Discovery of NDI-101150, A Highly Potent and Selective HPK1 Inhibitor for the Treatment of Cancer, Through Structure-Based Drug Design

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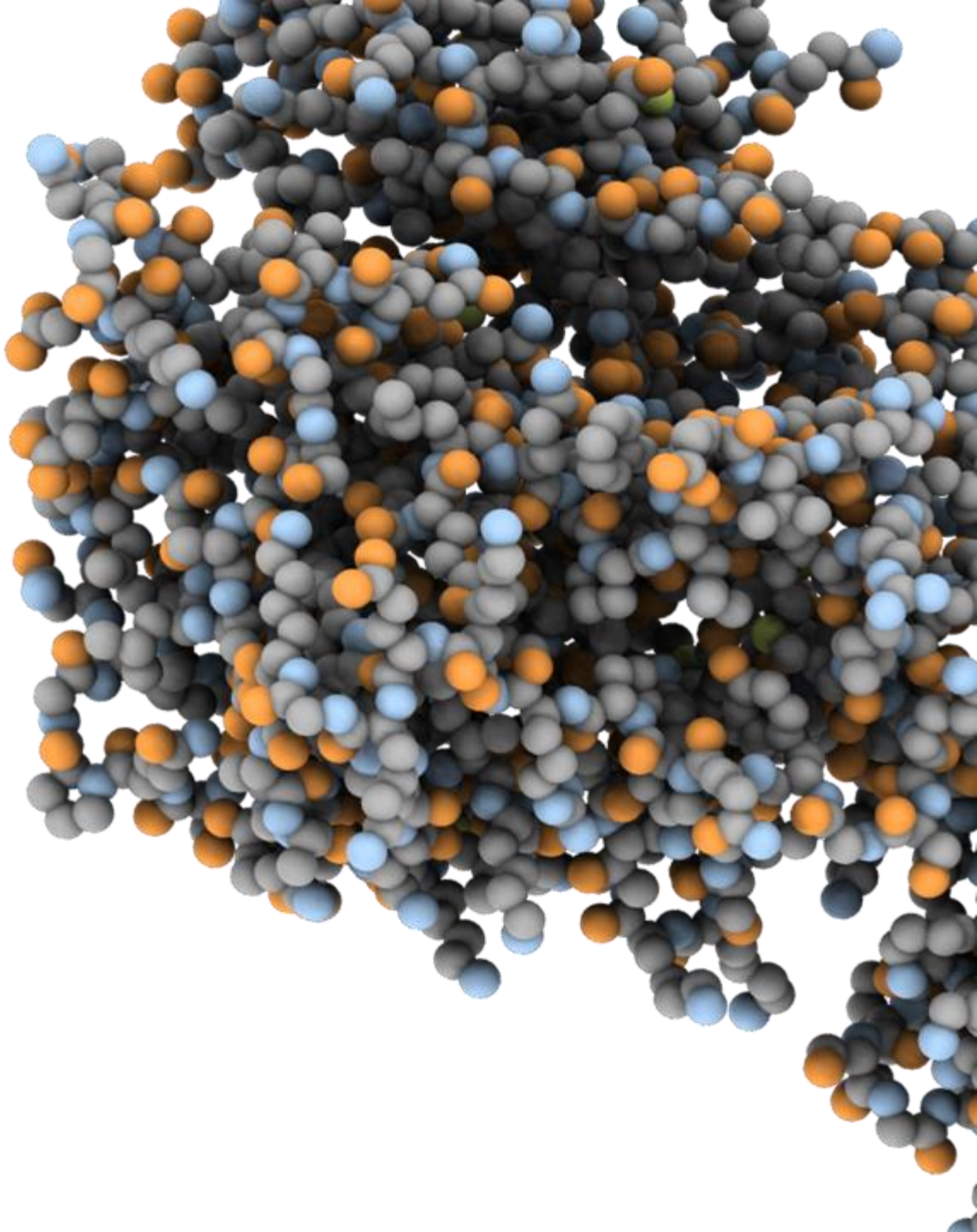
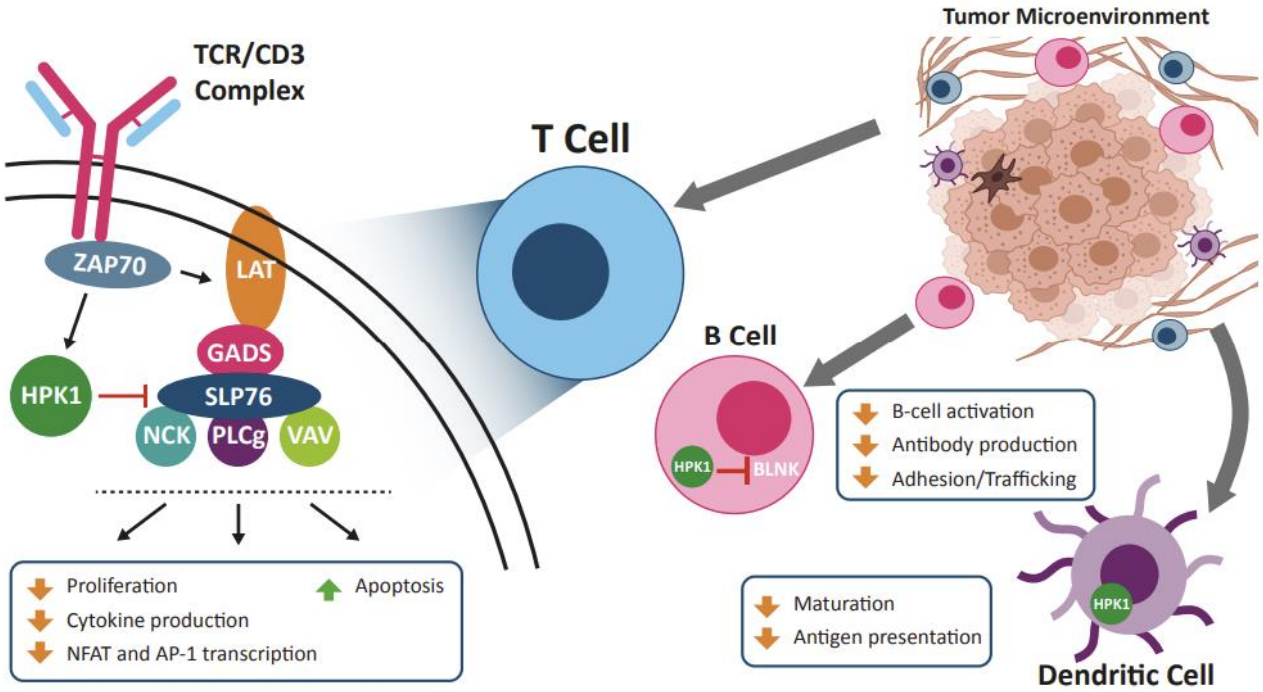
Nimbus Therapeutics

2024



Hematopoietic Progenitor Kinase 1 (HPK1, MAP4K1)

A Compelling Immuno-Oncology Therapeutic Target Due to Role in Broad Multi-Immune Activation



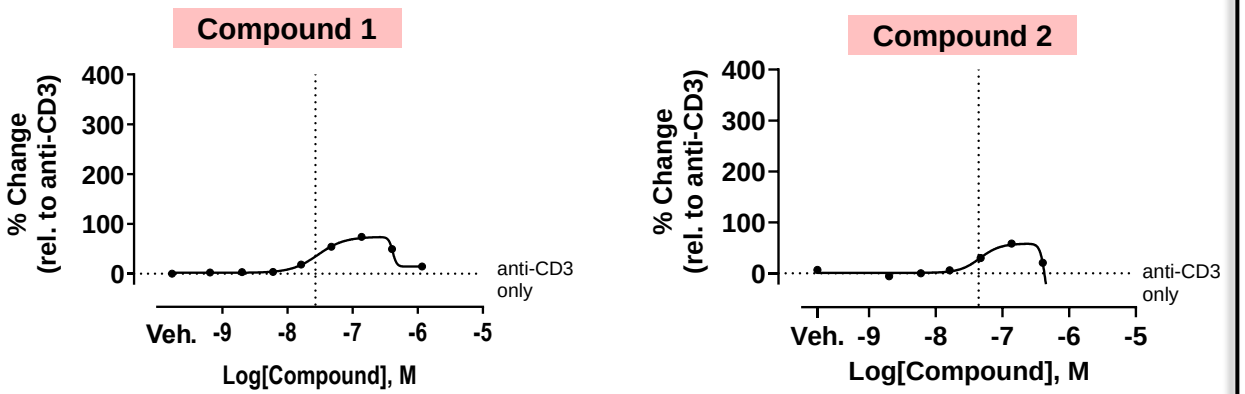
- Negative regulator of T cell, B cell, and dendritic cell-mediated immune response[1,2]
- Expression is restricted to hematopoietic cells
- Genetically validated target
  - HPK1$^{-/-}$ mice have enhanced anti-tumor T-cell response and are resistant to growth of Lewis lung carcinoma[1,2]
  - HPK1 kinase-inactive-knock-in mice show impaired GL261 tumor growth, associated with increased T-cell infiltration[1,2]
- The MAP4K family consists of 6 members with varied and compensatory roles in immune cell signalling and inflammation
- MAP4K1 and MAP4K4 are negative regulators and MAP4K3 (GLK) is a positive regulator of the T-cell activation
- **Key requirements for HPK1 inhibitor design is to get selectivity against**
  - **GLK and kinases involved in the propagation of TCR signalling, such as Src and other STE20-like family of kinases**

1. Alzabin S., et al. Cancer Immunology and Immunotherapy 2010, v59: 419-429.
2. Hernandez S., et al. Cell Reports 2018. v25: 80-94.

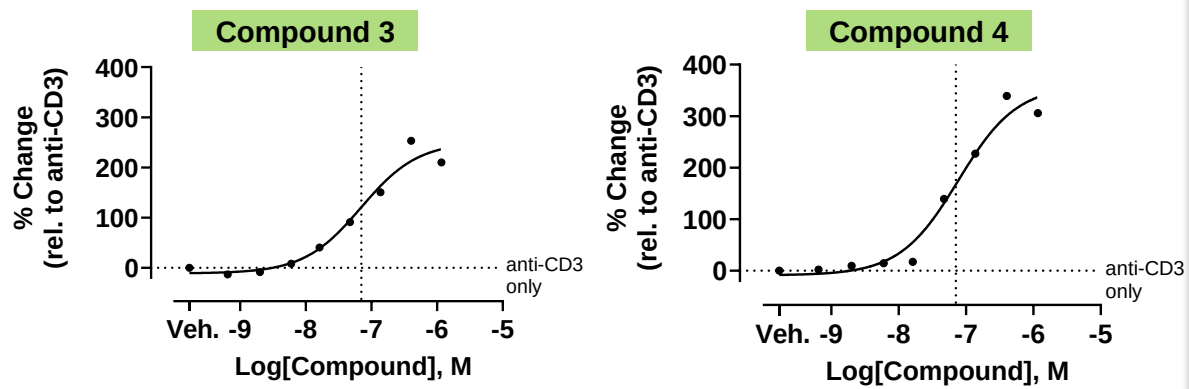
A High Degree of HPK1 Inhibitor Selectivity is Required for Cellular On-target Activity

IL-2 induction in primary mouse T cells: Compounds with superior GLK selectivity have improved functional activity compared to those with poor selectivity. **Potency alone is not enough to induce immune response you need GLK selectivity**

Potent HPK1 inhibitors with poor GLK selectivity



Potent HPK1 inhibitors with good GLK selectivity



Compound	Biochem HPK1 IC_{50} (nM) @ 1mM ATP	HPK1 cell IC_{50} (nM)	GLK Biochem selectivity (Fold vs HPK1 @ 1 mM ATP)
1	1.7	97	3
2	0.5	100	5
3	2	120	150
4	3	93	160

- EC_{50}s shift to the left (more potent) and the magnitude of IL-2 induction (peak response) increases with improved GLK selectivity

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Origins of Nimbus HPK1 Chemical Matter

Virtual Screen

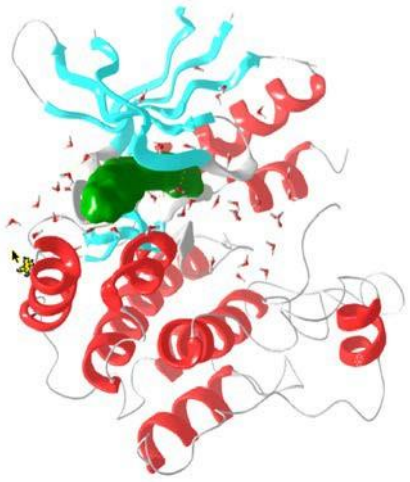
- 4 Receptor ensemble (3 HPK1 & 1 GLK) co-crystals
- Virtual library 11.5 M commercial compounds
- Filters used property space, Glide docking, Wscore
- 14 Hits Identified

Focus:

- SAR by catalog

Deprioritized due to poor potency of hits

Multiple HPK1/ GLK Proprietary Structures Obtained



Nimbus Library Hit

- Excellent affinity
- Poor Selectivity
- FEP/SBDD enabled. Multiple high resolution structures obtained

Focus:

- Improve selectivity
- Further ADME Profiling

Deprioritized in favor of the lead series due to moderate selectivity

Literature-Based Hit Generation

- Identify known literature compounds with potent HPK1 inhibition - Good affinity, Poor selectivity

Focus:

- Obtain an HPK1 co-crystal to build selectivity

Deprioritzed as we could not generate co-crystal with HPK1

Knowledge-Based Hit Generation

- Good affinity & selectivity
- FEP/SBDD enabled
- Lead Series

Focus:

- ADME Optimization

Prioritized due to excellent selectivity against GLK

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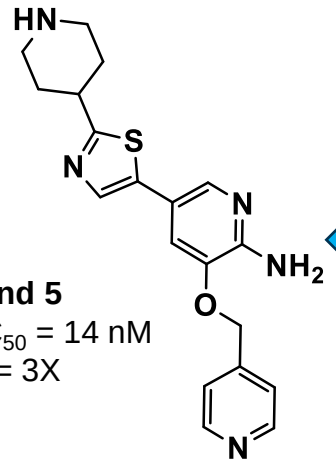
Knowledge Based Hit Generation

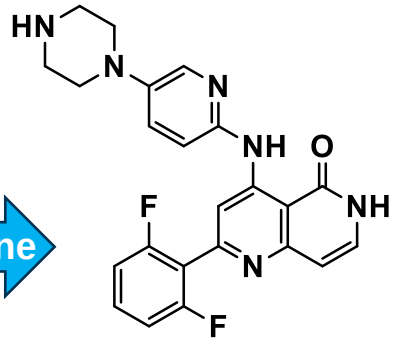
Literature



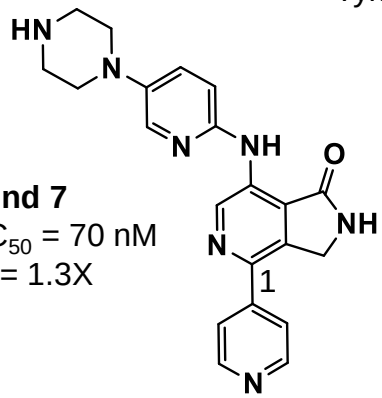
Compound 5
*HPK1 IC_{50} = 14 nM
GLK Sel = 3X
Tyk2 NA

Combine

Nimbus Hit

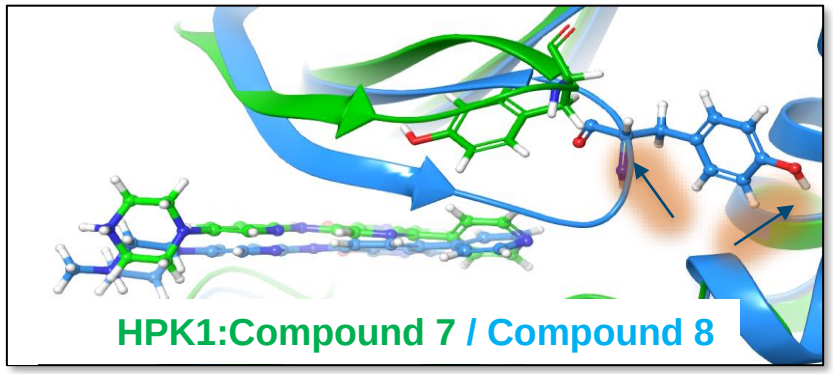


Compound 6
*HPK1 IC_{50} = 4 nM
GLK Sel = 0.17X
Tyk2 Sel = 0.14X



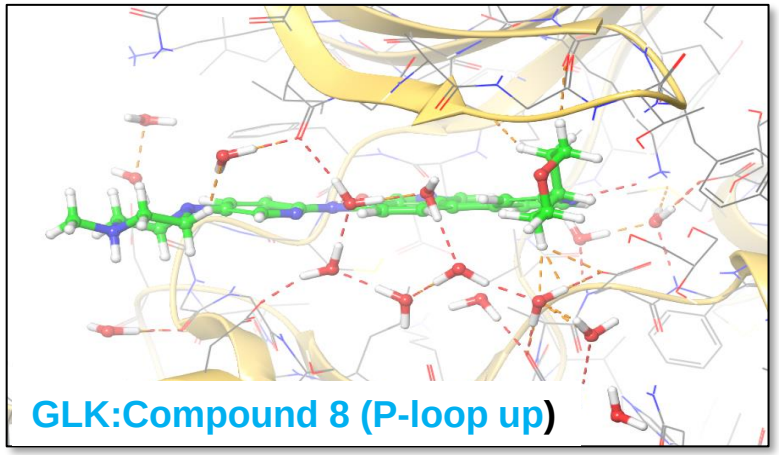
Compound 7
*HPK1 IC_{50} = 70 nM
GLK Sel = 1.3X
Tyk2 NA

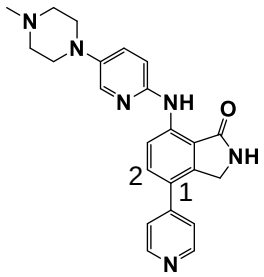
New series (aza isoindolinone) identified



Goal: Overlap of Tyr with C-1 substituent to improve HPK1 potency

P-Loop conformation affects orientation of identical residues
-Tyr 27 away from binding pocket in GLK: **Compound 8**
-Tyr 28 in binding pocket in HPK1:1 **Compound 7**





Compound 8
*HPK1 IC_{50} = 98 nM
GLK Sel = 1.4X
Tyk2 NA

Goal: Break the water network to improve selectivity against GLK

- P-loop interacts directly with water network
- GLK structures show highly defined network near C1/C2 vectors

*Assay run at Km ATP

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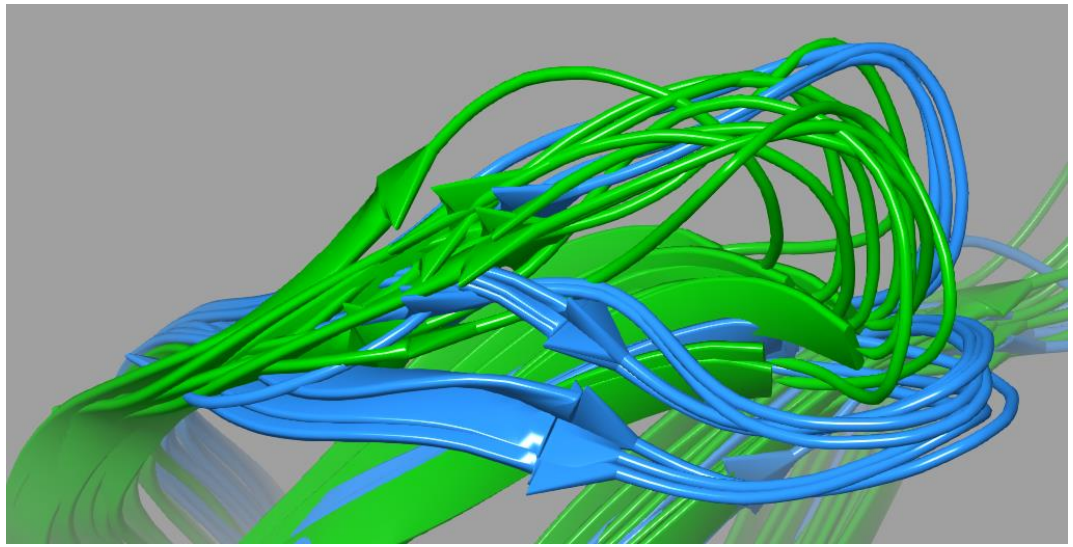
Significant Conformational Flexibility Observed in HPK1 and GLK X-ray Structures

HPK1	GLK
13 Co-crystals (2 constructs) C-Helix, activation and P-loops take on a wide range of conformations	31 Co-structures (5 constructs) Soakable system GLK P-loop alternates between two positions

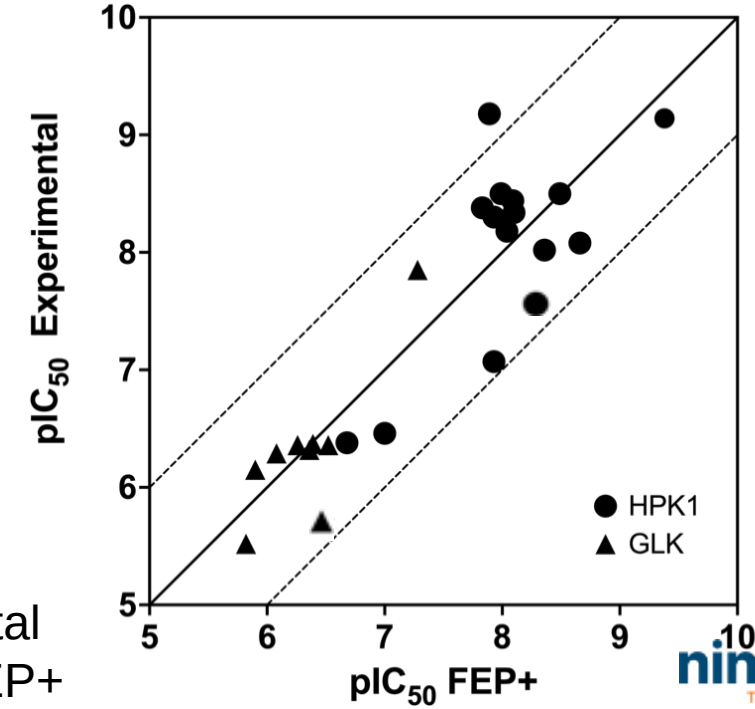
Overlay of HPK1 P-loop and GLK P-loop from co-crystals with different ligands



Flexibility of the P-loop creates challenges and opportunities

- Due to the dynamic nature of the P-loop use of FEP+ to increase potency and selectivity was challenging
- Enhanced flexibility of HPK1 provided a path to selectivity

Prospective FEP+ predictions for HPK1 and GLK plotted against experimental



High-resolution ligand bound crystal structures facilitated the use of FEP+

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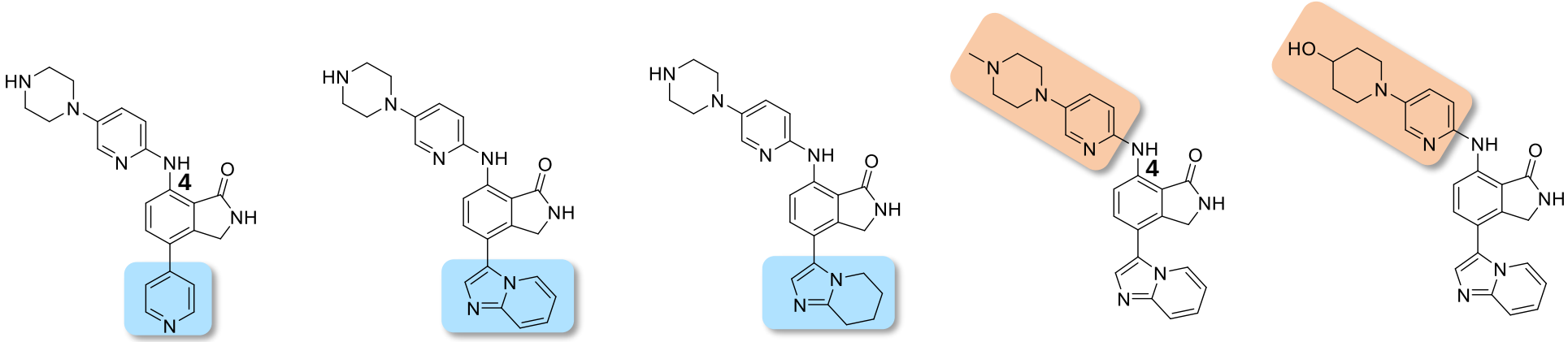
Identification of Potent and Selective Bicyclic Series - Target Interaction with Tyr28



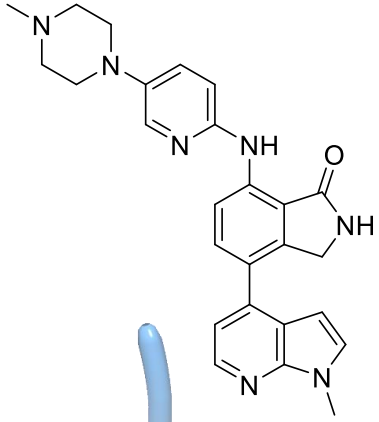
Assay	9	10	11	12	13
HPK1[a] IC_{50} (nM)	43	1.8	15	0.8	2.6
GLK Selectivity[b]	1	26	21	74	46
Caco-2 $P_{app, A-B}$[c] / ER	5.7 / 14	0.6 / 92	0.4 / 29	26 / 2.8	--
AChE IC_{50} (nM)	--	--	--	26	>10,000

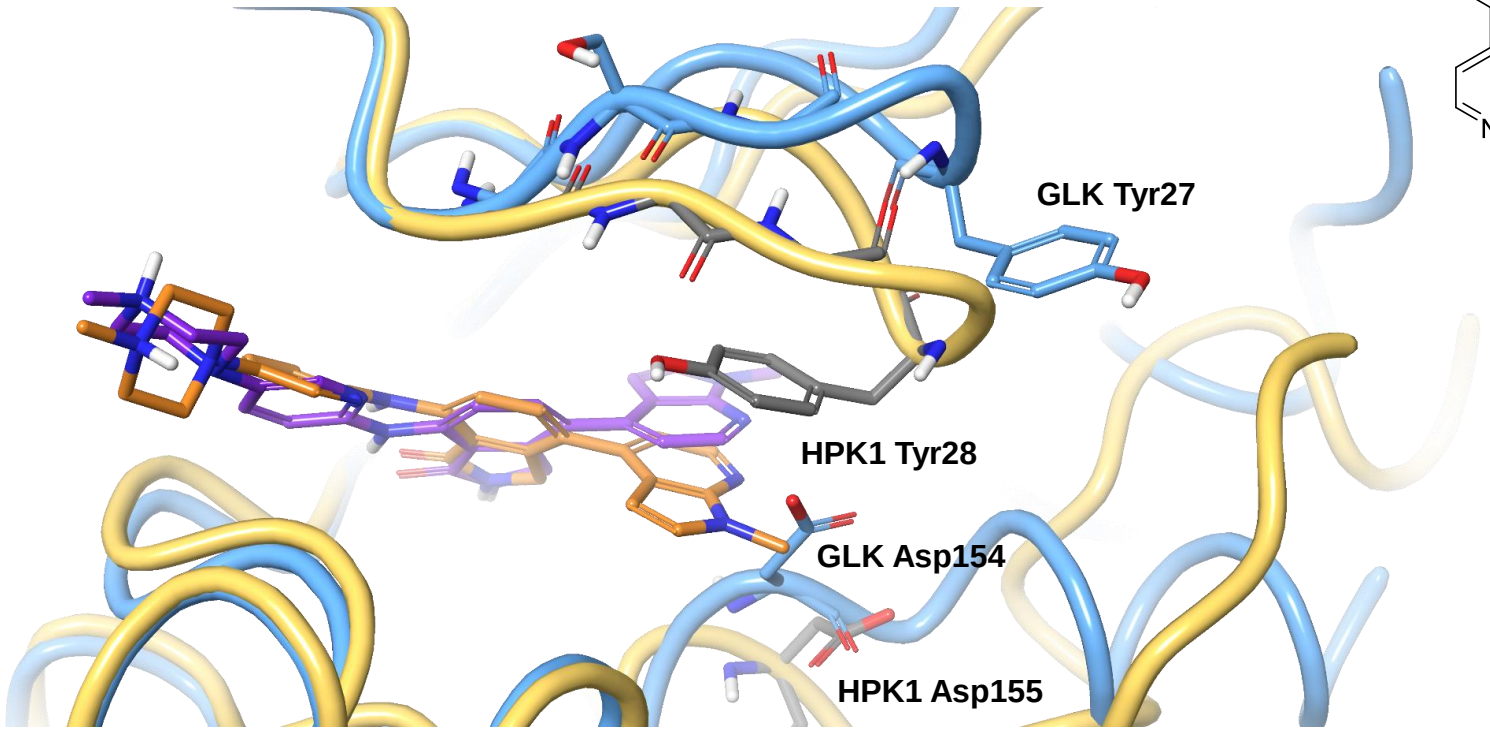
[a]Biochemical potency at 1 mM ATP; [b]1 mM ATP; [c]10^{-6} cm/s

- Replacement of monocyclic pyridine with bicyclic aromatic rings improved HPK1 potency and selectivity against GLK
- Cross-screening identified acetyl choline esterase (AChE) inhibition as a significant liability for the C-4 piperazine series.
  - Compound **12** exhibited human AChE IC_{50} of 26 nM
- Docking in a AChE literature structure showed that piperazine might be making a cation-π interaction with Trp86
  - Replacement of the basic piperazine compound **13** removed AChE inhibition (IC_{50} > 10,000 nM)

Structural Basis for GLK Selectivity

Compound 14
Caliper 1 mM ATP
HPK1 IC_{50} = 1.4 nM
GLK IC_{50} = 84 nM
Selectivity = 60x





HPK1 with bound Compound 14 (yellow ribbon, orange ligand)
GLK with bound Compound 14 (blue ribbon, purple ligand)

P-Loop confirmation affects orientation of identical residues Tyr 28 (HPK1 in the pocket) versus Tyr 27 (GLK away from pocket)
Gly-Gly-Gly HPK1 P-loop motif gives added flexibility over GLK with Gly-Ser-Gly

Flexibility of HPK1 P-loop allows Tyr28 π-π interaction with bicyclic
Tyr27 for GLK is orientated away from the pocked and no interactions are present with 14

Conventional DFG Asp position as observed in GLK not compatible with ligand out orientation and needs to move for HPK1

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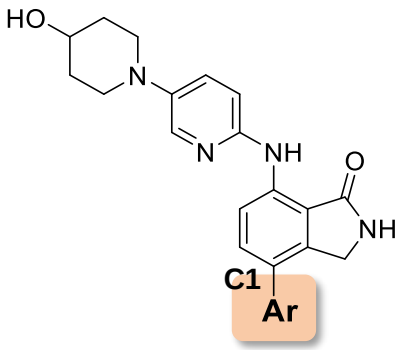
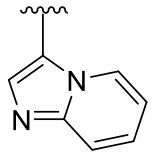
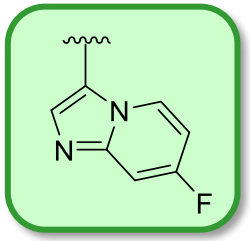
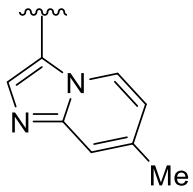
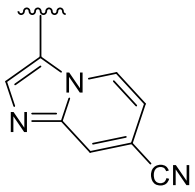
Identification of Potent and Selective C-1 Aryl Substituent

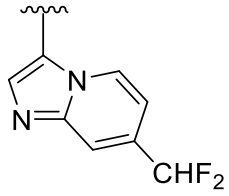


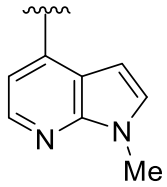


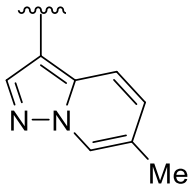


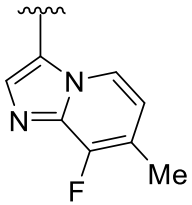












Assay	13	15	16	17	18	19	20	21
HPK1[a] IC_{50} (nM)	2.6	4.2	1.8	0.7	3.6	2.2	0.9	0.5
GLK Selectivity[b]	46	127	175	439	269	91	180	152
Cell pSLP76 IC_{50} (nM)	116	101	1800	225	204	218	56	46
hPPB fu (%)	1.8	1.5	<1	<1	<1	<1	<1	<1

[a]Biochemical potency at 1 mM ATP; [b]1 mM ATP

- Compounds are active in cell-based pSLP76 assay
- High plasma protein binding: few compounds have a measurable free fraction in human PPB measurements
- Compound **15** had the best balance of physical properties, GLK selectivity and cellular potency
  - At 75 mpk, it showed only 4 hours coverage of pSLP76 IC_{50}

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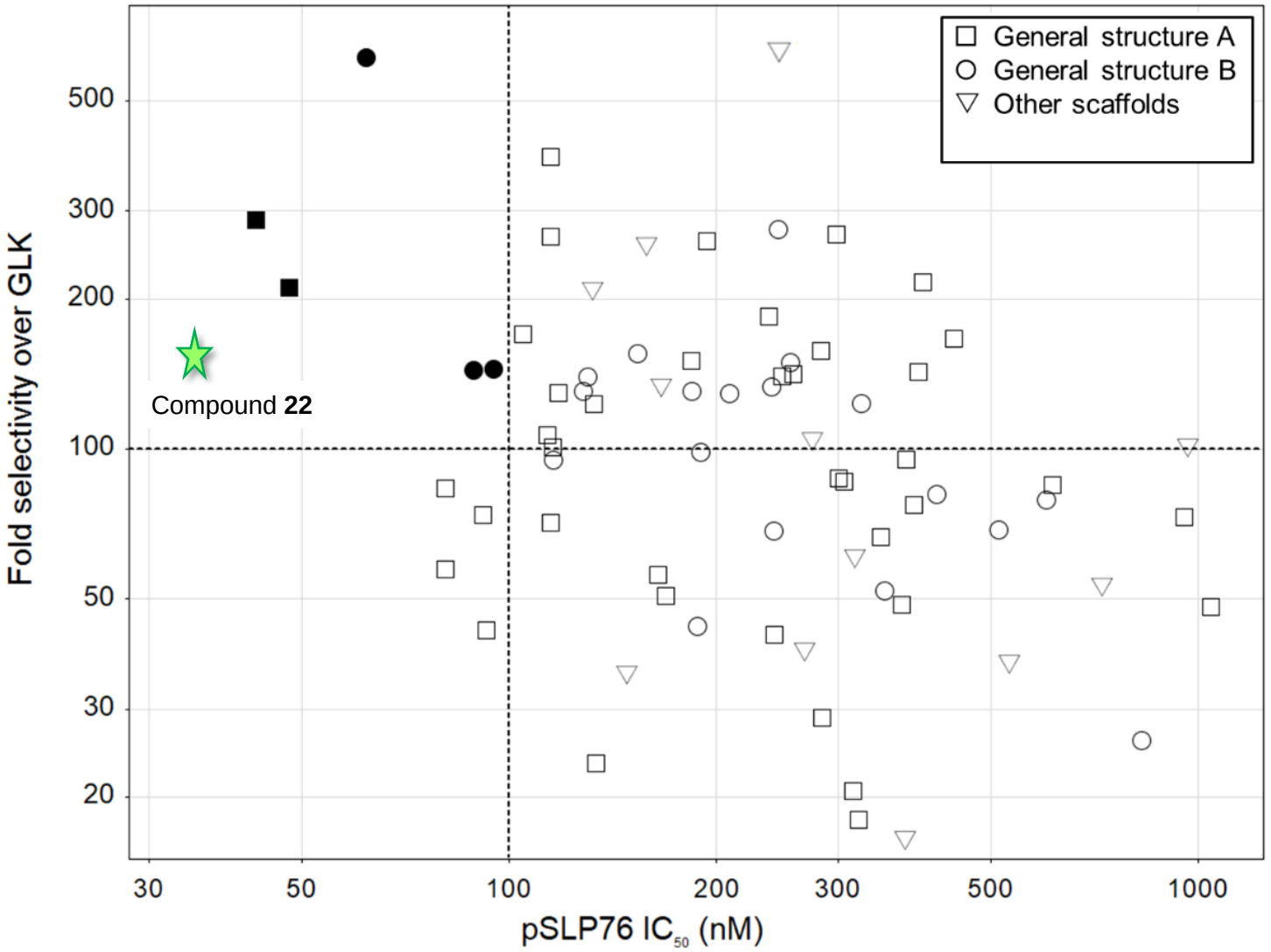
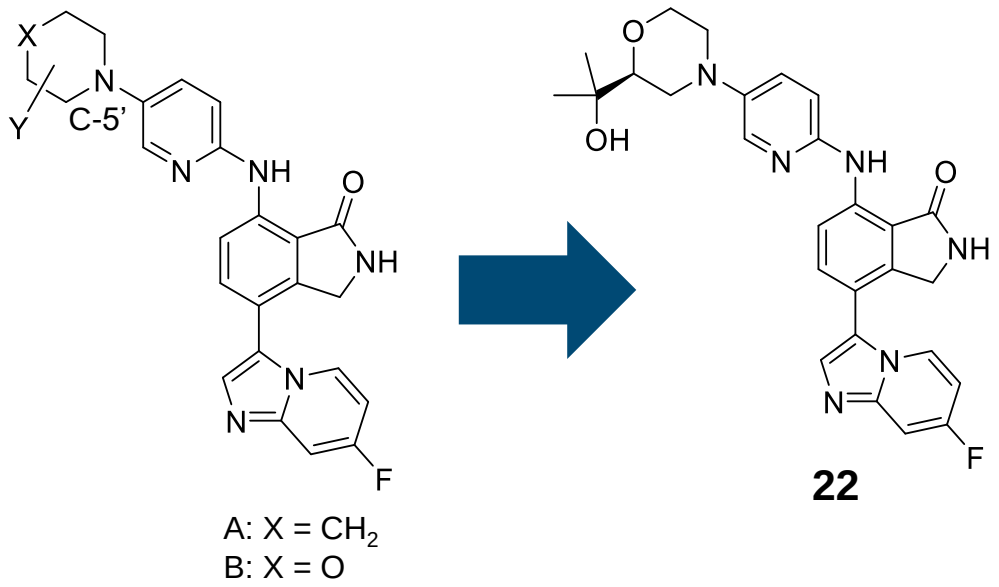
Identification of Potent and Selective Solvent Facing C-5' Substituent





Compound **22** identified as a lead compound

- >100-fold selectivity against GLK and good potency in biochemical and pSLP76 assay
- In a mouse PO PK study at 75 mpk showed 24 hour coverage of pSLP76 IC_{50}

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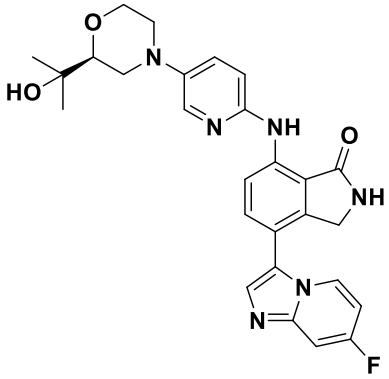
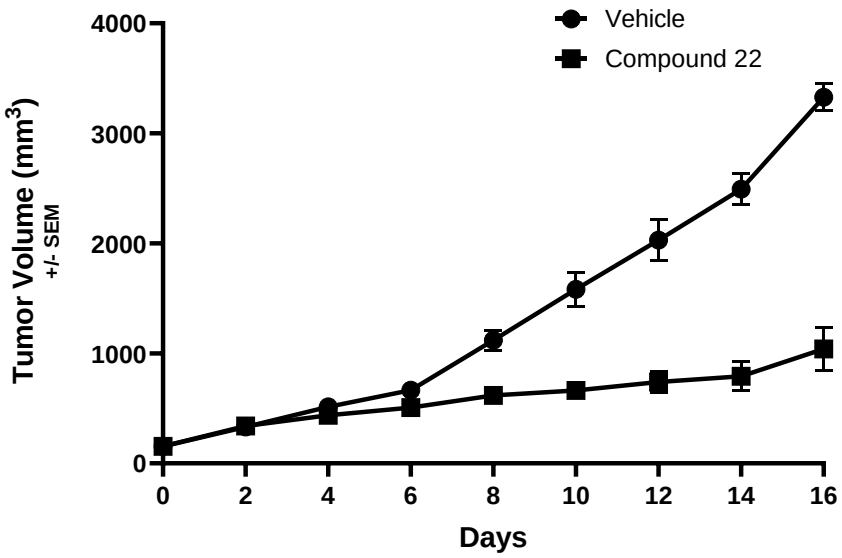
Early Frontrunner (Compound 22) Exhibited Suboptimal Predicted Human PK

Potent and selective HPK1 inhibitor



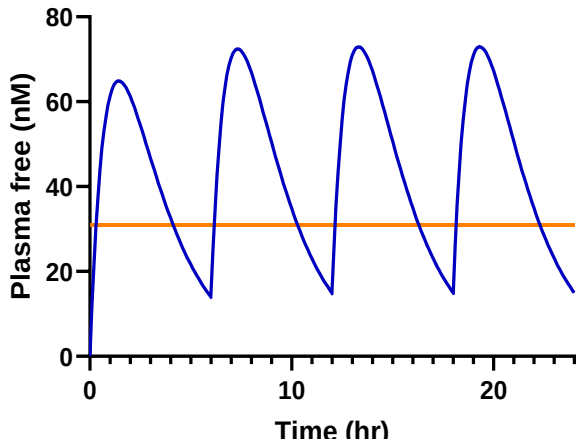


Assay	Compound 22
HPK1 IC_{50} 1mM ATP	0.7 nM
HPK1 pSLP76 IC_{50}	31 nM
Selectivity assessment (fold selectivity @ [1 mM ATP])	
GCK / MAP4K2	600
GLK / MAP4K3	130
HGK / MAP4K4	2800
KHS / MAP4K5	100
MINK	2600
TNIK	500

Assay	Compound 22
MDCK ($x10^{-6}$ cm/s) A-B / EER	52 / 15
Kinetic solubility (FeSSIF)	>200 µM
hHeps (mL/min/Kg) Clpred	< 8
Mouse Cl_{obs} (mL/min/Kg) (mHeps)	11 (36)
Rat Cl_{obs} (mL/min/Kg) (rHeps)	27 (26)
Dog Cl_{obs} (mL/min/Kg) (dHeps)	18 (17)
hPPB (%)	98.1
Rat Vss (L/kg)	0.90
Mouse Vss (L/kg)	0.50
Dog Vss (L/kg)	1.6

- CT-26 syngeneic mouse model **22** dosed at 75 mpk PO QD
- Robust and statistically significant tumor growth inhibition(69%) observed with 16h coverage of pSLP76 IC_{50}

600mg QID Compound 22 needed for 16h coverage of pSLP76 IC_{50}



Cl=7mL/min/kg
Vss=0.9L/kg,
hppb=1.9%
%F=30

Predicted high dose high frequency in humans

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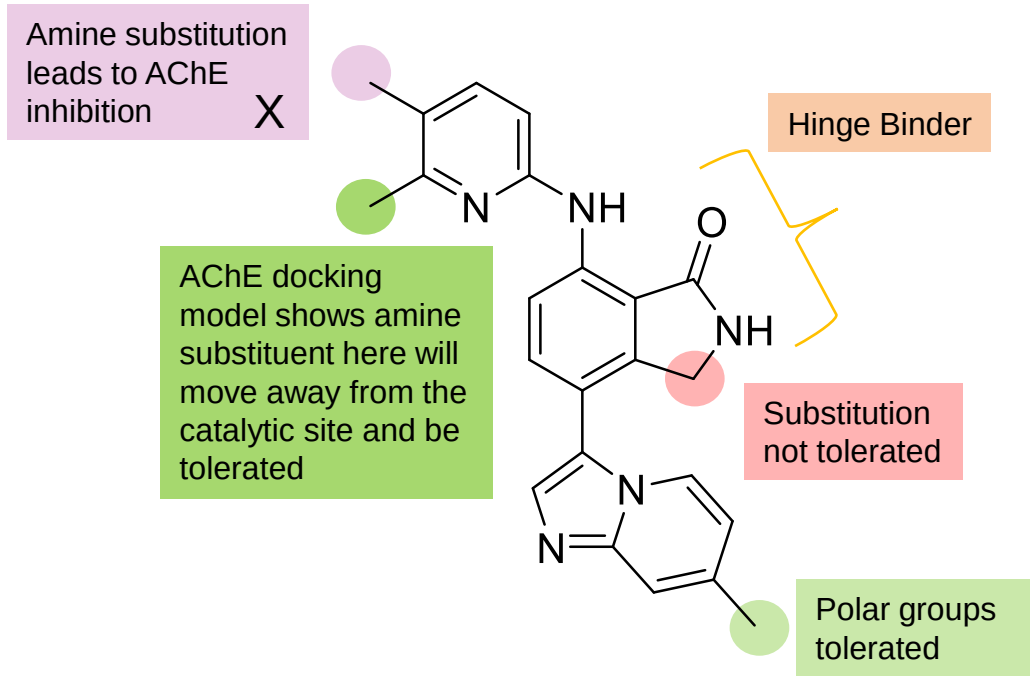
Designing for Dose: Increased Volume of Distribution(Vd) and Half-life to Improve Predicted Human Dose

What parameters can we change to get reasonable predicted human dose?

Assay	Target Profile	Phys Props
HPK1 IC_{50} 1mM ATP (nM)	<1	
HPK1 cell IC_{50} (nM)	< 50	
GLK selectivity fold	> 100	
pK_A	> 7.5	Basic amine
hPPB (fu) %	measurable	cLog P < 3
HLM Cl (mL/min/kg)	≤ 12	
MDCK Permeability A-B (10^{-6} cm/s)	≥ 15	HBD ≤ 2
Volume of Distribution Vss L/kg	> 5	pKA > 7.5

Back to the drawing board



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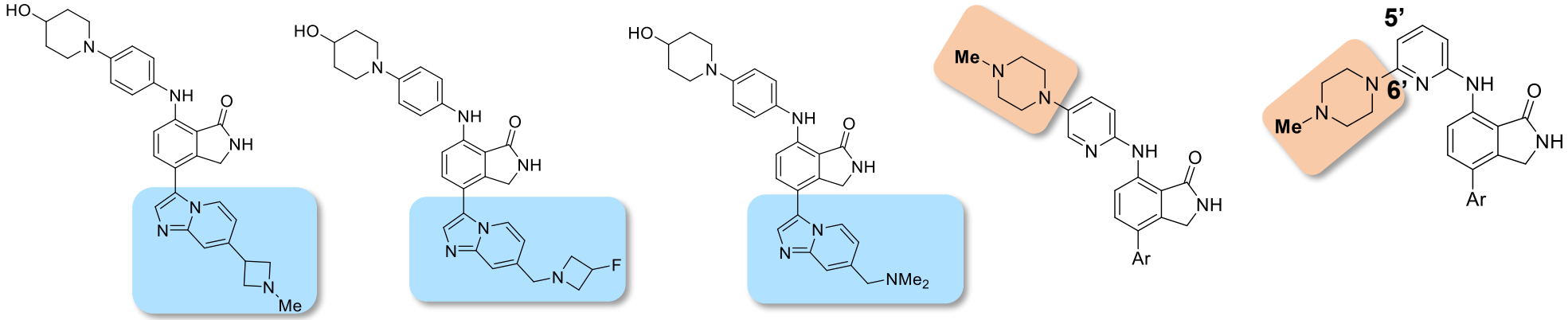
Medicinal Chemistry Strategy to ↑ Vd and $T_{1/2}$: Add Basic Amine while Maintaining Potency and Selectivity



Assay	23	24	25	Series 26	Series 27
HPK1[a] IC_{50} (nM)	2.4	336	174	<10	**<10**
GLK Selectivity[b]	498	--	--	>100	**>100**
AChE IC_{50} (nM)	1230	9950	2100	<50	**>10000**

[a]Biochemical potency at 1 mM ATP; [b]1 mM ATP;

- Structural analysis suggested the imidazopyridine group may accommodate polarity – but potency and AChE selectivity was suboptimal (compounds **23**-**25**)
- HPK1 potency was retained when the amine pointed towards solvent (substitution of the pyridyl)
- The 6' substituted amine series **27** maintained HPK1 potency and selectivity against AChE
  - Further exploration at the 6' position was pursued

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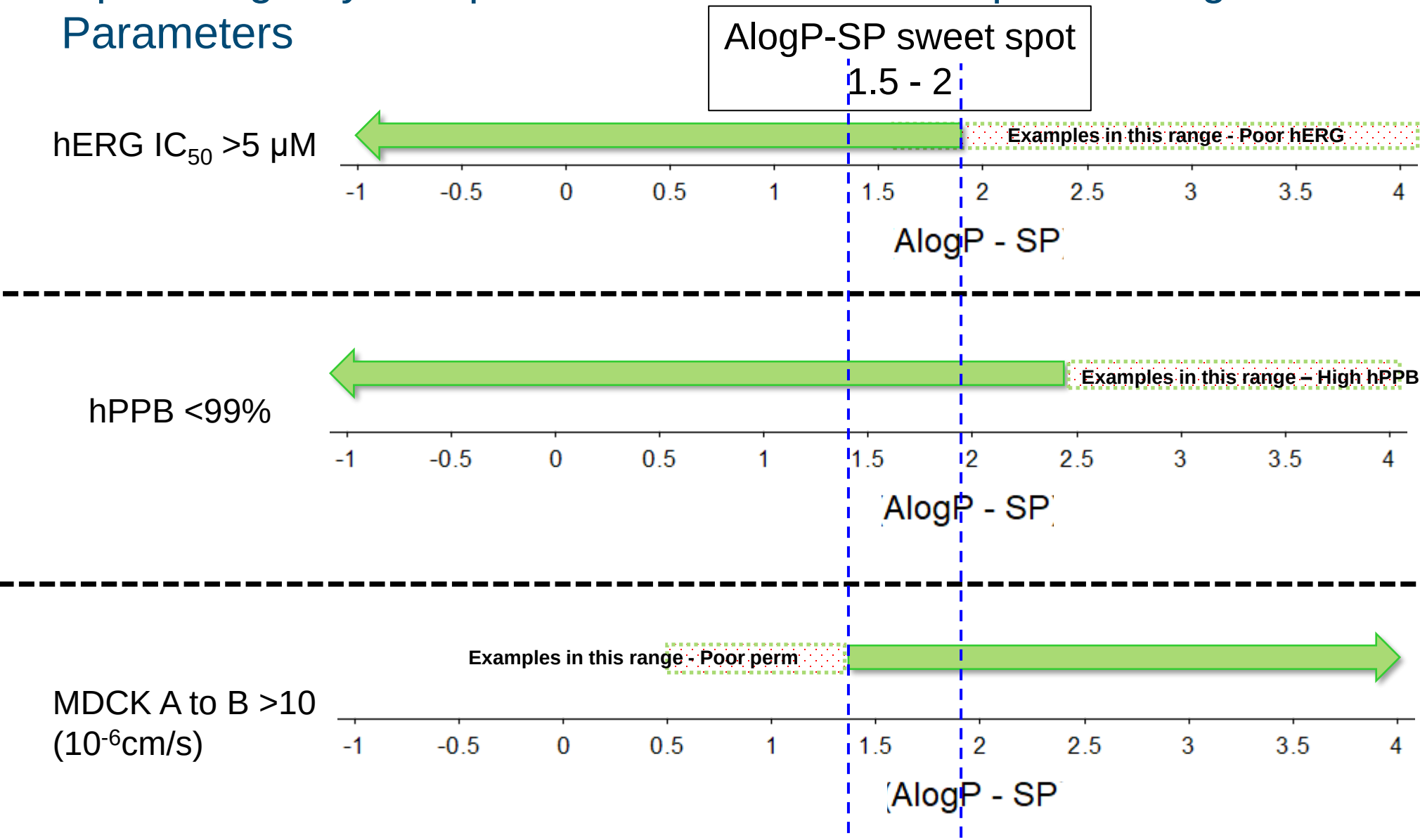
Optimizing Phys Props in the Basic Series - Impact of AlogP-SP on Different Parameters



AlogP-SP* used to establish correlations to ADME properties. To achieve all 3 parameters need to be in sweet spot

* cLogP surrogate from Schrödinger

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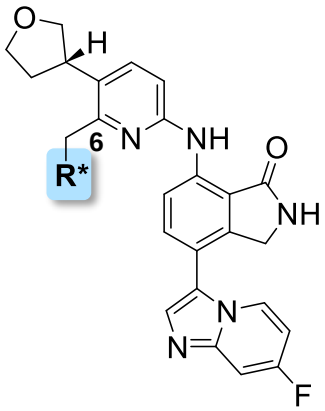
Identification of Development Candidate NDI-101150



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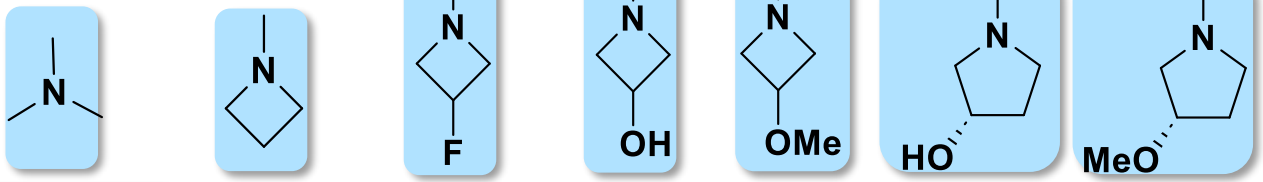
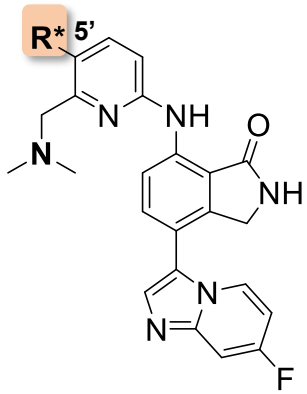


Assay	101150	28	29	30	31	32	33
HPK1[a]/pSLP76 IC_{50} (nM)	0.7/42	1.3/154	0.6/134	0.8/300	0.4/18	0.8/151	0.7/32
GLK Selectivity[b]	377	523	518	288	258	265	429
MDCK WT $P_{app, A-B}$[c]	32	6	5	3	30	8	75
hHep Cl_{pred} (mL/min/kg)	8	10	14	8	18	14	17

[a]Biochemical potency at 1 mM ATP; [b]1 mM ATP; [c]10^{-6} cm/s ; * stereochemistry arbitrarily assigned

- Dimethyl amine at C-6 shows best P_{app} and hHep Cl_{pred}



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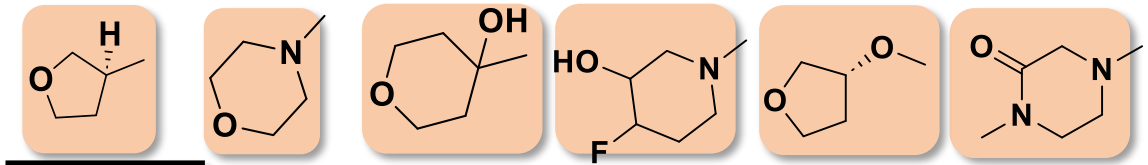

Assay	101150	34	35	36	37	38
HPK1[a]/pSLP76 IC_{50} (nM)	0.7/42	0.9/88	0.9/114	2.1/74	0.5/176	1.1/540
GLK Selectivity[b]	377	469	613	256	1176	402
MDCK WT $P_{app, A-B}$[c]	32	19	8	8	9	
hHep Cl_{pred} (mL/min/kg)	8	<8	<9	<9	12	
hERG PC IC_{50} (nM)	2829	7218	12700	7712	1093	
Rat PK Vss (L/kg)/Cl (mL/min/kg)/F%	5/34/20	9/98/<1	17/82/4	23/73/<1		

- Low rat oral bioavailability could be driven by low permeability or high clearance

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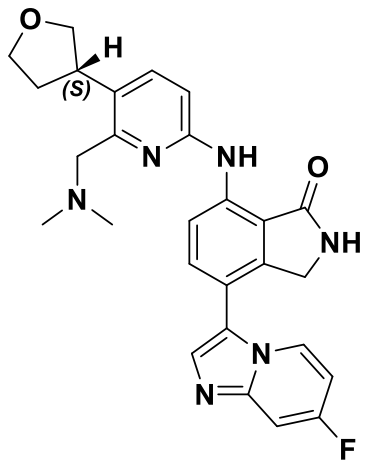
NDI-101150: Development Compound Profile



Assay	NDI-101150
Molecular Weight	486.5
Log P (measured)	2.6
Log D (measured)	2.0
pKa (measured)	8.9
Solubility (pH 7.4)	140 uM
MDCK P_{app} (x10^{-6} cm/s) / ER	32 / 0.7

HPK1 Assay	IC_{50} (nM)
Caliper @ 1 mM ATP	0.7
pSLP76	42
Kinase (Fold Selectivity)*	
GCK/MAP4K2	**8000**
GLK/MAP4K3	**377**
HGK/MAP4K4	**10740**
KHS/MAP4K5	**489**
MINK/MAP4K6	**10740**
TNIK/MAP4K7	**1336**
c-SRC	**3630**
FYN	**3110**
LCK	**2143**
SYK	**20000**
TYK2	**30000**

Assay	NDI-101150
Cyp Inhibition	1A2, 2B6, 3C8, 2C9, 2C19, 3A4M, 3A4T IC50 > 30 uM 2D6 IC50 = 17 uM No evidence of TDI at 50 uM
Cyp Induction	PXR activation assessed at 3/30uM no activation
hERG Manual PC IC_{50}	2800 nM

- In the GLP in vivo telemetry study in conscious monkeys no effects on cardiovascular function (HR, BP, ECG parameters)
- > 300-fold selectivity in 300+ kinase panel
- 4 hits in Eurofins panel. Secondary assays complete– no concerns.

* 1 mM ATP assay

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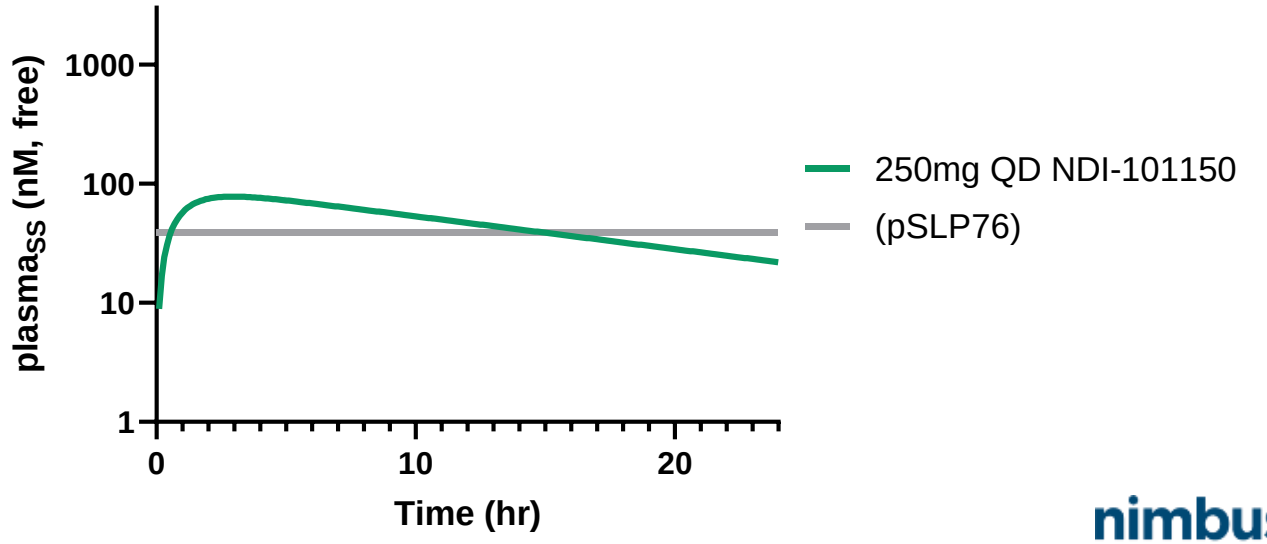
NDI-101150: Suitable In Vivo Exposure and Clearance from Discovery PK Studies

In vivo					In vitro		
Species	Cl (mL/min/kg)	V (L/kg)	Half life (hr)	F (%)	Microsomes (mL/min/kg)	Hepatocytes (mL/min/kg)	PPB (%)
Mouse	30	1.9	1.1	50	61	84	80
Rat	34	5.1	3	18-44	24	44	84
Monkey	16	3.3	6.1	14-24	36	22	87
Dog	43	13	6.8	33	26	24	73
Human	TBD	TBD	TBD	TBD	12-13	7	75

Early dose projection for 16 h pSLP76 coverage

V = 6 L/kg, Cl = 7.2 mL/min/kg, t1/2 = 11 h



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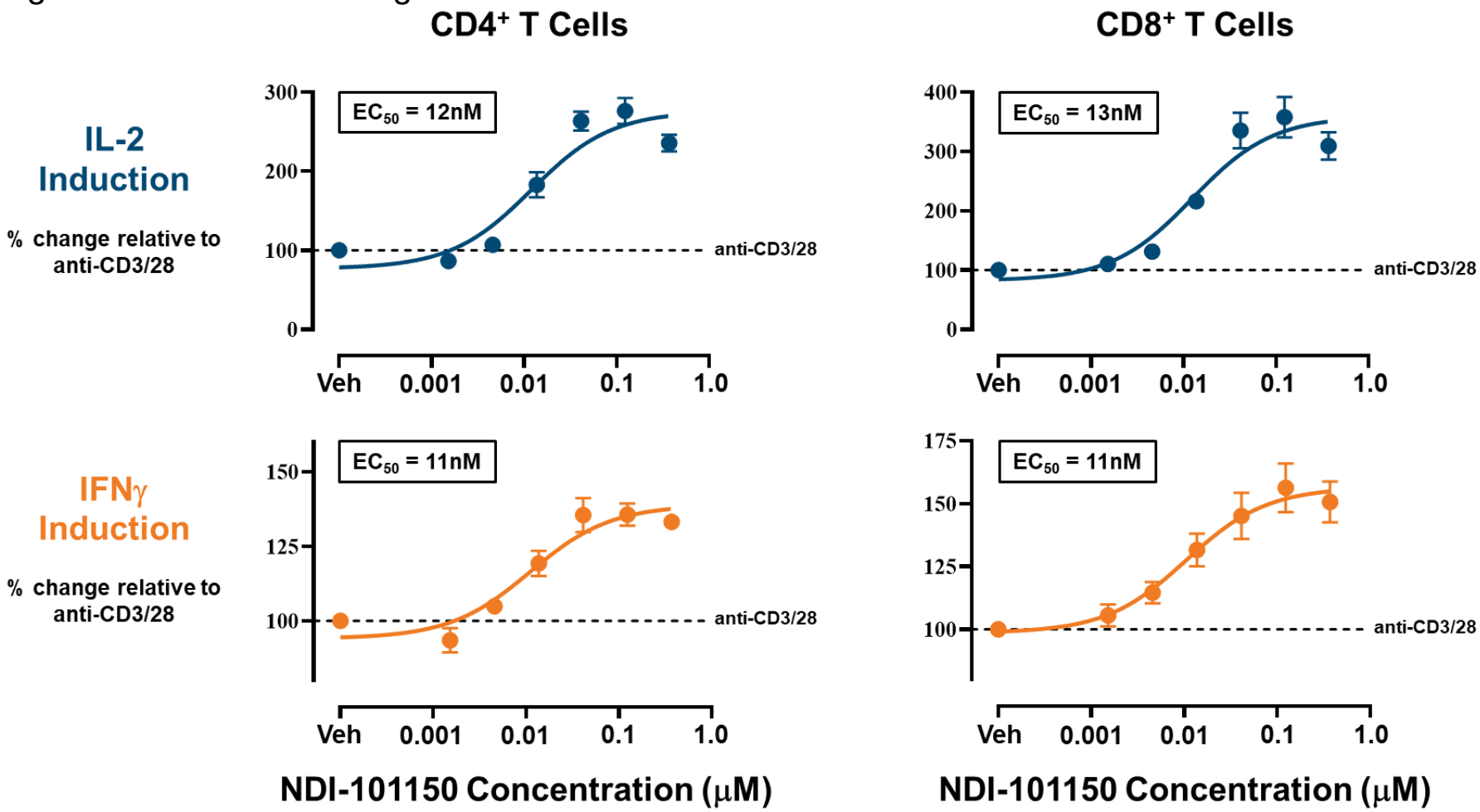
Nimbus HPK1 Inhibitor NDI-101150 Preclinical Biology

NDI-101150 mediates activation of not only T cells, but also B cells and dendritic cells

- In human T cell activation assays, NDI-101150 was able to enhance the activation of both CD4+ and CD8+ T cells by increasing levels of IL-2 and IFNg

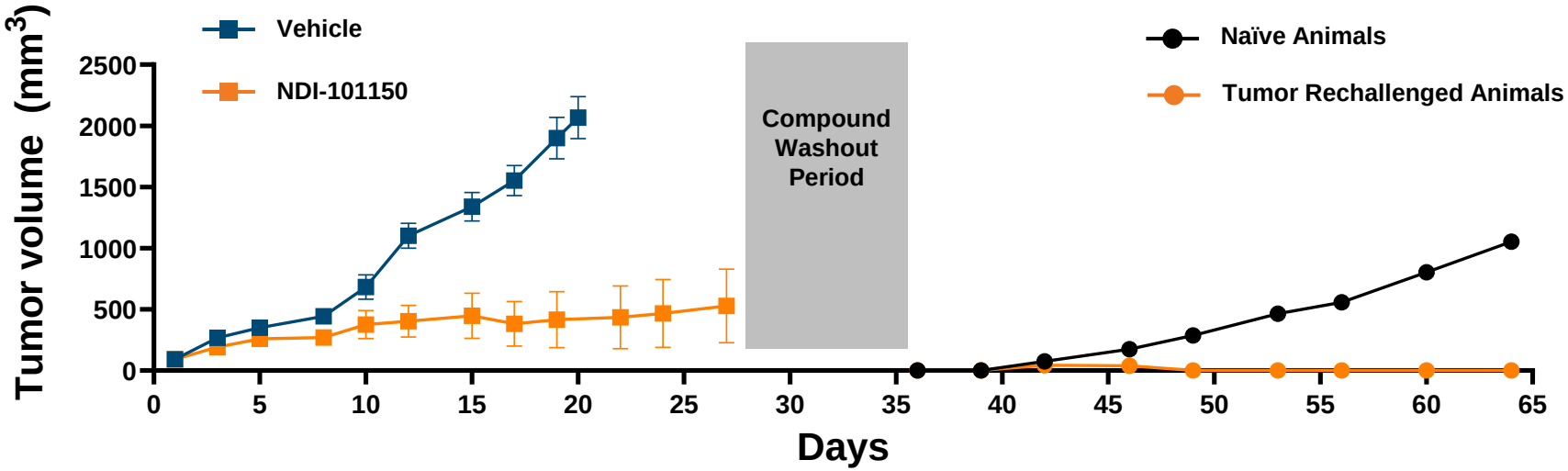


- Enhances IgG secretion by B cells
- Enhances activation and antigen presentation in bone marrow derived dendritic cells

MAP4K = mitogen-activated protein kinase kinase kinase.,Treg = regulatory T cells; PGE2 = prostaglandin E2, TGFβ = transforming growth factor beta; IgG = immunoglobulin G

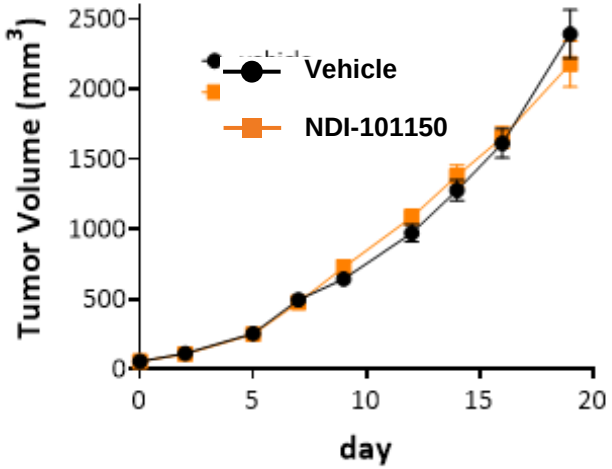
NDI-101150 Demonstrates Robust Efficacy in the EMT-6 Syngeneic Mouse Model, in an Immune-dependent Manner

Robust Efficacy & Durable Immune Memory in the Murine EMT6 Syngeneic Tumor Model



- NDI-101150 75mpk/day, po showed 7 complete responses (CRs) of 10 mice
- After rechallenge with tumor, NDI-101150 cured mice show complete tumor regression ***without any further dosing***

No Efficacy Observed in EMT-6 Xenograft Model



- NDI-101150 75mpk/day, po did NOT induce tumor growth inhibition in a xenograft version of EMT6 showing that HPK1 inhibition is mediated through the animals immune system

EMT-6 = experimental mammary tumor-6; mpk = mg/kg; po = orally; QD = every day

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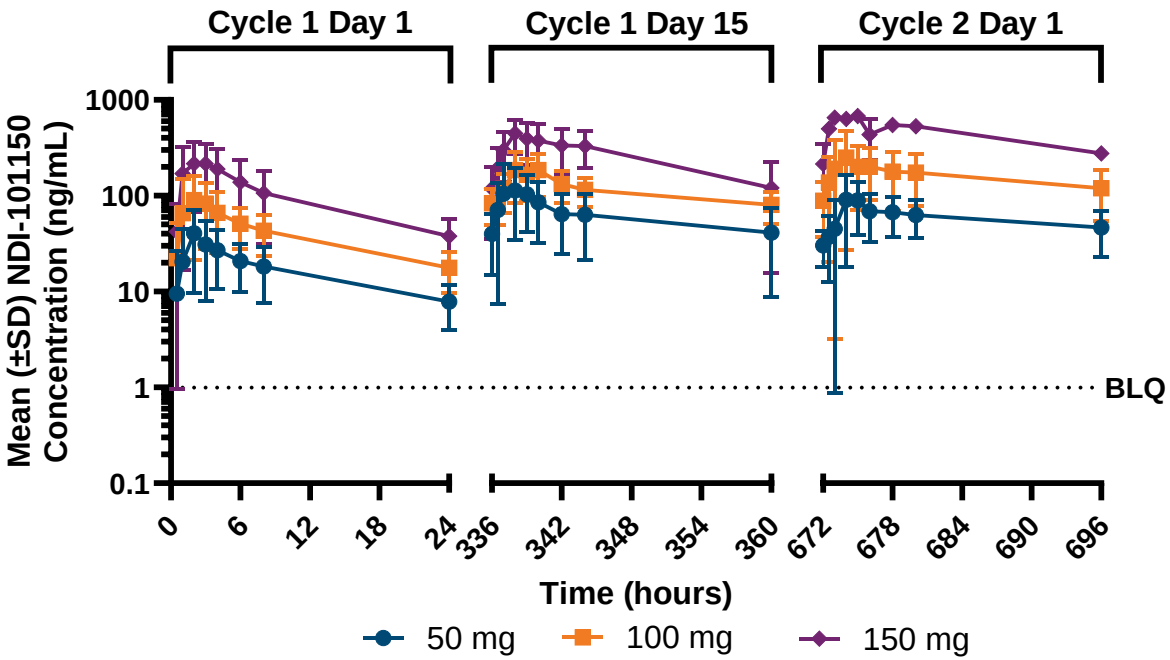
NDI-101150 is being investigated in a Phase 1/2 Clinical Trial

- Phase 1/2 multicenter, open-label trial (NCT05128487) designed to assess NDI-101150 as a monotherapy (50-200 mg dose) and in combination with 200 mg pembrolizumab in the treatment of adults with advanced solid tumors
- Primary objectives: recommended phase 2 dose(s) and maximum tolerated dose
- Secondary objectives: safety, pharmacokinetic profiles, and preliminary antitumor activity
- Preliminary results* presented at the 2024 ASCO Annual Meeting showed:
  - **Data from 44 patients in the dose escalation cohorts** (n=38 on monotherapy, n=6 on combination therapy) and 15 patients in the dose expansion cohorts
  - **NDI-101150 was well-tolerated with overall acceptable safety profile**
  - NDI-101150 plasma concentrations increased in a near dose-proportional manner
  - Steady-state plasma concentrations at all doses were sufficient to cover the pSLP76 IC50 for a duration consistent with preclinical efficacy modeling
  - **NDI-101150 showed an increase in activated CD8+ T cells and dendritic cell infiltration in on-treatment patient biopsies compared to archival biopsies, consistent with nonclinical studies of NDI-101150 showing immune cell infiltration and robust anti-tumor activity in murine syngeneic tumor models**
  - **Treatment with NDI-101150 monotherapy was associated with clinical benefit in five out of 30 (16.7%) response-evaluable patients, including one complete response, one partial response, and three cases of durable stable disease**

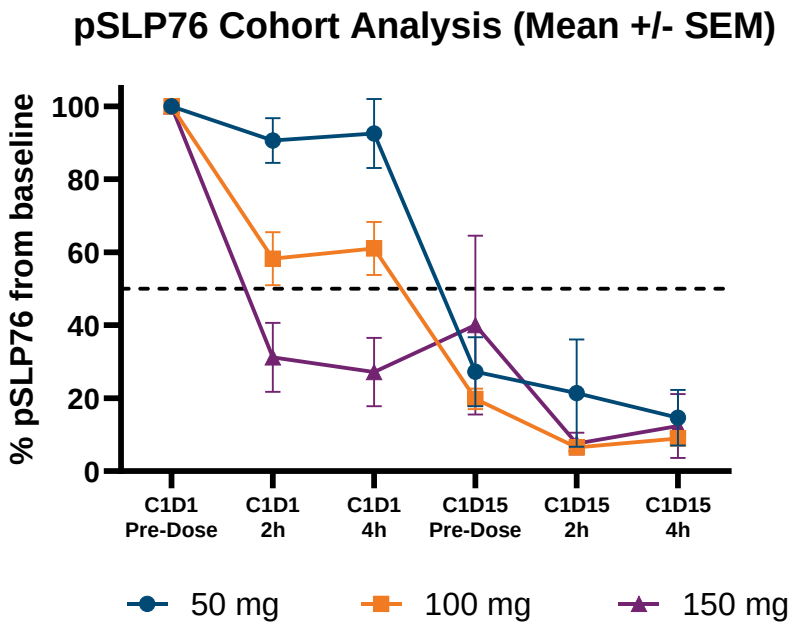
*Data cut-off: March 18, 2024
Noel MS, et al. Poster 3083 presented at ASCO Meeting 2024

Preliminary Monotherapy Pharmacodynamic and Pharmacokinetic Results from an Ongoing Phase 1a Dose Escalation Study of NDI-101150

Mean concentration on Cycle 1 Day 1, Day 15 & Cycle 2 Day 1



Change in percentage pSLP76 from baseline to Cycle 1 Day 15



- NDI-101150 showed a dose-dependent increase in plasma concentration and accumulation at steady state, with pSLP76 inhibited at all doses
  - Nearly dose proportional increases in mean exposure was observed on Cycle 1 Day 1
  - Steady state was achieved by Cycle 1 Day 15
  - Accumulation was observed between Cycle 1 and Cycle 2
  - PD results demonstrated >50% reduction of pSLP76 (proposed therapeutic target) in each cohort by Cycle 1 Day 15

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Summary

- Knowledge-based hit generation approach was the most successful of four hit generation campaigns
- SBDD applied to optimize both potency *and* selectivity
  - Multiple proprietary crystal structures of HPK1, GLK and KHS obtained
    - Co-crystal structures of near neighbor kinases applied to optimize selectivity
  - Protein/ligand structures guided SBDD and FEP despite highly flexible protein
  - Off-target undesired activity, e.g. AChE, hERG removed by modeling strategy
- Potent and Selective HPK1 inhibitors were optimized for their predicted human dose
  - Addition of basic groups to inhibitors to improve phys props (↑ volume of distribution, ↑ solubility, ↓ Cl_{int}) to improve predicted human dose and half life
- NDI-101150 was identified as a potent HPK1 inhibitor which showed
  - High selectivity both within the MAP4K family and in the wider kinome
  - Activation of T cells, B cells and dendritic cells, to mount a robust anti-tumor response
  - Robust preclinical activity, both as a monotherapy and with synergistic efficacy in CPI combinations
- NDI-101150 is currently being investigated in a Phase 1/2 clinical trial (NCT05128487)
  - NDI-101150 treatment associated with activated CD8+ T cells and dendritic cell infiltration in tumors
  - Treatment with NDI-101150 monotherapy resulted in preliminary evidence of clinical benefit and an acceptable safety profile

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