 1 mM ATP)	1,336
HGK (@ 1 mM ATP)	>10,000
MINK (@ 1 mM ATP)	>10,000
FOLD selectivity against immune cell kinases	
FYN (@ 1 mM ATP)	3,110
c-SRC (@ 1 mM ATP)	3,630
LCK (@ 1 mM ATP)	2,143
GCK (@ 10 µM ATP)	>8,000
SYK (@ 10 µM ATP)	>20,000

Figure 3. NDI-101150 Inhibits HPK1-Mediated Phosphorylation of BLNK in Purified Human B Cells



CD19+ B cells were purified from human donors and allowed to rest overnight. The following day, cells were treated with NDI-101150 or DMSO for 1 hour before stimulating with IL-21, IL-4, and anti-CD40 antibody for 30 minutes. Whole cell extracts were prepared and phospho-BLNK (Thr 152) was detected by western blot. The primary human B-cell phosphorylated BLNK (pBLNK) IC_{50} value of 48 nM was similar to the primary human T cell pSLP-76 IC_{50} value of 28 nM

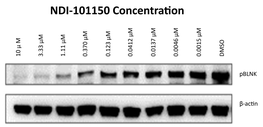
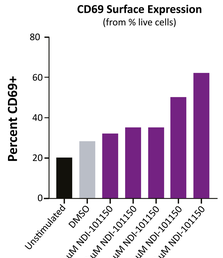
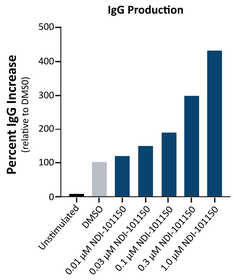


Figure 4. NDI-101150 Dose-Dependently Increases CD69 Expression, IgG Secretion, and Proliferation of Human B Cells

CD19+ B cells were purified from human donors and allowed to rest overnight. The following day, B cells were pre-treated with various concentrations of NDI-101150 or DMSO for 1 hour before stimulating with either CD40L or a combination of anti-CD40, IL-21 and IL-4. Expression of CD69 on the surface of activated B cells was determined by FACs at 2 hours post-stimulation (purple bars), IgG production from activated B cells was captured and quantified by ELISA at 4 days post-stimulation (blue bars), and proliferation of activated B cells was determined using CellTiter-Glo® at 5 days post-stimulation (orange bars).





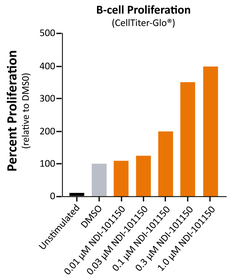
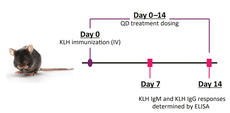


Figure 5. NDI-101150 Treatment Enhances KLH-Specific Antibody Production *In Vivo*

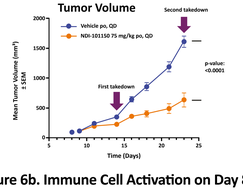


Treatment group	Immunization
Group 1: naïve animals	PBS
Group 2: vehicle, PO, QD	KLH
Group 3: NDI-101150, 75 mg/kg, PO, QD	KLH

Figure 6a-d. NDI-101150 - Treated Murine EMT-6 Tumors Show Hallmarks of B-Cell Activation and B-Cell Infiltration

Mice were injected SC with EMT-6 tumor cells into the right flank and were randomized on Day 0. On Day 8 and Day 17, serum IgG analysis, tumor RNA sequencing, tumor flow cytometry analysis and tumor multiplex IHC were performed

Figure 6a. NDI-101150 Induces Robust Tumor Growth Inhibition that is Accompanied by an Increase in Circulating Total IgG Levels in the EMT-6 Syngeneic Model



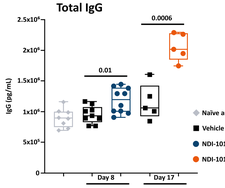
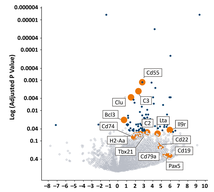


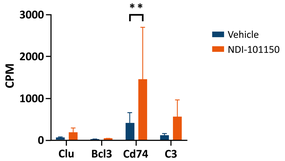
Figure 6b. Immune Cell Activation on Day 8

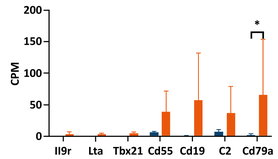
B-cell immunity-associated genes on Day 8
Volcano plot based on differential analysis between NDI-101150-treated tumor and vehicle-treated tumor on Day 8. Differential expressed genes are defined by log2 fold change >|1| and padj < 0.05



Day 8 GO: biological process	Adjusted p-value
Immune system process	7.31E-06
Lymphocyte activation	0.0001
Leukocyte activation	0.0005
Lymphocyte-mediated immunity	0.001
B-cell-mediated immunity	0.002
T-cell activation	0.004
Cytokine production	0.012
Immune effector processes	0.02
Leukocyte-mediated immunity	0.02
CD4+ αβ T cell differentiation	0.03

Expression of B-cell-mediated immunity-associated genes on Day 8
Two-way ANOVA, ** indicates padj < 0.01





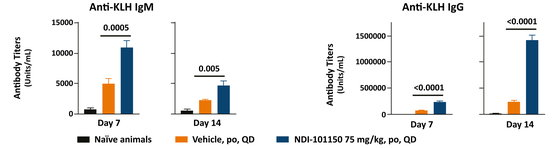


Figure 6c. NDI-101150 Increases Total lymphocyte and B Cell Infiltration into EMT-6 Tumors as Measured by FACS

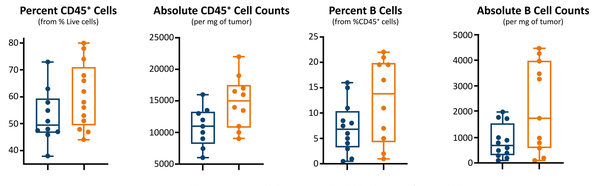
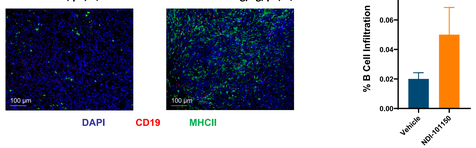


Figure 6d. Multi-Plex IF Staining Shows that NDI-101150 Treated EMT-6 Tumors Have Increased CD19+ B cells on Day 17

Two-way ANOVA; *indicates padj <0.05



CONCLUSIONS

- NDI-101150 is a highly potent and selective inhibitor of HPK1 achieving sub-nanomolar biochemical potency and greater than 300-fold selectivity across the MAP4K family.
- HPK1 inhibition results in a dose-dependent decrease in phospho-BLNK, a validated PD biomarker in B cells isolated from human peripheral blood mononuclear cells (PBMCs).
- NDI-101150 enhances the activity of human B cells ex vivo as shown by increasing pro-inflammatory cytokine secretion and proliferation as well as enhanced cell-surface expression of activation markers and antibody production.
- HPK1 inhibition results in increased antigen-specific antibody production in vivo in response to immunization with KLH.
- NDI-101150 induces robust tumor growth inhibition in the EMT6 syngeneic model, which includes induction of increased circulating antibody levels, enhanced B-cell activation and infiltration into tumors.
- NDI-101150 is currently being investigated in a first-in-human multi-center open-label phase 1/2 trial (NCT05128487) as monotherapy or in combination with pembrolizumab in patients with advanced solid tumors.

*Presenting author: David Ciccone: david.ciccone@nimbustx.com

Disclosures: David Ciccone, Fu-Shan Kuo, Scott Boiko, Scott Daigle, Samantha Carreiro, Alexander Benzell, Neelu Kaila, Gene Yau, Esha A. Gangolli, Denise Levasseur, Bhaskar Srivastava, Frank G. Basile and Christine Loh are employees of Nimbus Therapeutics.

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