Discovery and Optimization of CBL-B Inhibitors

NX-1607

Discovery On Target
Boston, MA
October 18, 2022
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Nurix’s DELigase Protein Modulation Discovery Platform

**DEL Discovery**

> 5 billion drug-like compounds that can be easily screened against hundreds of proteins to identify starting points therapeutic discovery

**Rational and Empirical Chemistry**

Structure Based Drug Design combined with chemistry automation enables broad exploration of lead-like chemical space for each program

**Direct-to-Cell Biology Capabilities**

High throughput cellular assays monitor protein levels and biological phenotypes to assess impact on biology

**Scaled Screening for in vivo exposure**

Capacity to screen for ideal in vivo drug exposure profile and assess impact on disease biology
Nurix Is Advancing Four Wholly Owned Clinical Programs with a Deep Pipeline of Proprietary and Partnered Novel Targets

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<thead>
<tr>
<th>MOA</th>
<th>Drug Program</th>
<th>Target/ Delivery</th>
<th>Therapeutic Area</th>
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<td>CBL-B</td>
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<td>Cell Therapy</td>
<td>Ex vivo CBL-B Inhibition</td>
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<td>Wholly owned</td>
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CBL-B is a Modulator of Immune Cell Activation

- CBL-B is an E3 ubiquitin ligase highly expressed in cells of the immune system

- CBL-B regulates T, B, and NK cell activation

- Blocking CBL-B removes a brake on the immune system

- *cbl-b* deficient mice demonstrate robust T cell and NK cell-mediated antitumor immunity

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Synergy with anti-PD-1

- IL-2 production
- Proliferation
- Central memory phenotype
- Anti-tumor activity
- Threshold of activation
- T cell exhaustion

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CBL-B inhibition
CBL-B is a Modulator of Immune Cell Activation

Inactivation or deletion of CBL-B results in hyperactive T cells and inhibition of tumor growth.

IL-2 secretion in KO and ligase inactive T cells *ex vivo*

Paolino et. al. *J. Immunology*, 2011

Ligase-dead or KO exhibit enhanced and equivalent response to either single- or double stimulation

Ligase-inactive *cbl-b* knock-in mice inhibit tumor growth (TC-1 syngeneic model).

Nurix Data
Inactive CBL-B is Autoinhibited

- When Y363 of CBL-B is not phosphorylated, the helix of the LHR domain packs against the TKB domain
- Incapable of binding Ub-E2
- Phosphorylation of Y363 requires dissociation of LHR-RING from TKB

Active CBL-B Binds Ub-loaded E2 Ligases

"Closed-state" (inactive)

"Open-state" (active, binds E2-Ub)

Phosphorylation

CBL-B

TKB

RING

LHR

36 427 982
Multiple Lead-Finding Approaches Afforded CBL-B Binders

- All three screening techniques afforded validated binders, confirmed by X-ray crystallography.

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<td>Lib size</td>
<td>300K</td>
<td>1X10^9</td>
<td>1600</td>
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<tr>
<td># of Series</td>
<td>1</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Hit Affinity</td>
<td>28 µM</td>
<td>2.4 µM</td>
<td>1800 µM</td>
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<td>Hit mwt</td>
<td>338</td>
<td>537</td>
<td>211</td>
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<tr>
<td>Hit LE</td>
<td>0.27</td>
<td>0.22</td>
<td>0.33</td>
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CBL-B HTS Triage Revealed a Singleton Hit

104 hits from Closed State HTS
   Next: Tested from purchased powders

~90% confirmed from powders
   Next: Hits re-tested from purified compounds

< 15 hits repeated from purified compounds
   Next: Hits (re)tested in a variety of assays

- Solubility, Biophysical assay (SPR), FRET Artifact assay, Gel-based CBL-B Phosphorylation assay

- Preliminary SAR and X-ray crystallography

Identified singleton hit, NRX-1
HTS Reveals a Singleton Hit

NRX-1 (racemic)

\[ \text{mwt} = 338 \]
\[ K_{\text{sol}} = 280 \mu M \]
\[ \text{cLogP} = 3.46 \]
\[ \text{PSA} = 60 \]

\[ \text{IC}_{50} \text{: } 28 \mu M \]

E2 binding assay and counter assays to examine Src activity or FRET artifacts indicate that NRX-1 is a CBL-B inhibitor

- SPR confirms NRX-1 binding affinity and stoichiometry to CBL-B
- SPR binding affinity and biochemical potency in close agreement
NRX-3 is a Specific Inhibitor of CBL-B

NRX-1
HTS Screening hit

NRX-2
Chiral SFC

NRX-3
Resolved Screening hit
E2-Ub: IC$_{50}$ = 12 µM
mwt = 338; LE = 0.29

E2-Ub binding

SPR

K$_D$: 16 µM
NRX-3 is an Intramolecular Glue

1. Kinase
2. E2/substrate

Phosphorylation locks CBL-B in the **ACTIVE** Conformation

Immune Response
NRX-3 is an Intramolecular Glue

1. Kinase

NRX-3 acts as an intramolecular glue forcing CBL-B in its folded INACTIVE state

Y363 HELIX RING

Closed State

TKB

Opened State

TKB

2. E2/substrate

Phosphorylation locks CBL-B in the ACTIVE Conformation

E2

pY363

Substrate protein

Immune Response

14
Crystal Structure Confirms Binding Mode as Intramolecular Glue

NRX-3 binds to closed-state CBL-B and prevents phosphorylation
Early SAR: Focus on Affinity and Properties

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<thead>
<tr>
<th></th>
<th>NRX-3</th>
<th>NRX-4</th>
<th>NRX-5</th>
<th>NRX-6</th>
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<tr>
<td>E2-Ub: IC₅₀ (µM)</td>
<td>12</td>
<td>0.23</td>
<td>0.092</td>
<td>0.088</td>
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<tr>
<td>Ligand Efficiency</td>
<td>0.29</td>
<td>0.33</td>
<td>0.36</td>
<td>0.37</td>
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<tr>
<td>Cellular Substrate Ub IC₅₀ (µM)</td>
<td>7</td>
<td>3</td>
<td>1.7</td>
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<tr>
<td>Microsomes h/m Clₘᵢₙ (mL/min/kg)</td>
<td>20/360</td>
<td>-/500</td>
<td>30/73</td>
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<tr>
<td>Plasma stability m/r T₁/₂ (min)</td>
<td>-</td>
<td>140/-</td>
<td>280/-</td>
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<tr>
<td>Papp MDCK (MDR1) A→B/B→A ratio</td>
<td>26/1</td>
<td>33/1</td>
<td>9/6</td>
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<tr>
<td>Ksol (µM)</td>
<td>250</td>
<td>300</td>
<td>270</td>
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<tr>
<td>LogD₇.₄</td>
<td>2.6</td>
<td>2.3</td>
<td>1.9</td>
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Early SAR: Focus on Affinity and Properties

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<th>NRX-6</th>
<th>NRX-7</th>
<th>NRX-8</th>
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<tr>
<td>E2-Ub: IC\textsubscript{50} (\mu M)</td>
<td>0.088</td>
<td>0.038</td>
<td>0.021</td>
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<tr>
<td>Ligand Efficiency</td>
<td>0.37</td>
<td>0.37</td>
<td>0.36</td>
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<tr>
<td>Cellular Substrate Ub IC\textsubscript{50} (\mu M)</td>
<td>1.7</td>
<td>0.78</td>
<td>0.79</td>
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<tr>
<td>Microsomes h/m Cl\textsubscript{int} (mL/min/kg)</td>
<td>30/73</td>
<td>-/67</td>
<td>7/26</td>
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<tr>
<td>Plasma stability m/r T\textsubscript{1/2} (min)</td>
<td>280/-</td>
<td>&gt;1000/163</td>
<td>&gt;1000/&gt;1000</td>
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<tr>
<td>Papp MDCK (MDR1) A→B/B→A ratio</td>
<td>9/6</td>
<td>7/7</td>
<td>2/14</td>
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<tr>
<td>Ksol (\mu M)</td>
<td>270</td>
<td>260</td>
<td>300</td>
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<tr>
<td>LogD\textsubscript{7.4}</td>
<td>1.9</td>
<td>2.4</td>
<td>1.7</td>
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</table>
The SAR for rat plasma stability was not predictable by chemists

First observed with low recovery in PPB assays
To assist with lead optimization, models were built based on the 104 experimental plasma stability data points available at the time.

Despite the low volume of data, both regression and classification models demonstrated high predictive power and provided key insights driving series progression.
NRX-8 Is a Specific Inhibitor of CBL-B

NRX-8 displays clean 1:1 binding stoichiometry with CBL-B and is clean in off-target screening.

CEREP Panel, <40% activity at 10 µM (N = 52)
NRX-8 Maintains Original Hit Binding Mode
NRX-8 Inhibits Substrate Ub and Stimulates IL-2 Induction

Substrate Ubiquitylation – BT20 cell line

-100 -50 0 % Ubiquitylation Activity

Log uM

-4 -3 -2 -1 0 1 2 3

Human T cell assay – IL-2 production

Fold change over CD3, CD28 alone

0 10 20 30 40

Log uM

-4 -3 -2 -1 0 1

2.5X

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<tr>
<th>Assay Description</th>
<th>NRX-8</th>
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<tr>
<td>IL-2 (2.5X over baseline response)</td>
<td>80 nM</td>
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<tr>
<td>Cellular Ubiquitylation of substrate (BT20 – MSD assay)</td>
<td>850 nM</td>
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Pharmacologic Inhibition of CBL-B Recapitulates Anti-Tumor Effects of Genetic Model of Ligase Inhibition

Ligase-inactive *cbl-b* knock-in mice inhibit tumor growth in TC1 Syngeneic Model

CT26 Syngeneic Model

- **Vehicle**
- **45 mpk BID**
- **90 mpk BID**
- **180 mpk BID**

Tumor volume mm$^3$ (Mean±SEM)

Days post implant

Tumor volume mm$^3$ (Mean±SEM)

Days

NRX-8

0 5 10 25 30
Over 10,000-fold Enzymatic Potency Improvement Achieved While Improving Molecular Properties

~10,000x potency improvement

Clinical candidate range
Single-Agent NX-1607 Induces Antitumor Response in Multiple Models

**NX-1607**

**Reduced Tumor Volume**

**Colorectal**

Day 25 Tumors

**NS**  

\[ p = 0.0063 \]

**NX-1607**

**Prolonged Survival**

**Triple-Negative Breast**

NX-1607 30 mg/kg day 7 to 46

\[ p < 0.0001 \]

**NX-1607**

**Reduced Tumor Volume**

**B Cell Lymphoma**

NX-1607 30 mg/kg day 16 to 28

Shaded area indicates dosing period
NX-1607 and Anti-PD-1 Synergize to Enhance Anti-tumor Effects and Survival of Mice in Multiple Tumor Models

Colorectal (CT26) Long-Term Survival

Colorectal (MC38) Long-Term Survival

Triple-Negative Breast (4T1) Day 28 4T1 Lung Metastases

- Vehicle
- NX-1607
- anti-PD-1
- NX-1607+anti-PD-1

Shaded area indicates dosing period: NX-1607 (30 mg/kg, PO daily) and anti-PD-1 twice a week at 10 mg/kg dosing period
Summary

- CBL-B regulates T, B and NK cell activation
- Multiple screening approaches afforded validated binders to CBL-B
- Plasma instability may be an under-appreciated liability for amide-containing compounds
- Pharmacological inhibition of CBL-B recapitulates the anti-tumor effects of the genetic model of ligase inhibition
- NRX-8 specifically binds to CBL-B and 'glues' the protein in a closed state, preventing phosphorylation and E2-Ub binding
- Dosing of NRX-8 (45 mg/kg BID) inhibits tumor growth in mice
- Further optimization resulted in NX-1607 with sub-nM affinity and optimal in vivo anti-tumor activity
- Phase 1 clinical trial of NX-1607 in relapsed or refractory tumors is currently ongoing
Leader in Targeted Protein Modulation

Thank you