A Potent Solution to a Low Affinity Problem

Potency Release Methods for an NK Cell Targeted Cytokine

CASSS CMC North American Strategy Forum

18-19 July 2022

Morgan Wilson
On behalf of the Inhibrx CMC Team

INHIBRX

INBRX-121 – Path to Potency Assays

Topics to Cover

Mechanism of Action

- INBRX-121 components and mechanism of action

INBRX-121 Assays

- Out-of-the-gate challenges
- Assay development strategy for a Clinical Phase 1 program

INBRX-121 Cell-Based Bioassay

- Key functional considerations of INBRX-121 and cell line design
- Cell line characterization strategy
- Pre-validation de-risking and assay performance

INBRX-121 ELISA

- Concept and development strategy
- Pre-validation de-risking and assay performance

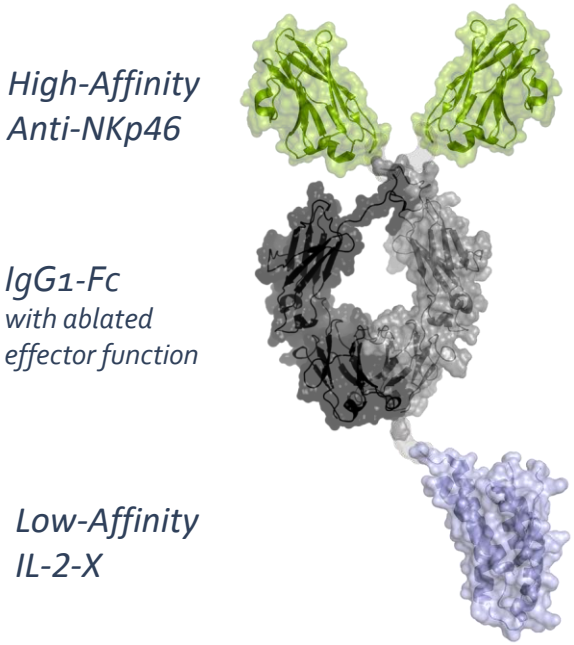
Early Structure/Function Assessment

- Hinge cleaved INBRX-121

INBRX-121 Introduction

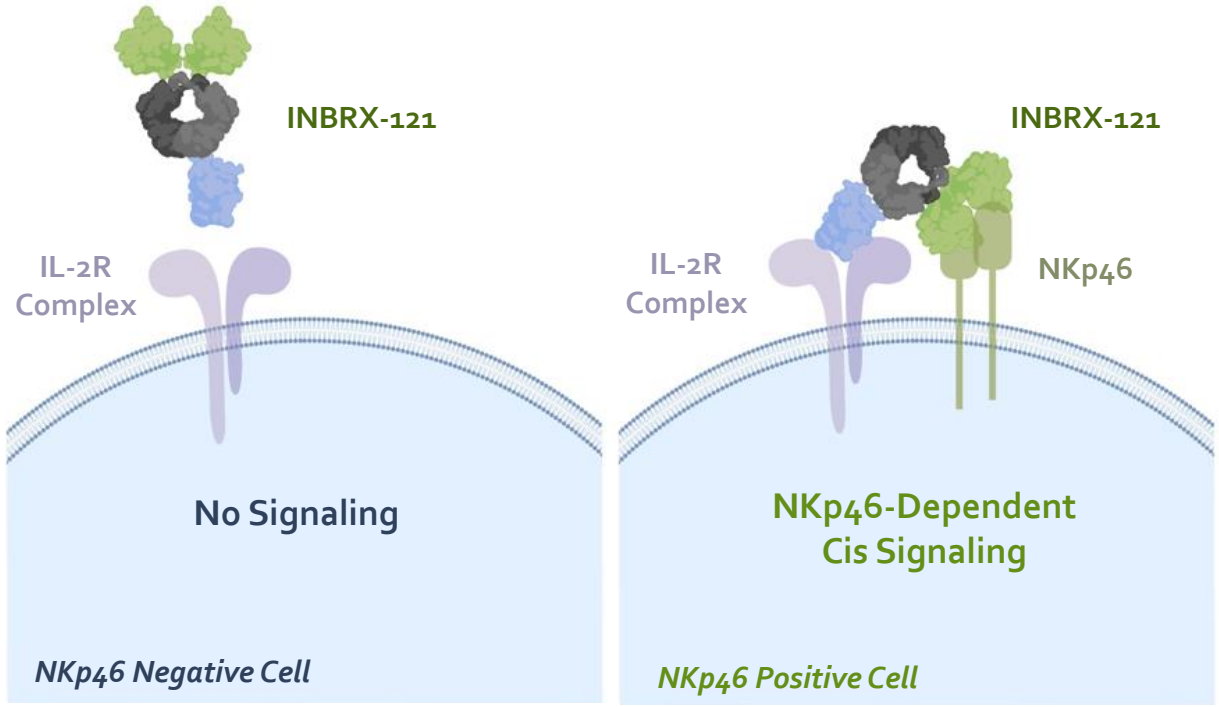
Components

- High-affinity single-domain antibody (sdAb) targeting NKp46 combined with an engineered IL-2 variant with reduced affinity* for the IL-2 receptor
- IgG1 Fc with ablated effector function



MOA

- The large affinity difference between the sdAbs and IL-2-X ensures specific modulation of intended target cells through cis-signaling, specifically enhancing NK cells without impacting the T cell subsets or vascular endothelial cells



Weak binding to IL-2 receptors on NKp46 negative cells (Tregs, vascular cells)

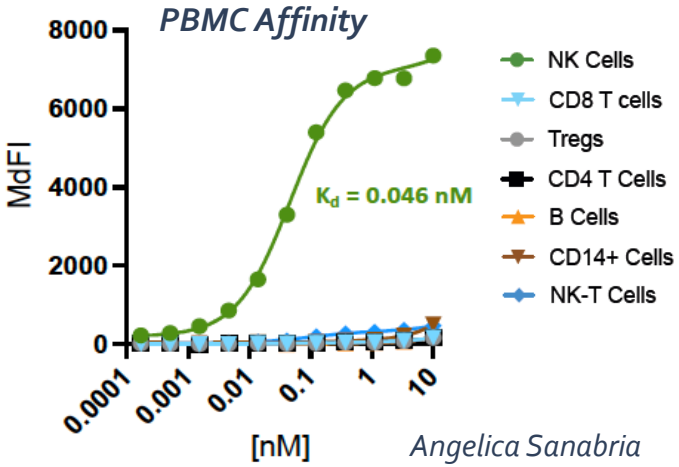
NKp46-dependent binding to IL-2 receptors on NKp46 expressing cells (NK cells)

*Discussed in detail on following slide

Immediate Challenges

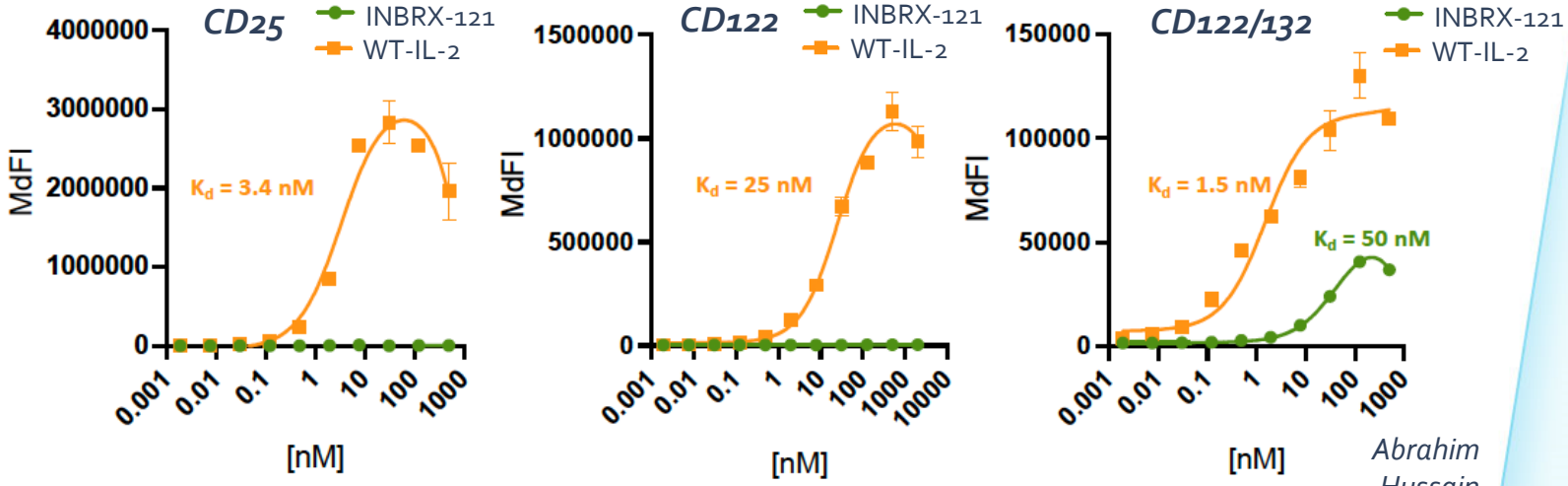
Low Affinity (Detuned) IL-2-X

NKp46 Binding *(High Affinity)*



Angelica Sanabria

IL-2-X Receptor Binding *(Low Affinity)*



Abrahim Hussain

Problem Statement

A detuned IL-2 is challenging to model with bioassays

Cell-Based Bioassay

Challenge

No commercial/ off-the-shelf options

Solution

Design INBRX-121-specific potency cell line

ELISA

Challenge

Low affinity IL-2 difficult to bind

Solution

Screen various approaches to binding assays

Assay Strategy

Clinical Phase 1 Program

Cell Line Generation

- Clone selection
- Characterization
- Banking

Cell-Based Bioassay

- Development
- DOE/Robustness
- Pre-qualification

Functional ELISA

- Development
- DOE/Robustness
- Pre-qualification

Time prior to assay validation

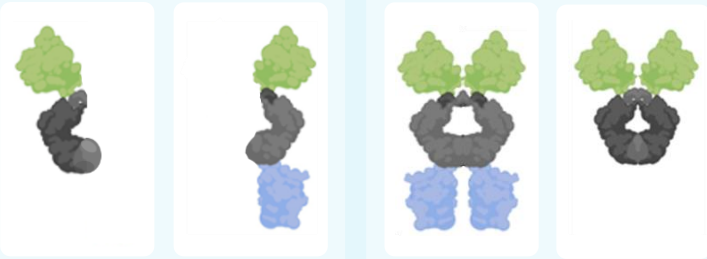
Functional Considerations

INBRX-121

Both assays must be able to model:

- NKp46 binding
- IL-2-X functional region
- Complete molecule

Potential PTMs / molecule variants should be considered during assay design



Half-molecules *Homodimers*

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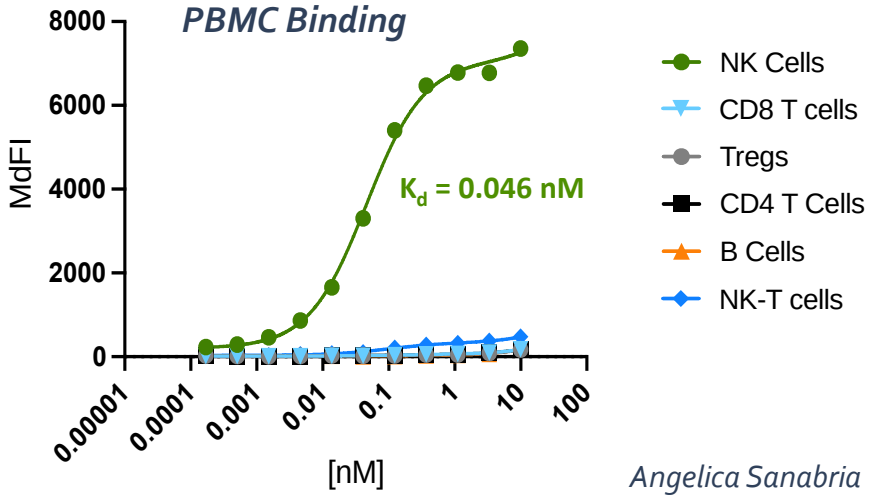
INBRX-121 Cell-Based Bioassay

Problem Statement	*No commercial/off-the-shelf options*
Solution	*Design novel INBRX-121-specific potency cell line*

Key Functional Considerations for Cell-Line Design

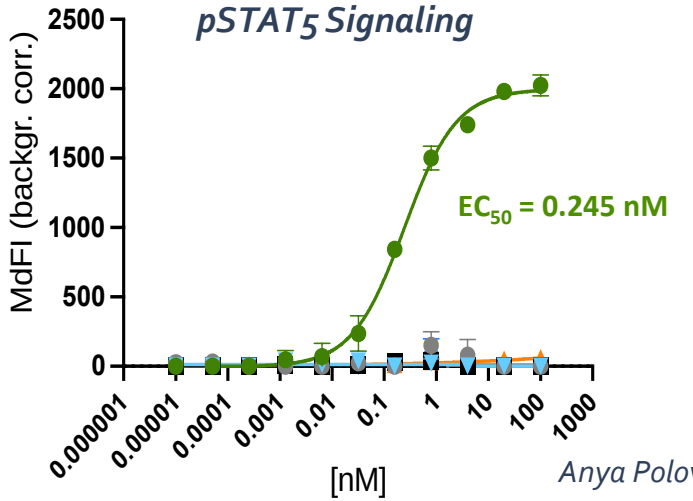
NKp46 Targeting



Angelica Sanabria

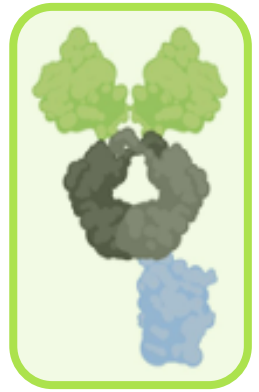
Binding of NKp46-specific sdAb part of INBRX-121 to PBMC subpopulation in healthy human donor blood

IL-2 Signal Transduction and Specificity



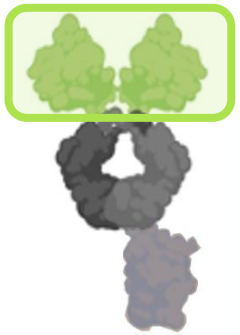
Anya Polovina

Healthy donor PBMC were stimulated with INBRX-121 dilutions; IL-2 signaling was quantified via pSTAT5



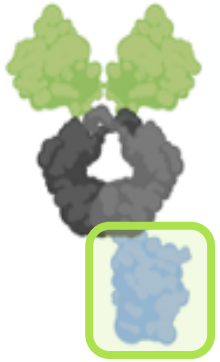
NK cell-specific pSTAT5 signaling is the result of

1. NKp46 targeting
2. Detuned IL-2-X binding to its receptor



High-Affinity Anti-NKp46

NK Cell specificity is driven by the NKp46-targting sdAb of INBRX-121



Downstream pSTAT5 signaling is driven by IL-2 binding

Low-Affinity IL-2-X

Required for Effective MOA Modeling

- ✓ NKp46 binding
- ✓ Downstream STAT5 signaling upon IL-2-X binding
- ✓ Specificity of NKp46 targeting combined with IL-2-X signaling

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Cell Line Design and Generation

Commercial Cell Line

HEK-Blue™ IL-2 cells from InvivoGen

- The binding of IL-2 to its heterotrimeric receptor leads to the JAK/STAT signal cascade and production of SEAP
- SEAP production can be colorimetrically quantified with the addition of substrate

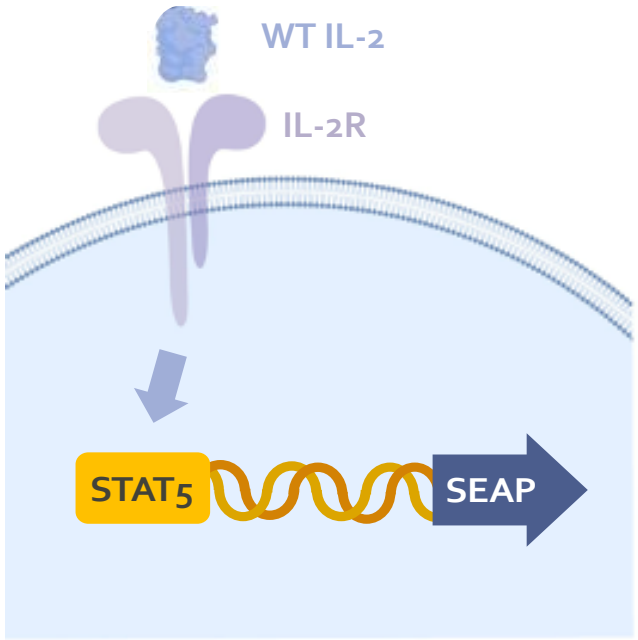
Augmented INBRX-121-Specific Cell Line

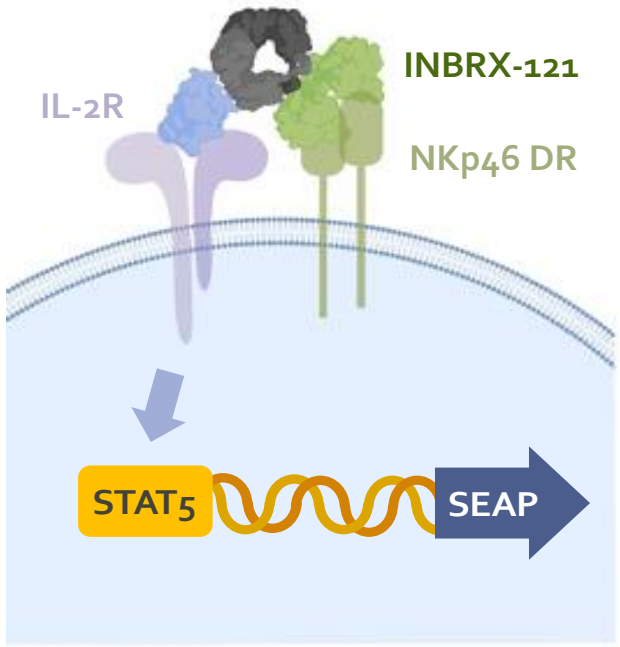
Addition of NKp46 "Dummy Receptor"

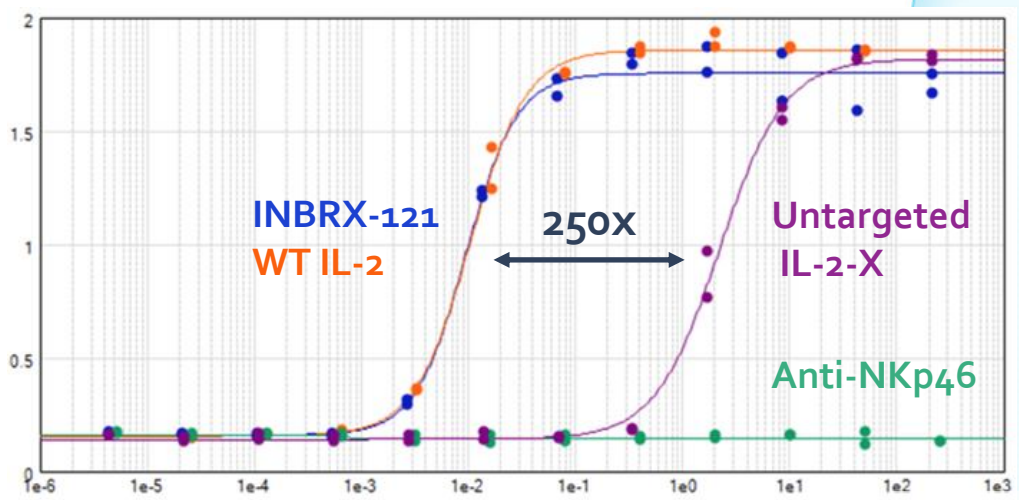
- NKp46 extracellular and transmembrane domain was stably engineered into the HEK-Blue™ IL-2 SEAP reporter gene line from InvivoGen

Specific Engagement of INBRX-121

- With the addition of NKp46 extracellular and transmembrane domains, we observed a 250x difference between INBRX-121 and untargeted IL-2-X
- This allows us to effectively model the affinity difference between the sdAbs and IL-2-X







Additional details and cell line history can be found in the appendix

Ying Li

Cell Line Characterization

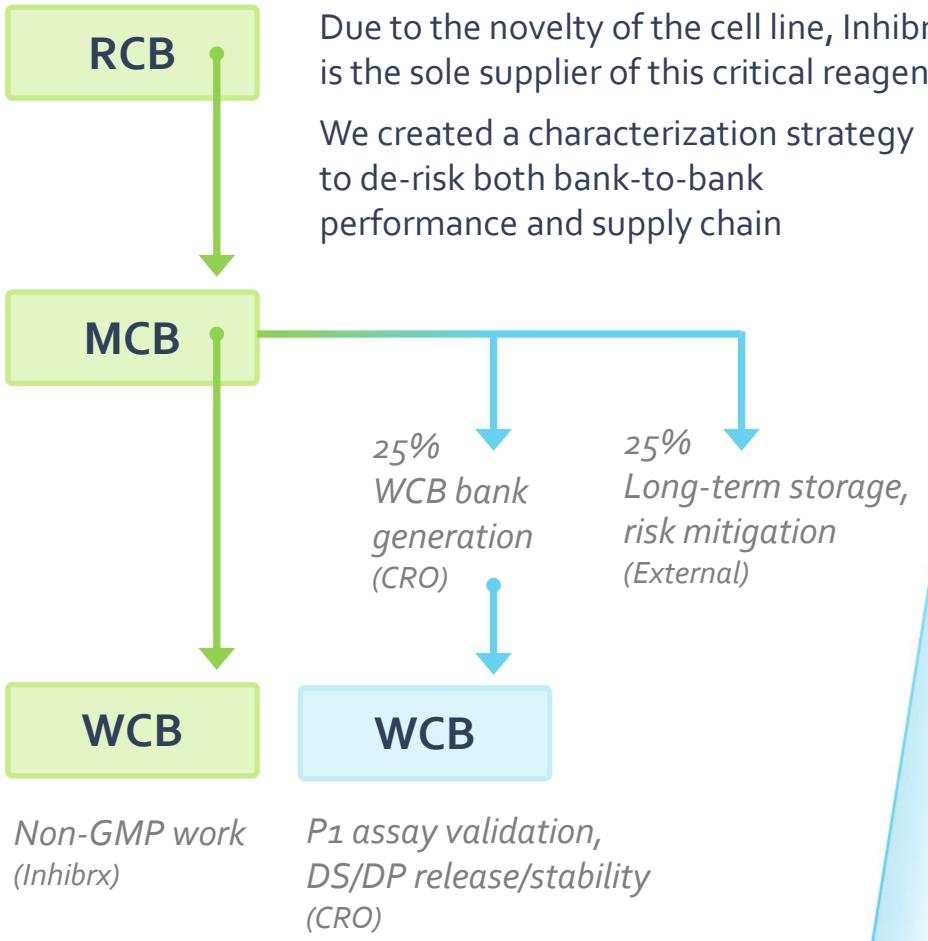
	RCB	MCB*	WCB*
Sequence transgene		Y	
Mycoplasma, Sterility, Human Virus Panel Screening *(External GMP testing)*		Y	
Post-banking functional check (1 week)	Y	Y	Y
Post-banking functional check (1 month)		Y	Y
Functional assessment *every 5 passages (to p25), 50-200% linear recovery and repeatability*		Y	Y
NKp46 receptor density quantification *every 5 passages (to p25)*		Y	Y
IL2 R receptor density quantification *CD25, CD122, CD132, every 5 passages (to p25)*		Y	Y
Bank-to-bank receptor density conformity		Y	Y

***Freezing Media and Banking Density Optimized**

Four test banks (5 vials/bank) were created to optimize:
- Cell banking density
- Freezing media
- Pre-banking harvest density

All test banks were assessed for:
- Post-thaw recovery
- Cell growth
- Assay function

Program Support: Cell Banks



Due to the novelty of the cell line, Inhibrx is the sole supplier of this critical reagent

We created a characterization strategy to de-risk both bank-to-bank performance and supply chain

INBRX-121 Cell-Based Bioassay Performance

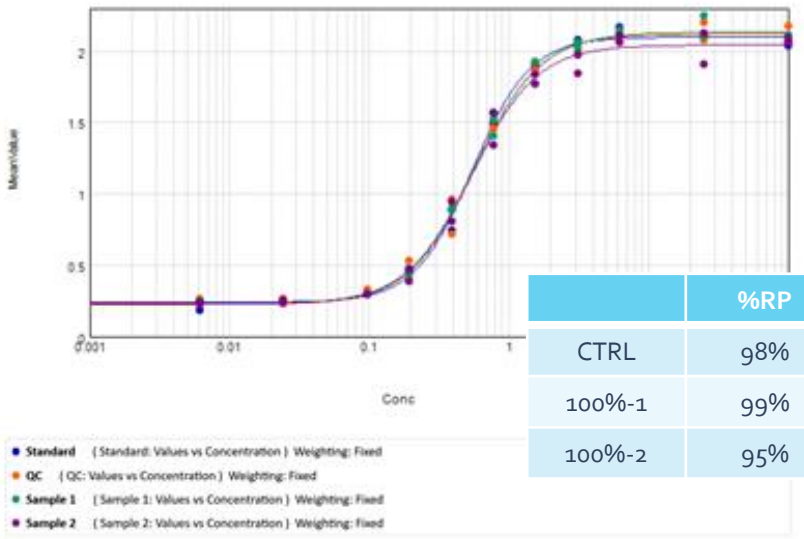
Example Plate Layout

- For our internal assay development, robustness, and pre-qualification, we place the control on the outer rows
- This helps our team to monitor potential edge effects over time and passage number

	1	2	3	4	5	6	7	8	9	10	11	12
A	CTRL– Pseudo Rep 1											
B	Sample 1 – Pseudo Rep 1											
C	STD – Pseudo Rep 1											
D	Sample 2 – Pseudo Rep 1											
E	Sample 1 – Pseudo Rep 2											
F	STD – Pseudo Rep 2											
G	Sample 2 – Pseudo Rep 2											
H	CTRL– Pseudo Rep 2											

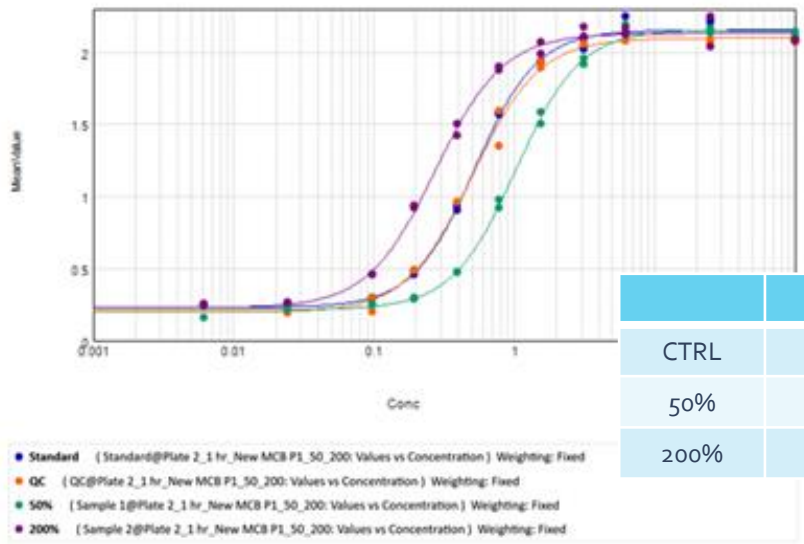
Representative Dose Response Curves

100% Repeatability



	%RP
CTRL	98%
100%-1	99%
100%-2	95%

50%, 200% Linear Recovery



	%RP
CTRL	96%
50%	51%
200%	187%

Ying Li

De-Risking CBBA Prior to Transfer and Validation

Robustness

Protocol

Day 1

- Add Cells
- Add INBRX-121
- Incubate o/n

Day 2

- Add supernatant to detection reagent*
- Develop detection
- Read on spectrophotometer

** Previously optimized from the vendor's protocol*

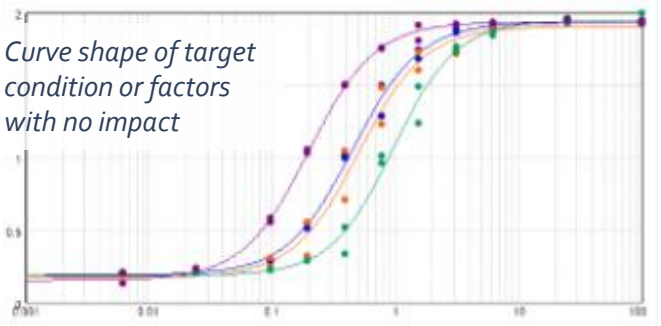
Robustness/DOE

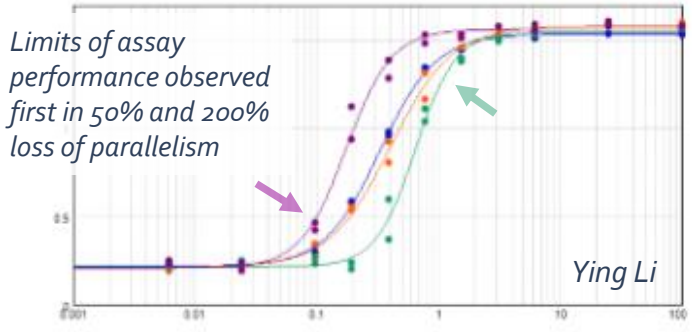
- Cell confluency: ±30% of target — *No impact*
- Cell density: ±20% of target — *No impact*

Key Factors

- Incubation: ± 10% of target → Locked at target ± 2.5%
- Supernatant: ± 20% of target → Locked at target
- Incubation: ± 60% of target → Locked at target ± 30%

Factors assessed with 50%, 100%, 200% linear recovery





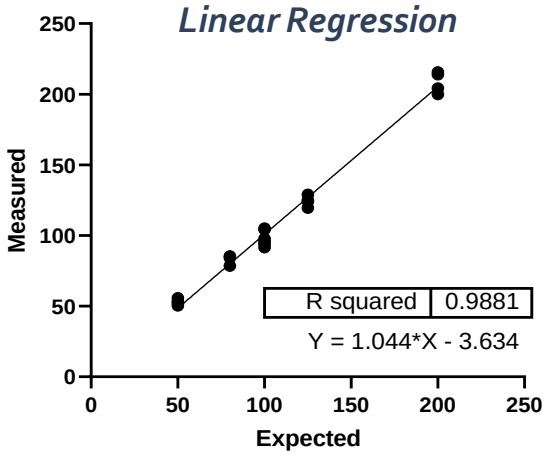
De-Risking CBBA Prior to Transfer and Validation

Assay Pre-qualification and Results

An in-house Inhibrx pre-qualification was performed to de-risk technical transfer and subsequent external validation:

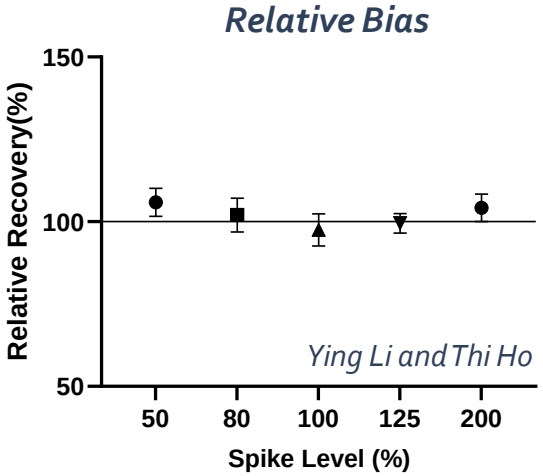
- 2 Analysts
- 5 Linear Recovery Levels: 50%, 80%, 100%, 125%, 200%
- 2 Linearity Repeats/Analyst
- 4 100% repeats/Analyst

Assay Pre-qualification Results



$R^2 = 0.988$

$Y = 1.04*X - 3.63$



Relative recovery: 98%-106%

Key Findings

Parameter	Results
Intermediate Precision	5% RSD
Specificity	Assay is specific, material used: - NKp46 targeting only - IL-2-X only
Stability Indicating	Assay is stability indicating, material used: - Blended Hinge-Cleaved Material - *(See discussion on Slide 20)*

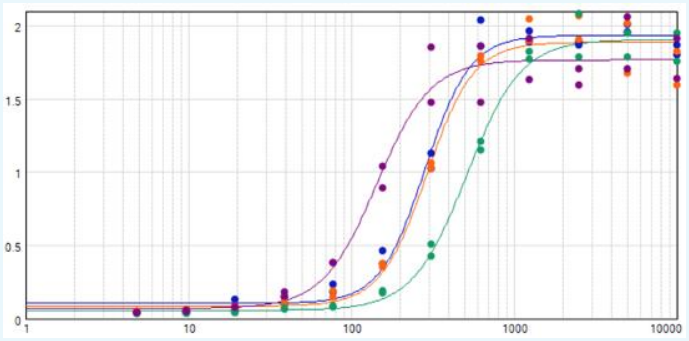
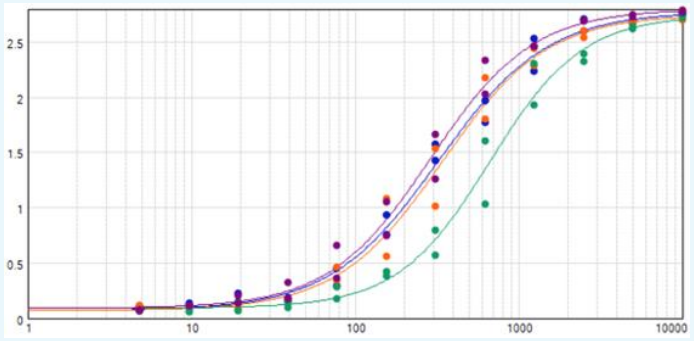
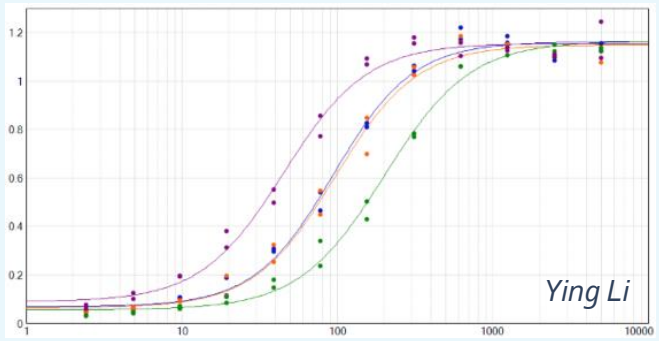
INBRX-121 Dual Domain ELISA

Problem Statement	*Low affinity IL-2-X is difficult to bind or detect in a plate-based binding assay*
Solution	*Screen non-traditional approaches to binding assays*

Plate-Based Assay Screening

- We considered only commercially available reagents for this assay screening
- For expediency, we used linear recovery samples to further hone our assay screening

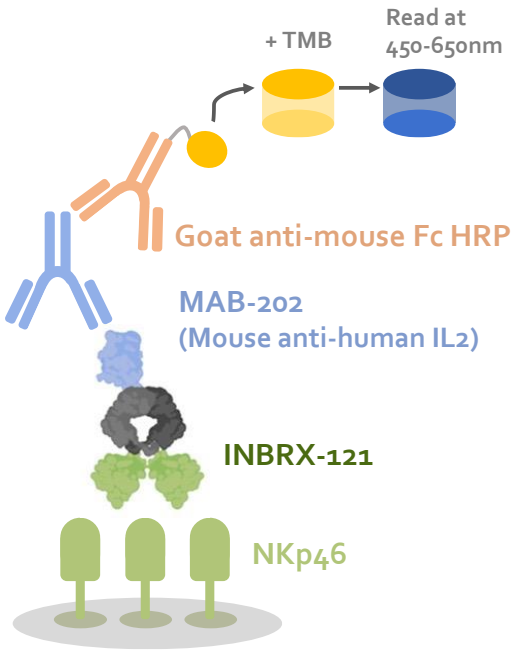
We screened several other assay formats and reagents. These are the only formats that generated reasonable curves

	Assay 1	Assay 2	Final Assay
Coat	NKp46	CD122 x CD132 hFc	NKp46
Detect	Biotinylated CD122 x CD132 hFc	Biotinylated NKp46	Anti-IL2 mAb (R&D systems , MAB-202)*
Secondary	Strep-HRP	Strep-HRP	Goat anti-mouse Fc HRP
Results			
Suitability	Not suitable Uncontrolled upper asymptote variability	Not suitable Failure in 200% linear recovery	Promising Accurate linear recovery, limited variability

***Anti-IL2 mAb, Clone MAB-202**
This mouse anti-human IL-2 antibody binds to the functional region and completely blocks the binding of IL-2 to its receptor

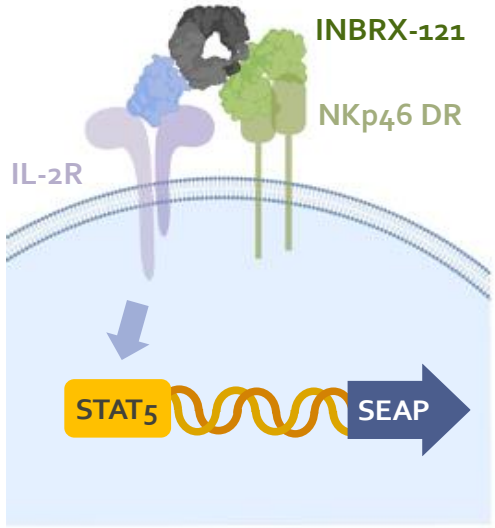
Clone MAB-202 and IL-2 Blocking Potential

Dual Domain ELISA

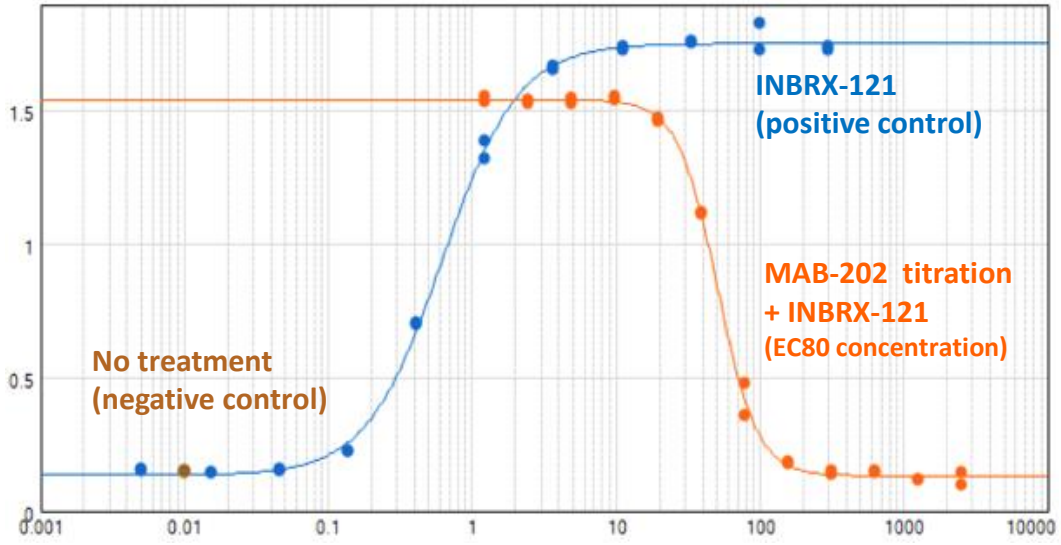


CBBA Refresher

Use INBRX-121 CBBA to assess MAB-202 functional region binding



Competitive Inhibition with CBBA



INBRX-121 (at an EC80 concentration) was pre-incubated with serially diluted MAB-202 prior to addition to the INBRX-121 CBBA reporter cells. *Ying Li*

Challenge

How can we prove MAB-202 binds to and blocks the functional region of IL-2-X?

Proof of Concept

If MAB-202 is blocking, it will:

- Prevent INBRX-121 from binding to IL-2 receptor
- No downstream signaling

The loss of STAT5-driven SEAP signal with increasing concentrations of MAB-202, which supports blocking of the functional region of IL-2

With the highest concentrations of MAB-202, complete loss of signal is observed, on par with low INBRX-121 concentrations and no-treatment negative control

INBRX-121 NKp46xIL2 Binding ELISA Performance

Example Plate Layout

- For our platform ELISAs, we used a more traditional plate layout
- Pseudo replicates are critical to reduce the inherent variability likely caused by the weak binding interactions of INBRX-121 with MAB-202

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD – Pseudo Rep 1											
B	Sample 1 – Pseudo Rep 1											
C	Sample 2 – Pseudo Rep 1											
D	CTRL– Pseudo Rep 1											
E	STD – Pseudo Rep 2											
F	Sample 1 – Pseudo Rep 2											
G	Sample 2 – Pseudo Rep 2											
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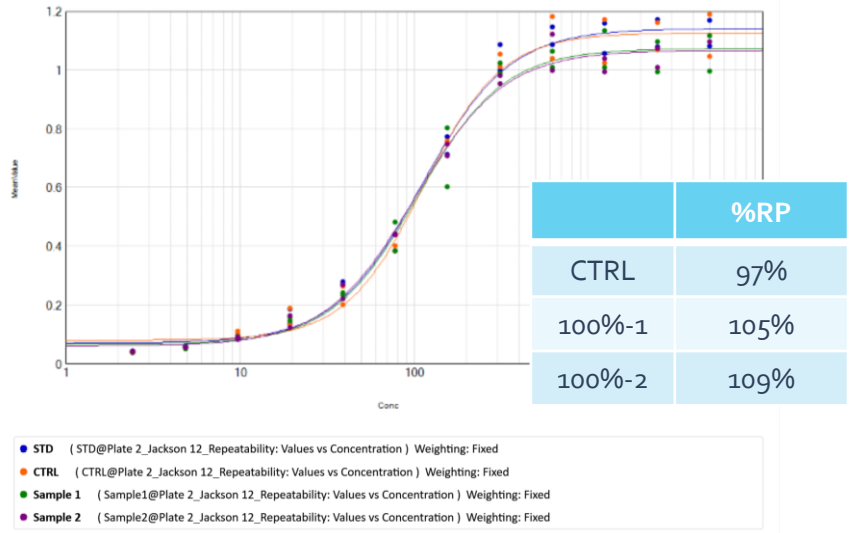
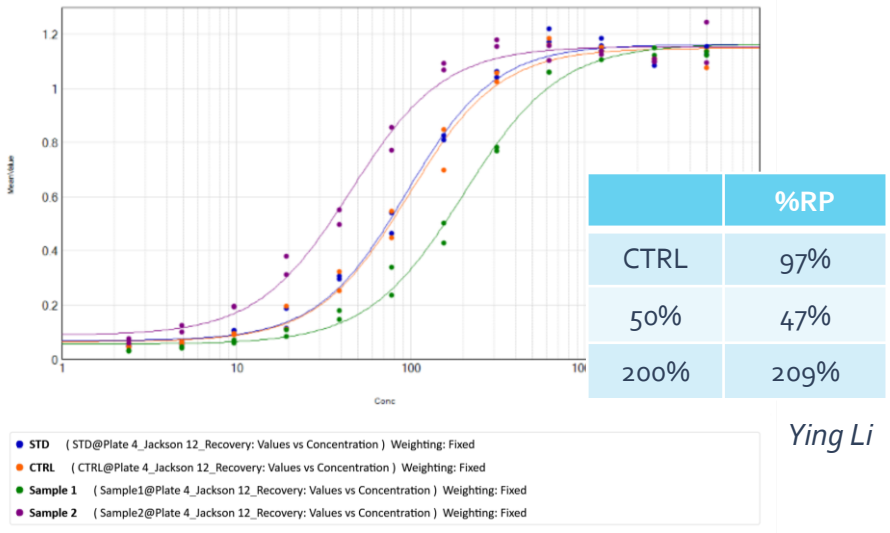
Representative Dose Response Curves

100% Repeatability



	%RP
CTRL	97%
100%-1	105%
100%-2	109%

50%, 200% Linear Recovery

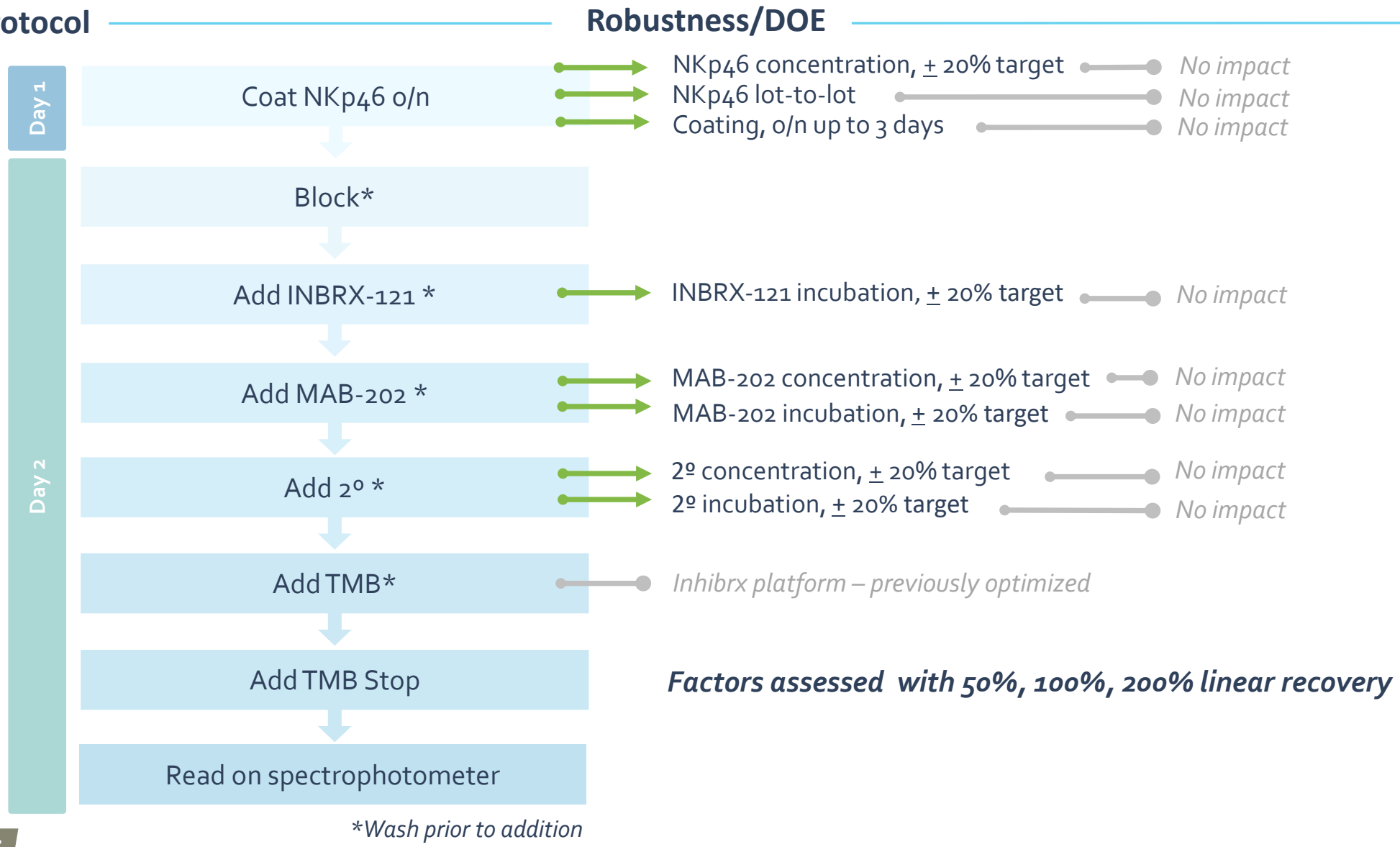


	%RP
CTRL	97%
50%	47%
200%	209%

Ying Li

De-Risking Dual Domain ELISA Prior to Transfer and Validation

Robustness



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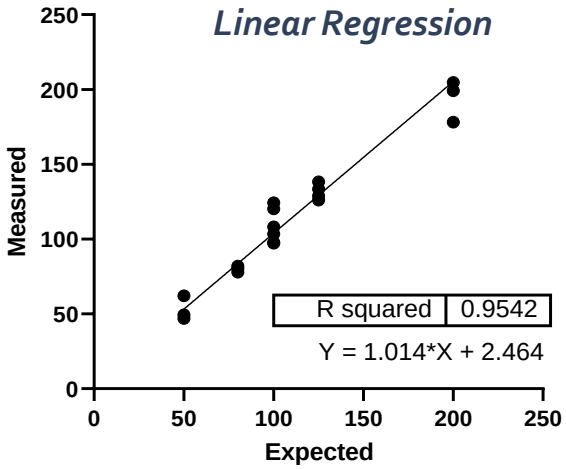
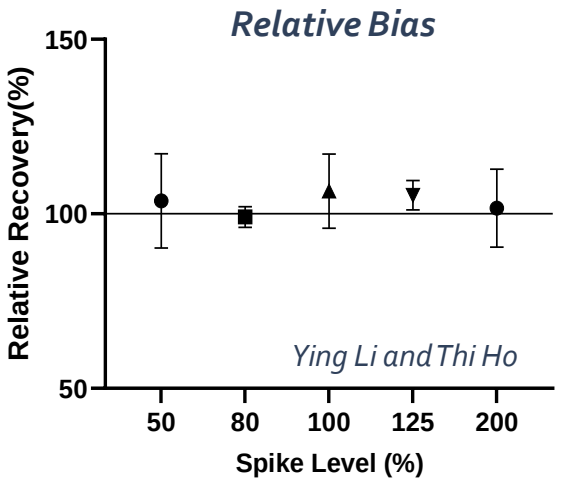
Assay Pre-qualification Results



$R^2 = 0.954$

$Y = 1.01*X + 2.46$



Relative recovery: 99%-107%

Key Findings

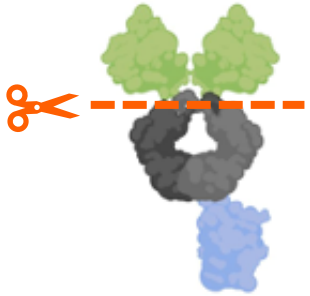
Parameter	Results
Intermediate Precision	10% RSD
Specificity	Assay is specific, material used: - NKp46 targeting only - IL-2-X only
Stability Indicating	Assay is stability indicating, material used: - Blended Hinge-Cleaved Material - *(See discussion on Slide 20)*

INBRX-121 Early Structure/Function

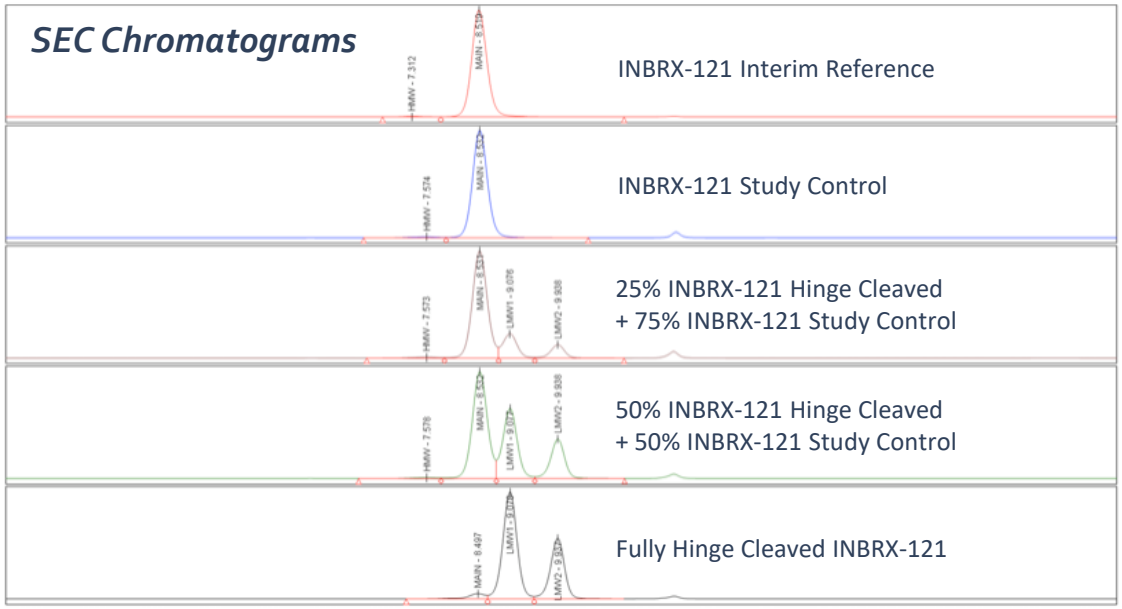
Structure-Function: Hinge Cleaved Samples

Concept

Due to program stage (pre-clinical), no relevantly stressed materials were available:



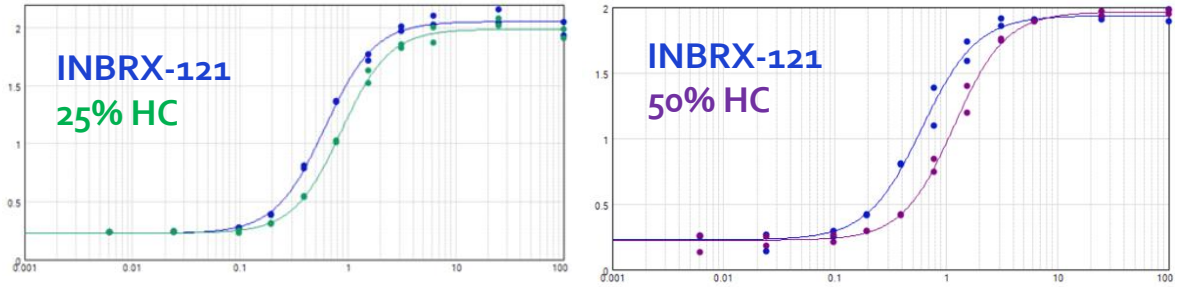
- Therefore, based on the MOA of INBRX-121, we decided to proteolytically cleave it at the hinge with IdeS
- Fully cleaved material was then blended with untreated INBRX-121 (study control)



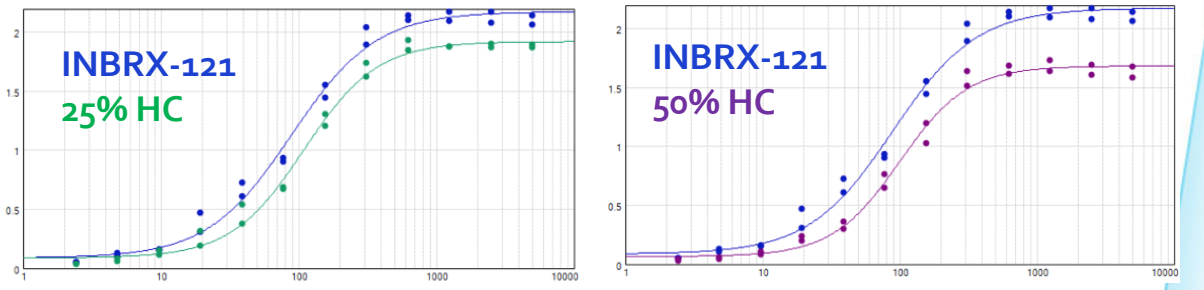
Yao Chen

Results

CBBA



ELISA



Ying Li

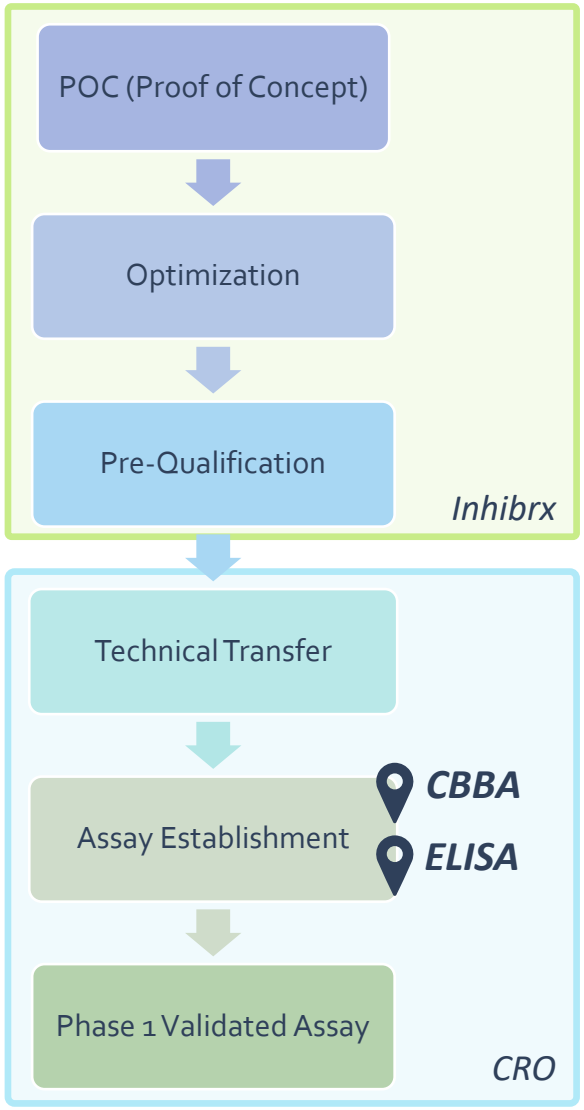
Results Summary

	CBBA %RP *Avg N=2*	ELISA %RP *Avg N=3*
25% HC	67%	70%
50% HC	51%	53%*

** Estimated relative potency due to lack of parallelism*

INBRX-121 Assays Ready for Phase 1 Validation

Current Status



Key Designations	CBBA *NKp46 x IL2 QuantiBlue Reporter Gene*	Dual Domain ELISA *Anti-IL2*
Pre-Qualification	**Key Findings** • *5% RSD* • *Specific to intact INBRX-121* • *Stability indicating*	**Key Findings** • *10% RSD* • *Specific to intact INBRX-121* • *Stability indicating*
Critical Reagents	**De-Risked** ✓ *Cell line characterization complete* ✓ *Cell line licensing complete* ✓ *Vector licensing complete* ✓ *Sterility, mycoplasma, human virus panel testing complete*	**De-Risked** ✓ *Commercially available reagents* ✓ *No impact of lot-to-lot*
Documentation	**Enabled Technical Transfer** *(2 sites)* ✓ *Protocol complete* ✓ *MDR complete* ✓ *Cell Banking and Characterization Document complete*	**Enabled Technical Transfer** *(2 sites)* ✓ *Protocol complete* ✓ *MDR Complete*
Validation	*Planned* ***Q4 2022***	*Planned* ***Q3 2022***

Next Steps for INBRX-121 Potency Assays

The CBBA and Dual Domain ELISA will be used for release and stability testing under GMP conditions. Using these assays we will:

Stage 1

Monitor changes to INBRX-121 while on stability

Stage 2

Understand the impact of minor forms of INBRX-121

Theoretical Minor Forms

Half Molecules

- IL-2-X

+ IL-2-X

Homodimers

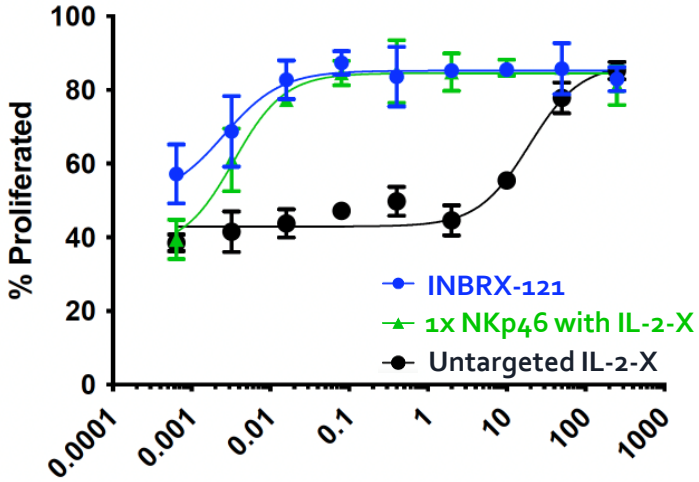
Knob-knob

Hole-hole

To date, no half-molecules or homodimers have been observed with current INBRX-121 single cell clone material

Potential Impact

In vitro NK cell Proliferation (Human)



1x NKp46 w/ IL-2-X

Chelsie Macedo

Heathy human donor blood was labeled with a proliferative dye and treated with INBRX-121. NK cells were identified with specific cell lineage markers and NK proliferation was quantified via flow cytometry

This is an example of potential limitations of the CBBA

- Both assays will be used in parallel for further product characterization, and to understand assay limitations
- If one assay is insensitive to molecule change, we have an orthogonal method as an alternative

Appendix

INBRX-121 Cell-Based Bioassay

Cell Line Generation History

- The NKp46 x IL-2 QuantiBlue reporter gene cell line is a weakly adherent HEK293 line
- Upon IL-2 stimulation, HEK-Blue™ IL-2 cells trigger the activation of STAT5 and the subsequent secretion of SEAP. The levels of STAT5-induced SEAP can be readily monitored using QUANTI-Blue ™
- Insert: NKP46 signal peptide, extracellular domain and transmembrane domain. Intracellular domain was replaced with a fluorescent compound to prevent NKP46 driven intracellular signaling
- Cells were transfected using a commercially available protocol
- Transfected cell pools were then harvested and labeled with a conjugated antibody according to the target of interest. Cells single sorted into 96-well culture plates (confirmed visually 1 cell/well) based on expression
- Clonal cell cultures were expanded and were screened for citrine expression. ICD fluorescent compound positive clones were expanded into flasks. Selected cell lines were tested for NKP46 expression and reporter gene function.

Cell Line Media and Culturing Conditions

Growth Media for NKP46 transfected cells

- DMEM-based with FBS and selection antibiotics

**Other lots/vendors have been tested with this cell line – no differences in expression or performance were observed*

Seeding Density for Culturing

- 2-, 3-, and 4-day culturing conditions were established

Seeding Density for Cell Banking

- 2-, and 3-day culturing conditions were established
- These conditions differ from regular culture conditions and were optimized for bank consistency and post-thaw recovery