NDI-101150 is a Potent and Highly Selective HPK1 Inhibitor that Both Synergizes with and Differentiates from anti-PD1 Immune Checkpoint Blockade

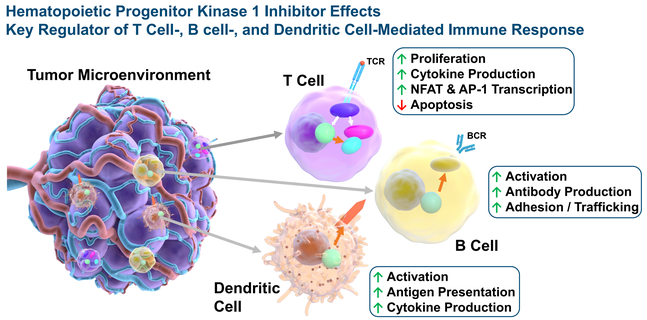
David Ciccone[1], Fu-Shan Kuo[1], Lili Chai[2], Scott Daigle[1], Neelu Kaila[1], Gene Yau[1], Mark Carlson[1], Bhaskar Srivastava[1], Peter Tummino[1], and Christine Loh[1]

[1]Nimbus Therapeutics, Boston, MA, USA; [2]ICE Biosciences, Beijing, China

BACKGROUND

Introduction

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 for many cancers [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.



Methods

Mechanistic activity of HPK1 inhibition alone or in combination with anti-PD1 checkpoint blockade was studied in vitro across multiple lymphoid and myeloid cell populations. These studies include human T cell exhaustion experiments, human mixed lymphocyte reactions, human B cell and dendritic cell activation, human T and B cell RNA sequencing, as well as various human T cell immunosuppressive assays. Anti-tumor efficacy and pharmacodynamic immune modulatory effects of NDI-101150 alone or in combination with immune checkpoint blockade therapies were analyzed across multiple murine syngeneic tumor models.

Results

HPK1 inhibition with NDI-101150 has a differentiated pharmacology profile from PD1 blockade. NDI-101150 was able to overcome the immunosuppressive effects of prostaglandin E2, adenosine, and TGF-β on human T cell activation. HPK1 inhibition also directly limited the suppressive capacity of T regulatory cells. In contrast, anti-PD1 treatment was not able to overcome the immunosuppressive activity associated with any of these mechanisms. In vivo, HPK1 inhibition significantly enhanced the production of antigen-specific antibodies in response to immunization with both T-dependent and T-independent antigens, while anti-PD1 treatment had minimal to no effects on antibody production. In the EMT-6 syngeneic model, 7/10 mice receiving NDI-101150 exhibited complete tumor regressions, compared to only 1/10 from an anti-PD1 cohort. Furthermore, NDI-101150 established a robust and durable immune memory response as 100% of treated mice showed complete rejection of tumor growth upon subsequent re-challenge.

HPK1 inhibition with NDI-101150 also shows robust effects in combination with PD1 blockade in vitro and in vivo. Both NDI-101150 and anti-PD1 were able to reinvigorate exhausted human T cells, restoring cytokine production and proliferative effects in mixed lymphocyte reactions. Furthermore, a combination of NDI-101150 and anti-PD1 synergized to enhance the activity of exhausted human T cells to an extent exceeding that observed with naïve human T cells in similar experiments. In vivo, while NDI-101150 or anti-PD1 treatments alone induced tumor growth inhibition in the murine CT-26 syngeneic tumor model, NDI-101150 and anti-PD1 in combination mediated complete tumor regressions in several mice. Furthermore, NDI-101150 seemed to induce a durable immune memory response as evidenced by the combination-treated animals showing complete rejection of tumor growth upon subsequent re-challenge, without any further dosing of NDI-101150.

NDI-101150 Possesses a Highly Selective Kinase Inhibitory Profile

Essential for Efficacy and Therapeutic Index

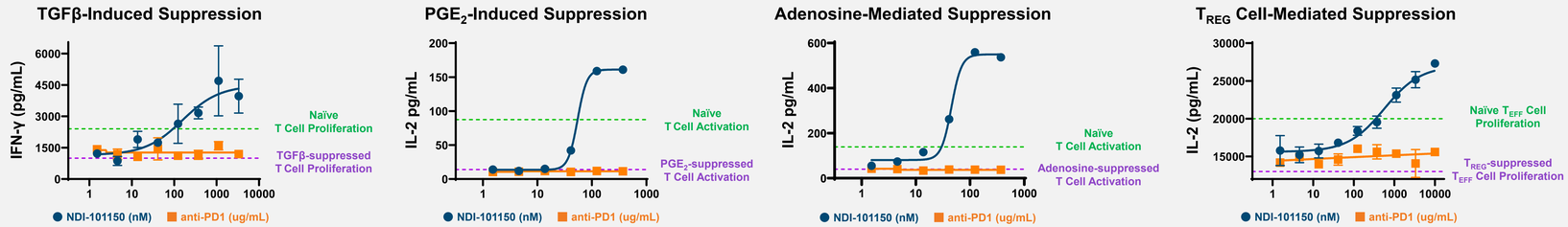
NDI-101150	
Potency	
HPK1 biochemical IC_{50}	0.7 nM
HPK1 cellular IC_{50}	41 nM
FOLD selectivity against MAP4K family	
GLK (@ 1 mM ATP)	377
KHS (@ 1 mM ATP)	489
TNIK (@ 1 mM ATP)	1,336
HGK (@ 1 mM ATP)	>10,000
MINK (@ 1 mM ATP)	>10,000
FOLD selectivity against immune cell kinases	
FYN (@ 1 mM ATP)	3,110
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

- **NDI-101150 shows high degree of selectivity**
  - Against the kinome, including closely related MAP4K family members
  - Against kinases involved in positive signaling from immune receptors
- **Enables desired pharmacology profile**
  - Specifically inhibit HPK1 to release the "brake" on immune receptor signaling
  - Without inhibiting kinases that "accelerate" immune receptor signaling
- **Better therapeutic window**
  - Avoid disrupting the functional activity of other kinases

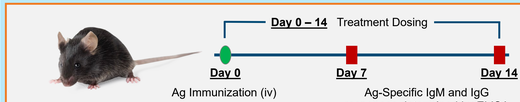
RESULTS

Differentiated Pharmacology Profile of NDI-101150 Compared to anti-PD1 Treatment

HPK1 Inhibition with NDI-101150 can Overcome Multiple Immunosuppressive Mechanisms Commonly Associated with Tumor Microenvironments while anti-PD1 Treatment has NO Effect



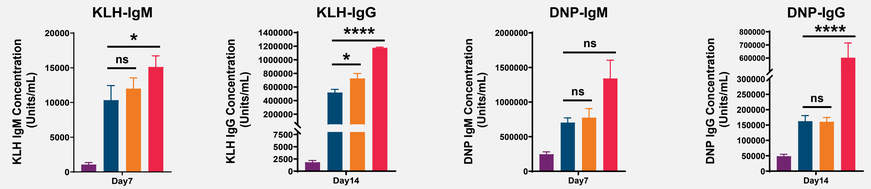
NDI-101150 Treatment Enhances Antigen-Specific Antibody whereas anti-PD1 Treatment has NO Effect



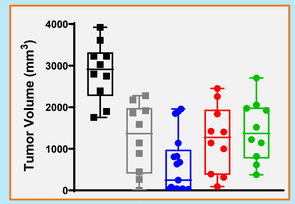
Treatment	Immunization (Ag)
Naïve Animals	PBS
Vehicle, po	KLH or DNP-Ficoll
anti-PD1 10mpk, ip, BIW	KLH or DNP-Ficoll
NDI-101150 75mpk/day, po	KLH or DNP-Ficoll

KLH, a **T-Dependent Antigen**, requires T cell help for B cell-mediated antibody production

DNP-Ficoll, a **T-Independent Antigen**, does not invoke T cell help for a B cell antibody response



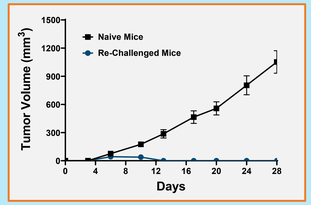
HPK1 Inhibition with NDI-101150 Induces Complete Responses in 6 Mice and Establishes a Durable Immune Memory Response in the Murine Syngeneic EMT-6 Model



Treatment Group	CRs	P-Value (t-test)
Vehicle, po, QD		
anti-mPD1 10mpk, ip, BIW	1	0.0002
NDI-101150 75mpk/day, po	6	< 0.0001
NDI-101150 45mpk/day, po		0.0001
NDI-101150 15mpk/day, po		0.0003

CRs, Complete Response (NO measurable tumor)

- **All 6 mice with CRs were enrolled in a tumor re-challenge study**
  - Treatment dosing was discontinued; animals experienced a 7 day washout
  - CT26 tumor cells were then injected into the opposite flank and tumor growth monitored for 28 days
- **All 6 animals completely rejected tumor growth while tumors grew normally in naïve mice**



Synergistic Activity of NDI-101150 in Combination with anti-PD1 Treatment

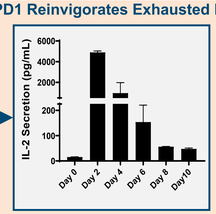
HPK1 Inhibition with NDI-101150 in Combination with anti-PD1 Reinvigorates Exhausted Human T Cells

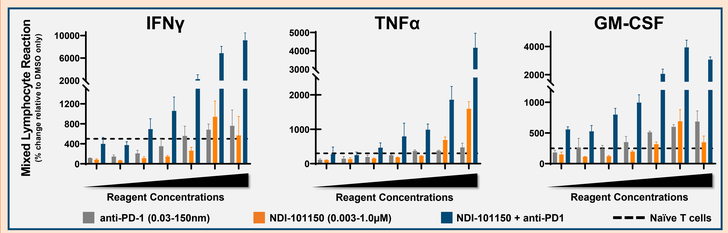
T cells were harvested from PBMCs isolated from 3 human donors and rested overnight

T cells were stimulated with anti-CD3/28 for 24hrs then allowed to rest for an additional 24hrs

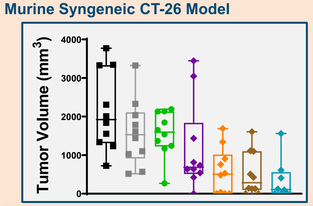
Supernatants were harvested for IL-2 analysis

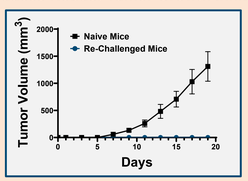
Repeat stimulation/rest cycle for a total of 10 days





NDI-101150 in Combination with anti-PD1 Induces Robust Tumor Growth Inhibition and Durable Immune Memory in the Murine Syngeneic CT-26 Model

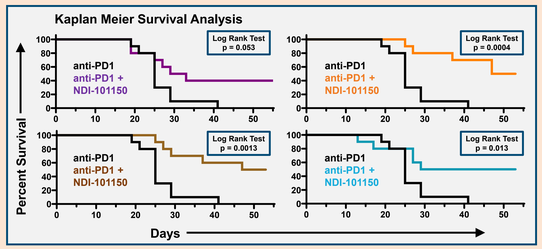




Treatment	CRs	P-Value (t-test)
Vehicle, po		
anti-PD-1 10mpk, ip, BIW		0.19
NDI-101150 75mpk/day, po		0.13
anti-PD-1 10mpk, ip, BIW NDI-101150-2 15mpk/day, po	2	0.38
anti-PD-1 10mpk, ip, BIW NDI-101150 45mpk/day, po	4	0.007
anti-PD-1 10mpk, ip, BIW NDI-101150 75mpk/day, po	4	0.004
anti-PD-1 10mpk, ip, BIW NDI101150 105mpk/day, po	3	0.002

CRs, Complete Response (NO measurable tumor)

- **All 13 mice with CRs were enrolled in a tumor re-challenge study**
  - Treatment dosing was discontinued; animals experienced a 7 day washout
  - CT26 tumor cells were then injected into the opposite flank and tumor growth monitored for 20 days
- **All 13 animals completely rejected tumor growth while tumors grew normally in naïve mice**



CONCLUSIONS

Pharmacological inhibition of HPK1 with NDI-101150 represents a powerful system-wide immunomodulatory approach with the potential to enhance anti-tumor immunity in patients failing to respond to currently approved immune checkpoint therapies or in combination with immune checkpoint blockade therapies [3]. NDI-101150 is currently being tested in a Phase 1/2 trial (NCT05128487) in patients with advanced solid tumors [4].

REFERENCES

1. Alzabin S., et al. Cancer Immunology and Immunotherapy 2010, v59: 419-429.
2. Hernandez S., et al. Cell Reports 2018. v25: 80-94.
3. Ciccone et al., SITC 2023, meeting poster 1340.
4. Sommerhalder et al., SITC 2023, meeting poster 751.

Presented at the AACR Annual Meeting 2024, April 5-10, 2024 - San Diego, CA

Supported by Nimbus Therapeutics, Boston, MA