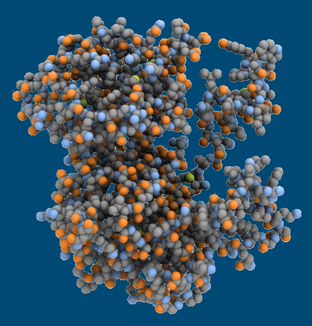

NDI-101150 Is a Potent and Highly Selective Hematopoietic Progenitor Kinase 1 (HPK1) Inhibitor That Promotes a Robust and Broad Anti-Tumor Immune Response

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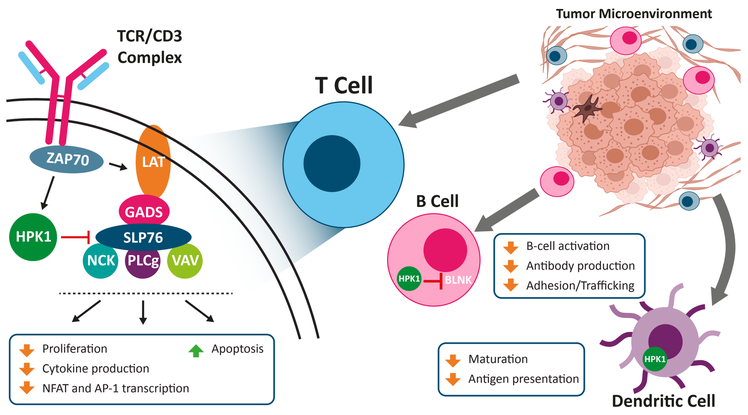
BACKGROUND

- Hematopoietic progenitor kinase 1 (HPK1) is a member of the MAP4K family of protein serine/threonine kinases that negatively regulates activation signals in multiple immune cells and is an attractive therapeutic target in many cancers (**Figure 1**).[1,2]
- Using structure-based drug design, we developed a highly selective HPK1 inhibitor, NDI-101150, with nanomolar potency and physiochemical properties suitable for once daily oral administration.
- NDI-101150 enhanced the activity of B cells ex vivo, increased antigen-specific antibody production in vivo, and induced robust tumor growth inhibition in an EMT-6 syngeneic model.[3]
- Preliminary clinical data indicate that NDI-101150 has antitumor activity and good tolerability in patients with advanced solid tumors.[4] We report the effects of NDI-101150 on a range of lymphoid and myeloid cells, as well as its antitumor and pharmacodynamic immunomodulatory activity.

Figure 1: HPK1 is a Compelling Immuno-Oncology Therapeutic Target



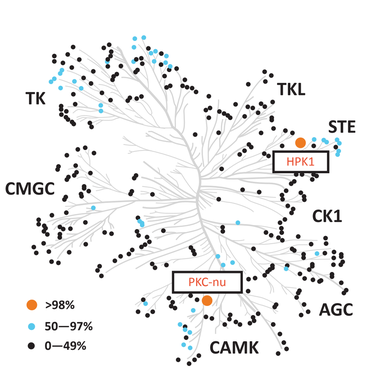
AP-1, activator protein 1; BLNK, B-cell linker protein; GADS, GRB2 related adaptor protein downstream of Shc; HPK1, hematopoietic progenitor kinase 1; LAT, linker for activation of T cells; NCK, non-catalytic region of the tyrosine kinase; NFAT, nuclear factor of activated T cells; PLCg, phospholipase C, gamma 1; SLP76, SH2 domain containing leukocyte protein of 76kDa; TCR, T cell receptor; ZAP70, zeta-chain-associated protein kinase 70

- Negative regulator of T cell-, B cell-, and dendritic cell-mediated immune responses
- Tissue-specific expression – only in hematopoietic cells
- Genetically validated target:
  - HPK1–/– mice are resistant to growth of Lewis lung carcinoma and have enhanced antitumor T cell responses
  - HPK1 kinase-inactive knock-in mice show impaired GL261 tumor growth, which is associated with increased T cell infiltration

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 (SAR) studies. NDI-101150 was identified as a highly potent HPK1 inhibitor that shows high selectivity against T 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 MAP4K 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 SLP76 and BLNK in Purified Human Lymphocytes

T Cells: PBMCs were isolated from human donors and treated with NDI-101150 or DMSO for 1 hour before stimulating with anti-CD3/28 for 30 minutes. Phospho-SLP76 (S376) was detected by FACS in both $CD4^+$ and $CD8^+$ T cells.

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.

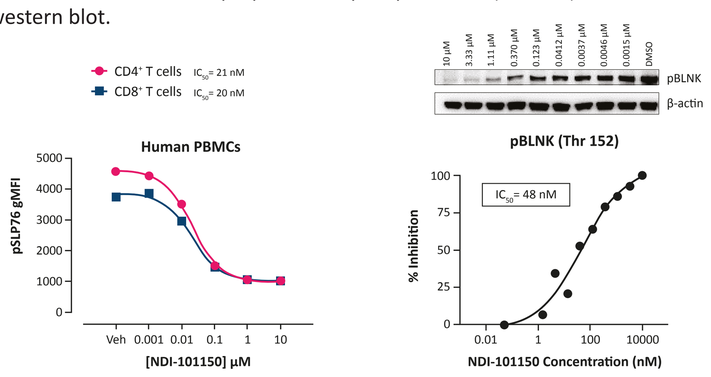
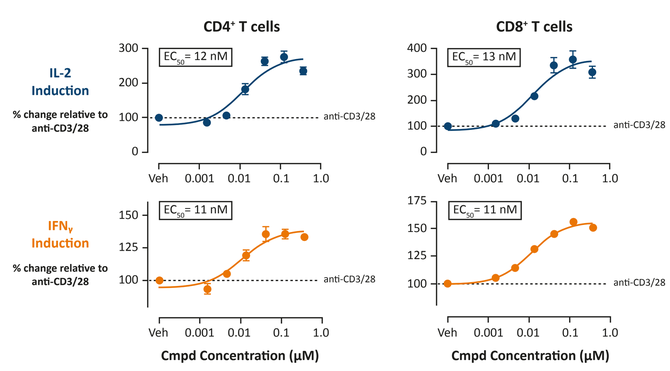


Figure 4. NDI-101150 Enhances IL-2 and IFNγ Production by Human T Cells

$CD4^+$ and $CD8^+$ T cells were purified from human donors and stimulated with anti-CD3/28 in the presence of NDI-101150. Cytokine production was assayed by MSD.



METHODS/RESULTS

Figure 5. NDI-101150 Reinvigorates Exhausted Human T Cells and Demonstrates Synergy with anti-PD1

T cells purified from human donors were exhausted in vitro through 5 iterative cycles of stimulation with anti-CD3/28 for 24 hrs followed by resting for 24 hrs. Exhausted T cells were then used in MLR experiments in the presence of NDI-101150, anti-PD1, or both, and cytokine production was measured by MSD.

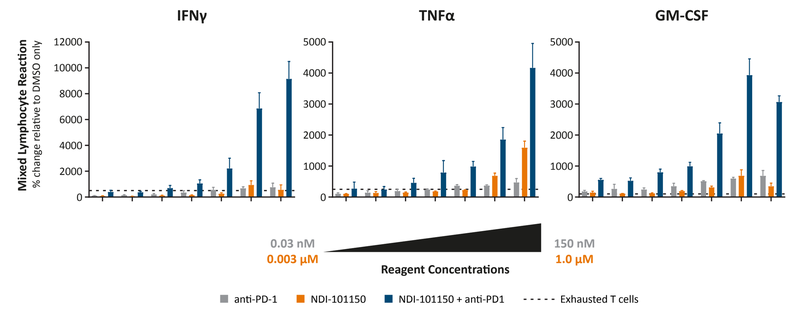


Figure 6. HPK1 Inhibition Overcomes Multiple Immunosuppressive Mechanisms

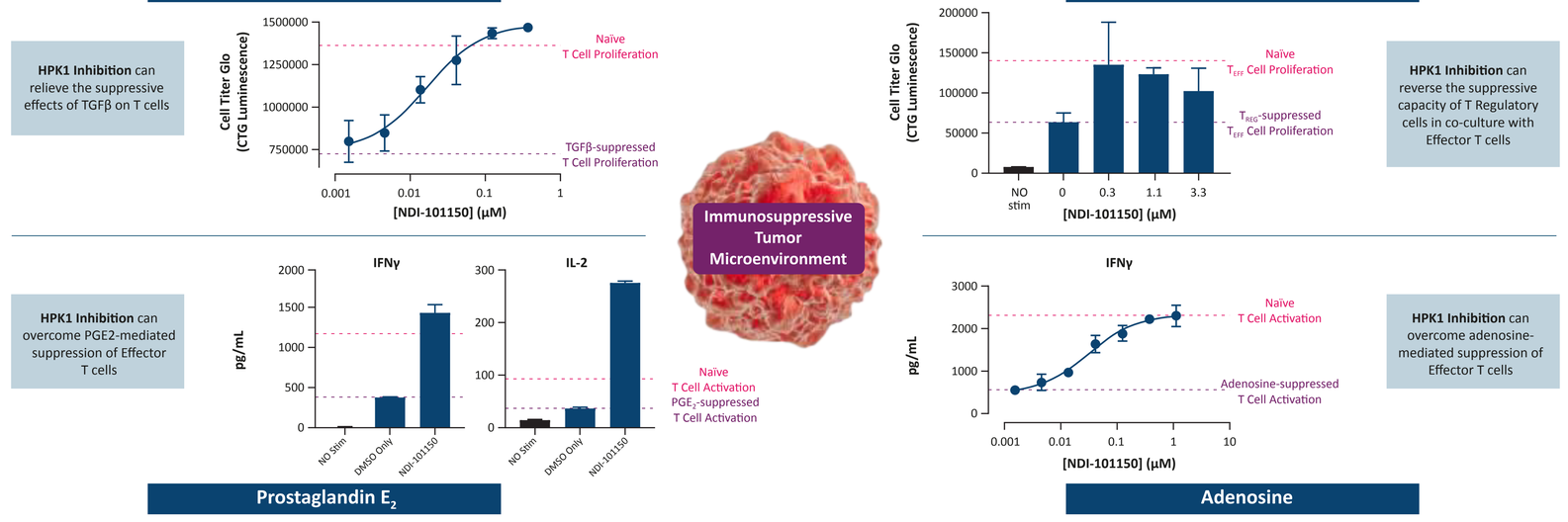


Figure 7. NDI-101150 Treatment Enhances the Activity of Human B Cells

B cells were purified from human donors, pre-treated with various concentrations of NDI-101150 or DMSO for 1 hour, and then stimulated. Expression of CD69 on the surface of B cells was determined by FACS at 2 hours post-stimulation (purple bars), IgG production from B cells was quantified by ELISA at 4 days post-stimulation (blue bars), and proliferation of B cells was measured using CellTiter Glo® at 5 days post-stimulation (orange bars).

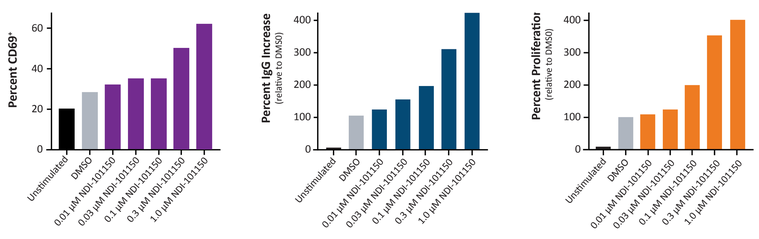


Figure 8. NDI-101150 Enhances Cytokine Secretion, Co-Stimulatory Receptor Expression, and Antigen-Presentation Capacity of DCs

Cytokine production via ELISA (orange bars) and costimulatory receptor expression via FACS (light blue bars) were measured from bone marrow-derived dendritic cells (BMDCs) matured in the presence of various concentrations of NDI-1011502 or DMSO. BMDCs pulsed with OVA peptide and cultured in the presence of NDI-101150 were assayed for their ability to activate purified OTI $CD8^+$ T cells (blue bars).

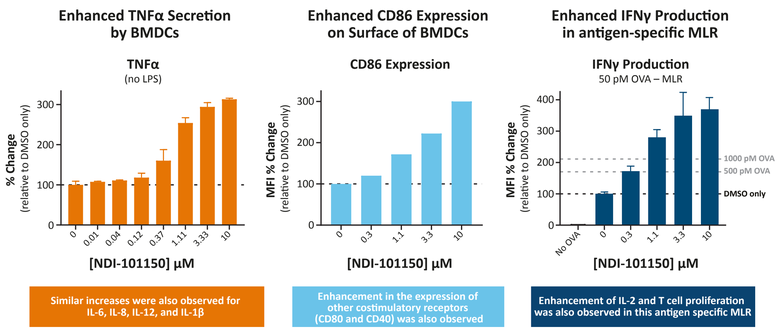
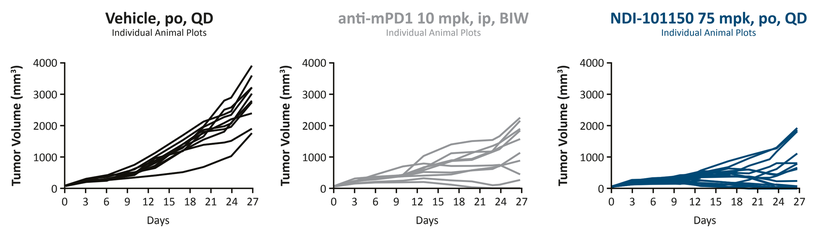


Figure 9. NDI-101150 Treatment Induces Robust Tumor Growth Inhibition and a Durable Immune Memory Response in EMT-6 Syngeneic Mouse Model

NDI-101150 treatment resulted in multiple complete responses (CRs, no measurable tumor) in the EMT-6 syngeneic model. Compound-dosing was discontinued in these animals and after the 5-day washout period, animals were re-challenged with EMT6 cells in the opposite flank and tumor growth was monitored for an additional 28 days (no further treatment was administered).



Treatment Group	P-Value (T-test)	CRs
Vehicle, po, QD		
Anti-mPD1 10 mpk, ip, BIW	0.0002	1
NDI-101150 75 mpk, po, QD	< 0.0001	6

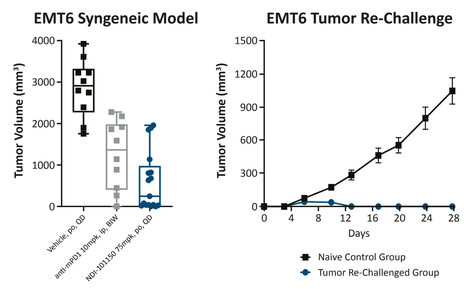
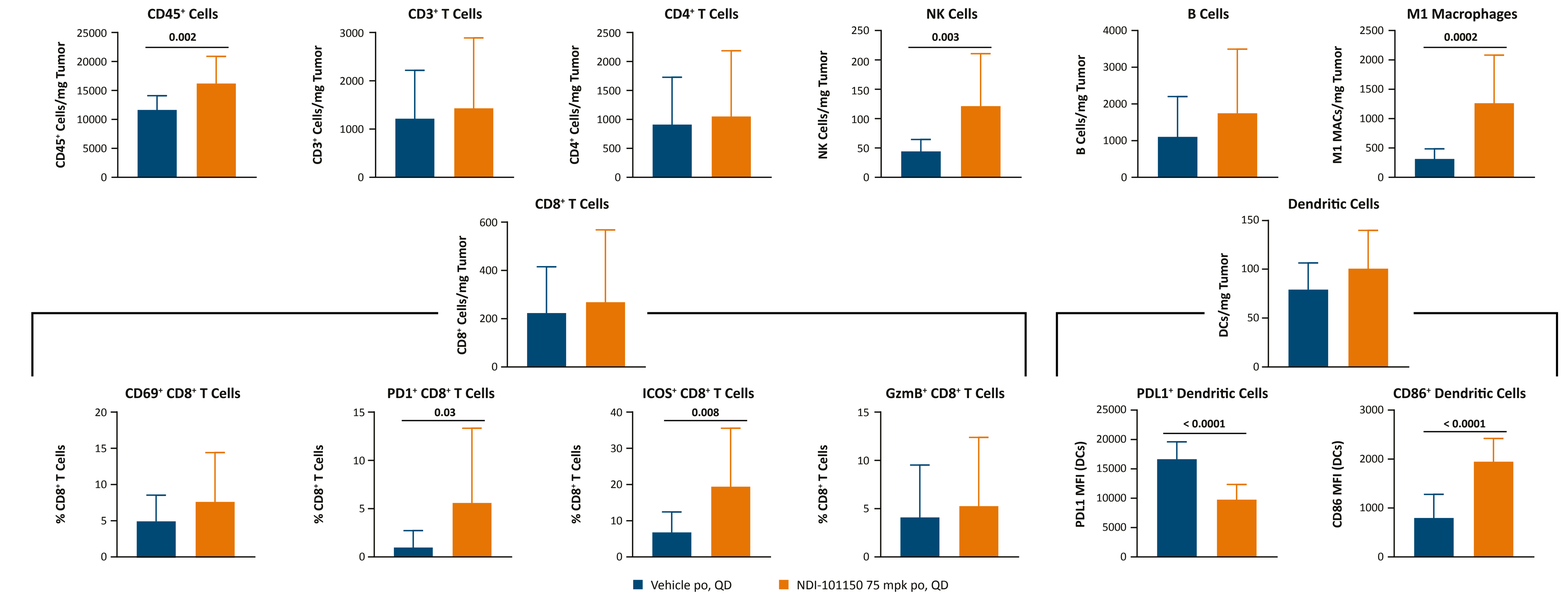


Figure 10. NDI-101150-Treated Murine EMT-6 Tumors are Infiltrated with Cytotoxic $CD8^+$ T cells, $CD19^+$ B cells, and Dendritic Cells

Mice were injected SC with EMT-6 tumor cells into the right flank and randomized once a mean tumor volume of 100 mm^3 was reached. Tumor-infiltrating lymphocytes were identified and characterized by FACS and multiplex immunofluorescence.



CONCLUSIONS

- The data we present support the use of NDI-101150 in creating a powerful multifaceted antitumor immune response alone or in combination with immune checkpoint inhibitor therapies
- 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.

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Disclosures
DC, F-S K, SB, SD, SC, NK, GY, EAG, DL, BS, FGB, and **CL** are employees of Nimbus Therapeutics (Nimbus Discovery Inc. on behalf of Nimbus Saturn Inc.)

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