# Using B-Body™ Bispecific/Multispecific Antibodies to Modulate Anti-tumor Immune Responses

Bonnie J Hammer, Ph D

August 29th, 2018

# Multispecific Antibodies

- Why use bispecific/multispecific antibodies?
  - Redirecting immune cells to tumors—T cells, NK cells, Macrophages
  - Increased specificity—SNIPER™ Treg Depleter
  - Novel mechanisms of action—Precision OX40 Agonists

- What makes a good bispecific/multispecific?
  - IgG like – low immunogenicity risk and longer half lives
  - High expression levels
  - Easy to purify – pure product is essential for the clinic
  - Standard manufacturing protocols – no custom GMP facility

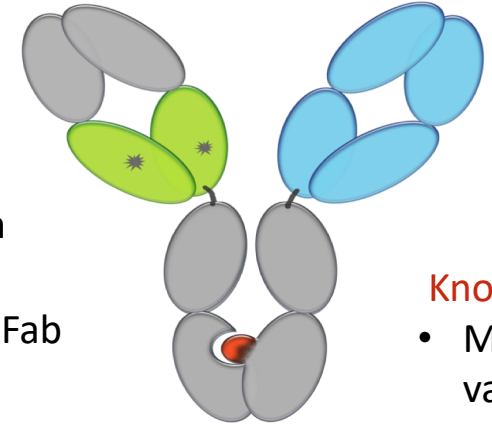


**Multispecific Antibodies Create New Opportunities in IO**

# B-Body™ Design Strategy

**Plug-N-Play Variable Domains**

- Enables direct import from all discovery sources
- Rapid in-format Discovery



CH1/CL Domain Substitution

- Derived from human antibody domain
- Multiple sets of orthogonal mutations
- Thermostability comparable to native Fab
- Proprietary separation technique for discovery

First Fab Arm

- Wild type fab architecture

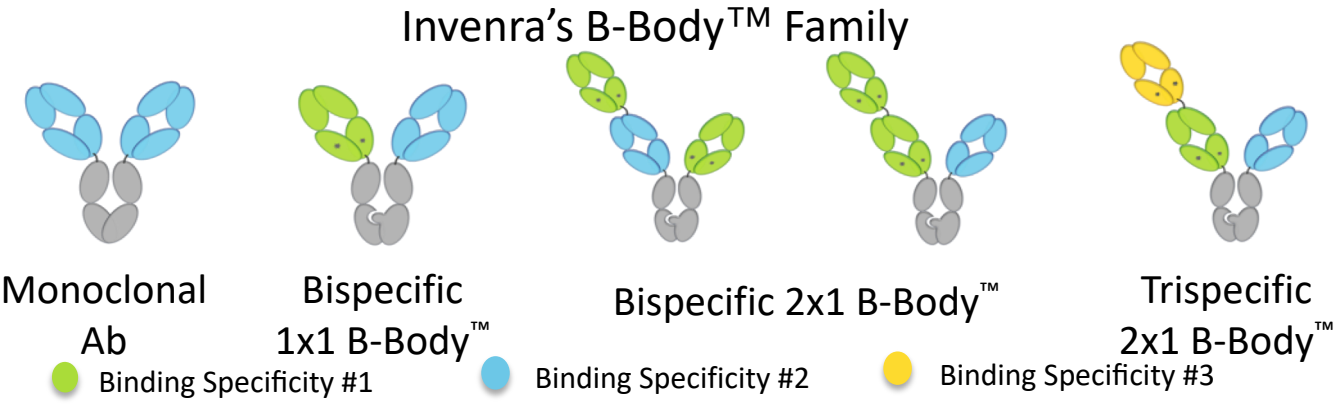
Knob-in-Hole-like Mutation

- Mutation set is clinically validated



**Maximizes Performance in Discovery and Manufacturing**

# Invenra's B-Body™ Platform

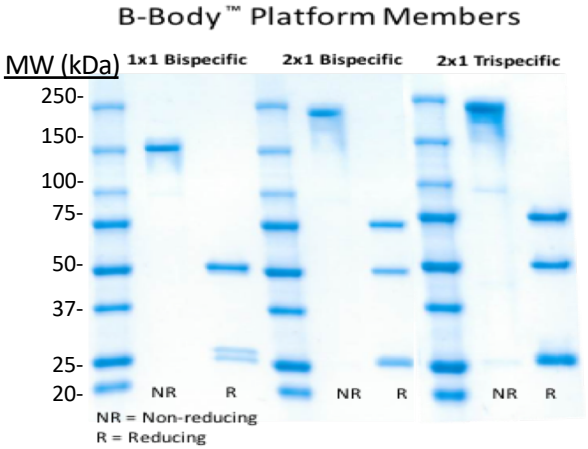



- The B-Body™ platform enables high throughput functional discovery <u>in the final therapeutic format</u>
  - In-format discovery avoids missing rare combinations of affinity, epitope, and architecture required for first-in-class therapeutics
- B-Bodies™ are designed for compatibility with <u>standard manufacturing</u> processes required for therapeutic development

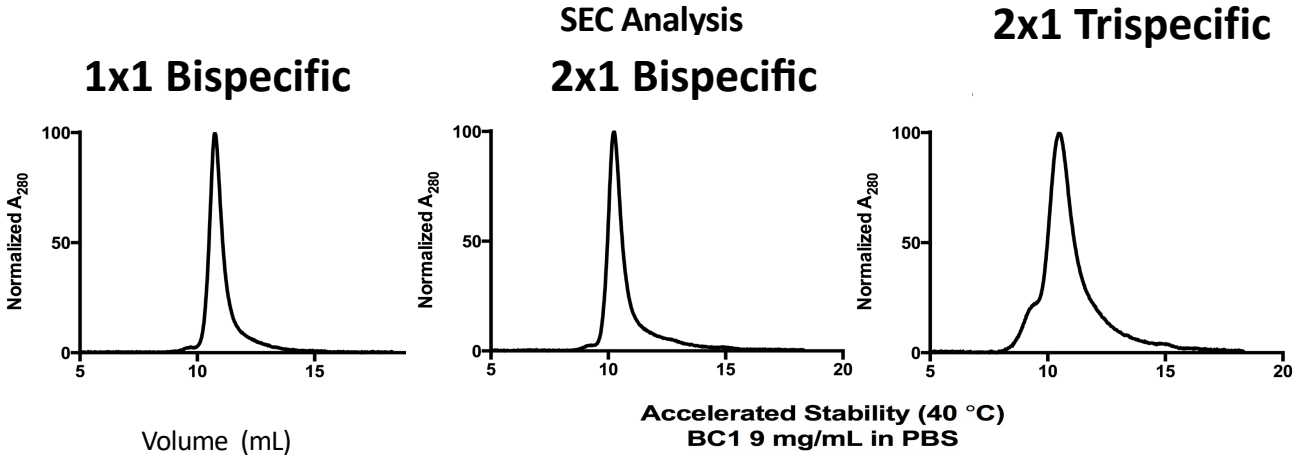


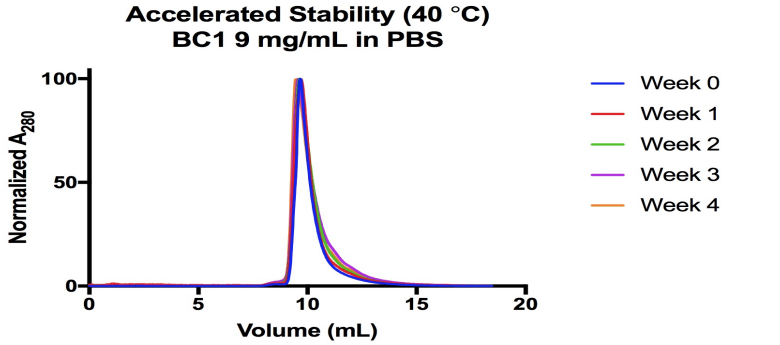
**High Performance in Both Discovery and Mfg Delivers New IO Drugs**

# Analysis of the B-Body™ Platform




SEC Analysis



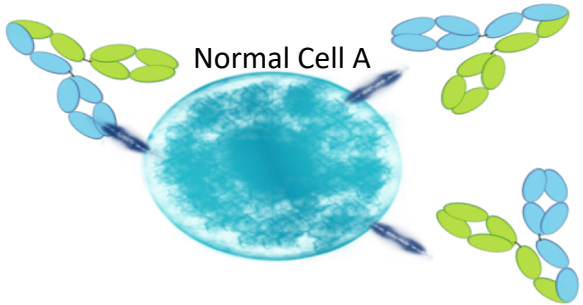

- High purity & homogeneity after purification
- Purification strategies compatible with standard platform processes
- Long term stability
- Thermostability



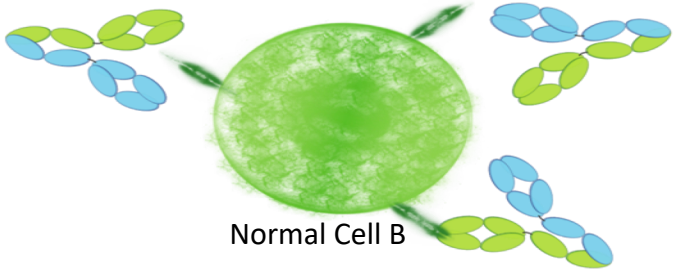
**Robust B-Body™ Assembly Facilitates Single-Step Bispecific Purification**

# SNIPER™ Treg Depletion

# SNIPER™ Theory

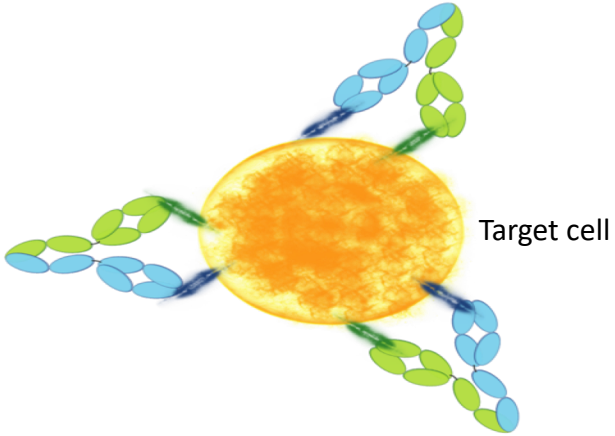



Monovalent binding; No avidity




Preferential targeting

<




Concurrent bivalent binding; Strong avidity



**SNIPER™ Bispecific Antibodies Can Be Highly Specific**

# Uses for SNIPER™ Technology

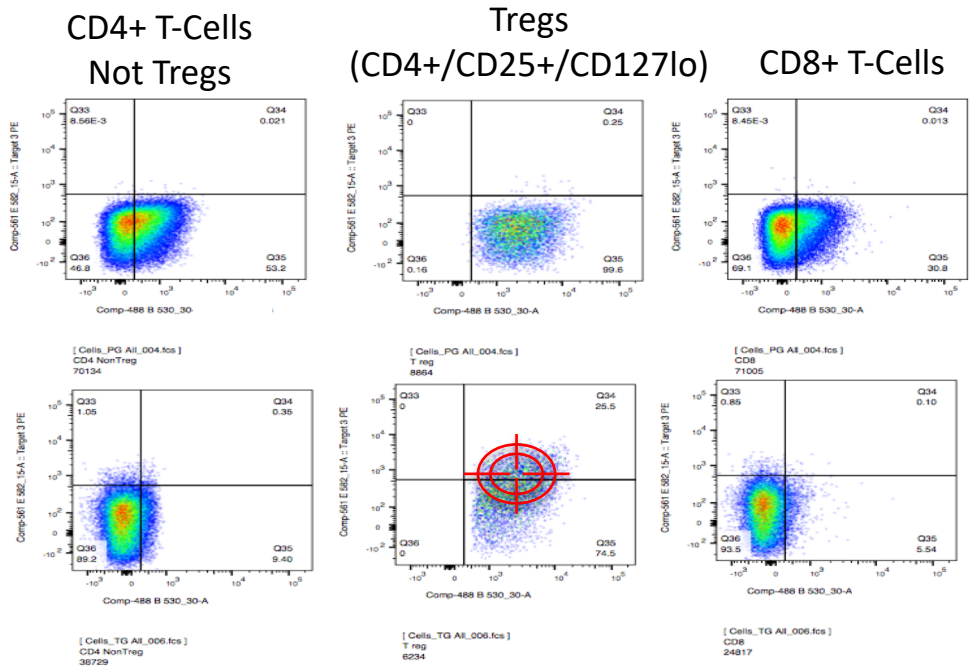
- SNIPER™ Proof-of-Principle Studies
  - Use SNIPER™ to specifically eliminate Tumor Tregs while avoiding effector T-cells and peripheral Tregs for treatment of cancer

- Future Uses:
  - SNIPER™ ADCs – Utilize combinations of tumor antigens for more precise tumor targeting and killing
  - SNIPER™ Redirection – B-Body™ trispecifics enable the redirection of effector cells (T-cells, NK cells, etc) based on combinations of tumor antigens
  - SNIPER™ Localization – B-Body™ SNIPERs can localize therapeutics to specific environments in the body



**SNIPERs™ Open New Therapeutic Options**

# SNIPER™ Target Validation




- Invenra has validated 5 targets that enable multiple combinations for unique targeting of Tumor Tregs in patient matched tumor/blood samples
- Confirmed that double positive Tregs are rare in the periphery of Healthy and Patient Donors



Confirm Double Positive Tregs in Tumor and not Blood

# Monovalent vs Bivalent Affinity

Target 2 Candidates

| KD (nM)* | | Ab-1 | Ab-2 | Ab-3 | Ab-4 | Ab-5 | Ab-8 | Ab-9 | Ab-11 | Ab-12 | Ab-13 | Ab-16 | Ab-17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 40 | 33 | 32 | 10 | 32 | 30 | 77 | 56 | 500 | 35 | 500 | 500 |
| Ab-1 | 106 | 0.88 | 0.95 | 0.97 | 0.34 | 0.74 | 0.46 | 0.87 | 0.45 | 0.67 | 0.36 | 0.59 | 1.30 |
| Ab-2 | 62 | 1.72 | 1.82 | 1.76 | 0.90 | 1.22 | 0.99 | 1.80 | 0.84 | 3.86 | 0.71 | 2.70 | 5.60 |
| Ab-4 | 66 | 0.47 | 0.65 | 0.65 | 1.68 | 0.70 | 0.67 | 0.79 | 0.50 | 1.66 | 0.42 | 1.25 | 1.99 |
| Ab-5 | 105 | 1.08 | 1.26 | 1.15 | 0.65 | 0.98 | 0.88 | 1.50 | 0.55 | 3.50 | 0.51 | 2.23 | 4.25 |
| Ab-7 | 83 | 0.29 | 0.29 | 0.39 | 0.23 | 0.23 | 0.25 | 0.29 | 0.18 | 0.17 | 0.15 | 0.20 | 0.45 |
| Ab-8 | 135 | 0.58 | 0.73 | 0.82 | 0.51 | 0.64 | 0.56 | 1.00 | 0.38 | 2.00 | 0.29 | 1.46 | 2.00 |
| Ab-9 | 79 | 0.39 | 0.52 | 0.57 | 0.38 | 0.59 | 0.50 | 0.59 | 0.31 | 0.93 | 0.55 | 0.85 | 1.70 |
| Ab-10 | 116 | 0.38 | 0.45 | 0.47 | 0.43 | 0.52 | 0.46 | 0.47 | 0.32 | 0.65 | 0.36 | 0.53 | 1.00 |

Target 1 Candidates

Monovalent

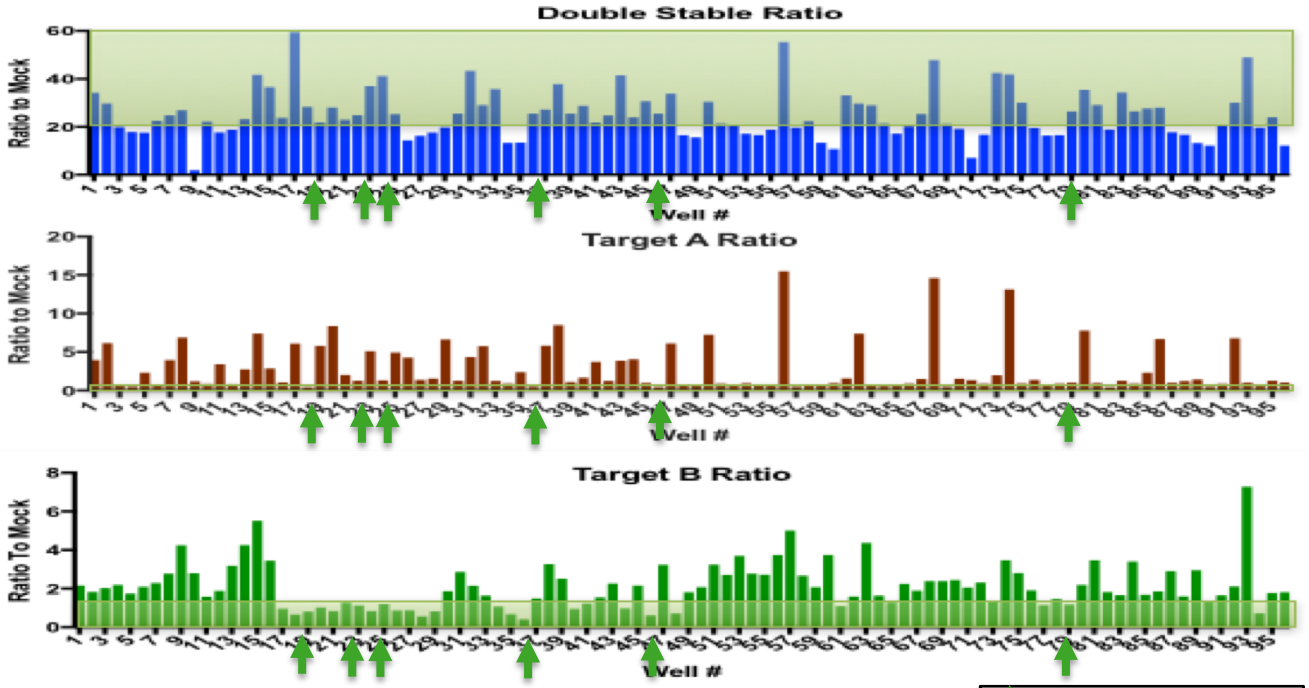
Monovalent

*KD estimates based on single concentration measurement



Low Affinities Can Generate High Avidity

# Target Cell Binding




Note: B-Body™ binding tested at 7 nM

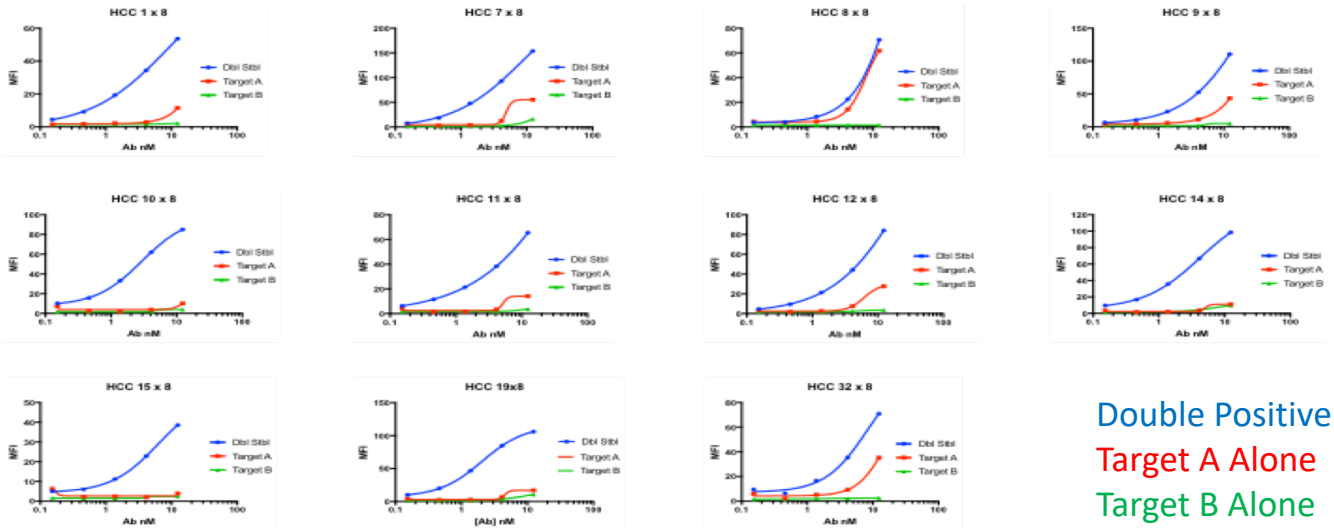
Top Candidates

We compared the cell binding of 96 purified B-Body™ candidates to double-positive and single-positive cell lines to identify SNIPER ™ candidates with the best selectivity.



Six Candidates Identified From Cell Binding

# Human SNIPER™ Discovery

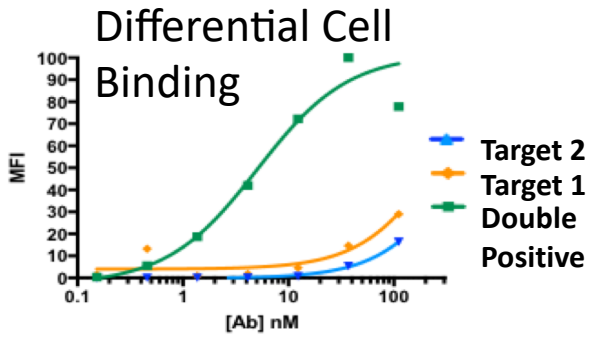



- Utilized matrix screening and dose response experiments to identify the best combination of binding pairs on double positive cell lines



Lead Selection Based Upon Differential Binding

# Human SNIPER™ Lead Candidate

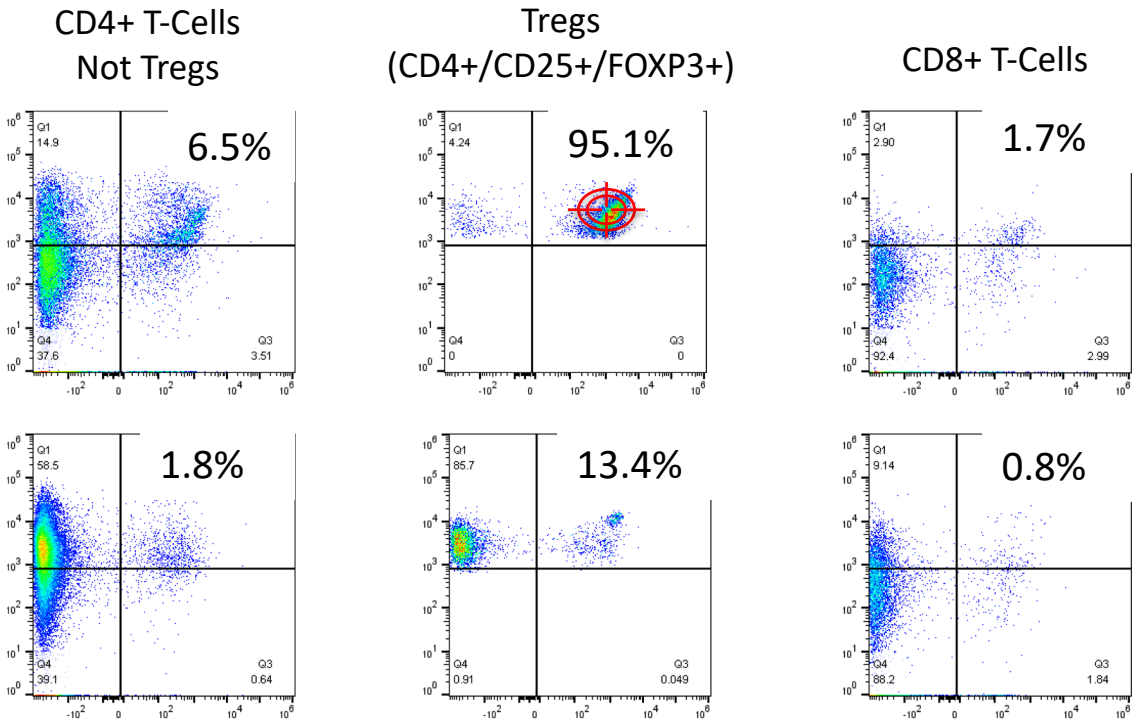



- Lead Candidate chosen based on:
  - Cell binding – Largest differential between single positive and double positive cell lines
  - Single positive cell lines with affinity >100 nM
  - Initial developability profile – High performance of parent IgGs in assays



Lead Candidate Ready for Primary Cell Screening

# Differential Targeting of Tumor Tregs



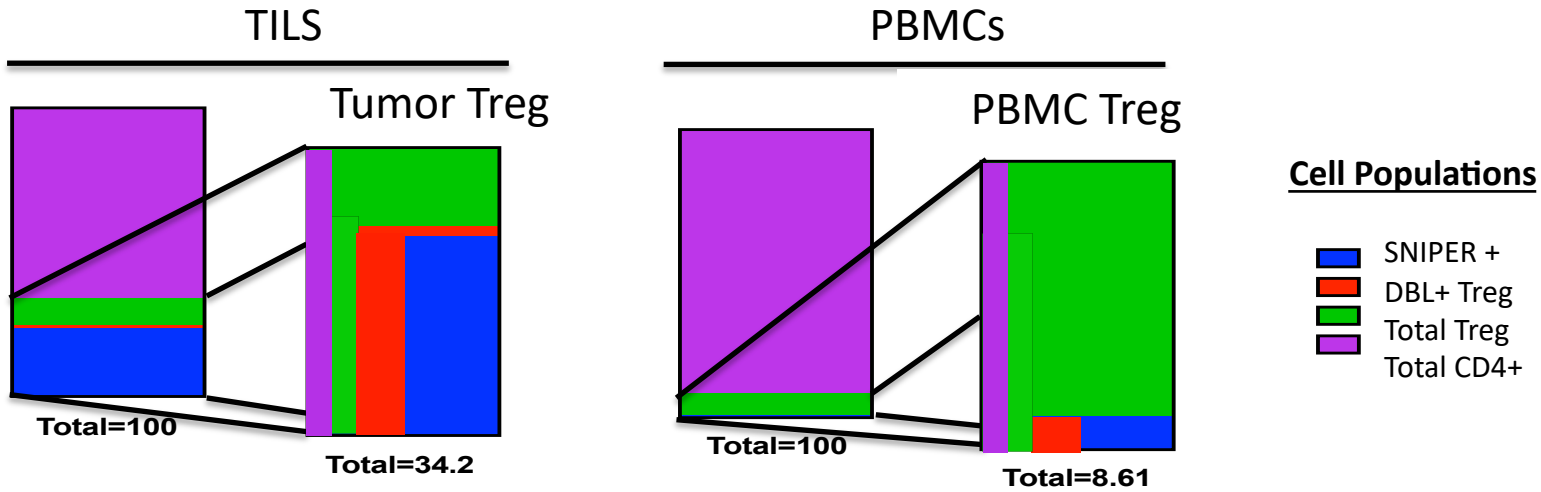

- Analyzed the binding of our Human SNIPER™ to matched NSCLC Tumor & PBMCs samples from two patients
- Goal – Target only the double positive tumor Treg population



Tumor Tregs are Preferentially Bound

# Patient 1: Tumor Treg Targeting

Lead candidate tested on primary human lung tumor samples with matched PBMCs




- 95% of the double positive Tregs were targeted in the tumors
- 98% of the double positive Tregs were targeted in the PBMCs
- 88% of the circulating Tregs were spared
- Great potential to reduce autoimmunity by reducing targeting of peripheral Tregs



**SNIPERs™ Specifically Target Tumor Treg Population**

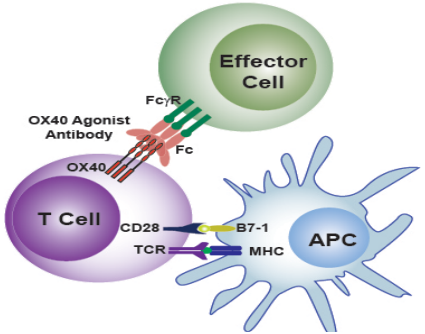
# SNIPER™ Lead Summary

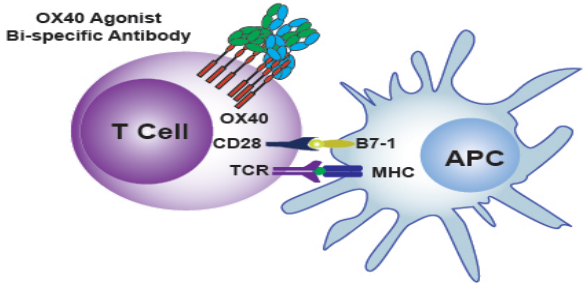
| Assay | Lead | Backup |
|---|---|---|
| Primary Cell Activity | +++ | ++ |
| Yield/Purity | +++ | +++ |
| Homogeneity | +++ | +++ |
| Accelerated Stability | +++ | +++ |
| Acid Stability | ++ | ++ |
| Stability at 25 mg/ml | +++ | TBD |
| Thermostability | +++ | +++ |
| Analytical HPLC (HIC, SMAC, & CIC) | +++ | +++ |
| Cyno Cross-reactivity | +++ | +++ |

- Next Steps:
  - Continue MOA Studies
  - Scale up for rat PK Studies
  - CMC
    - Cell-line development
    - Preclinical assays

# Multispecific OX40 Agonist Antibody

# The Disconnect of Clinical OX40 Agonists



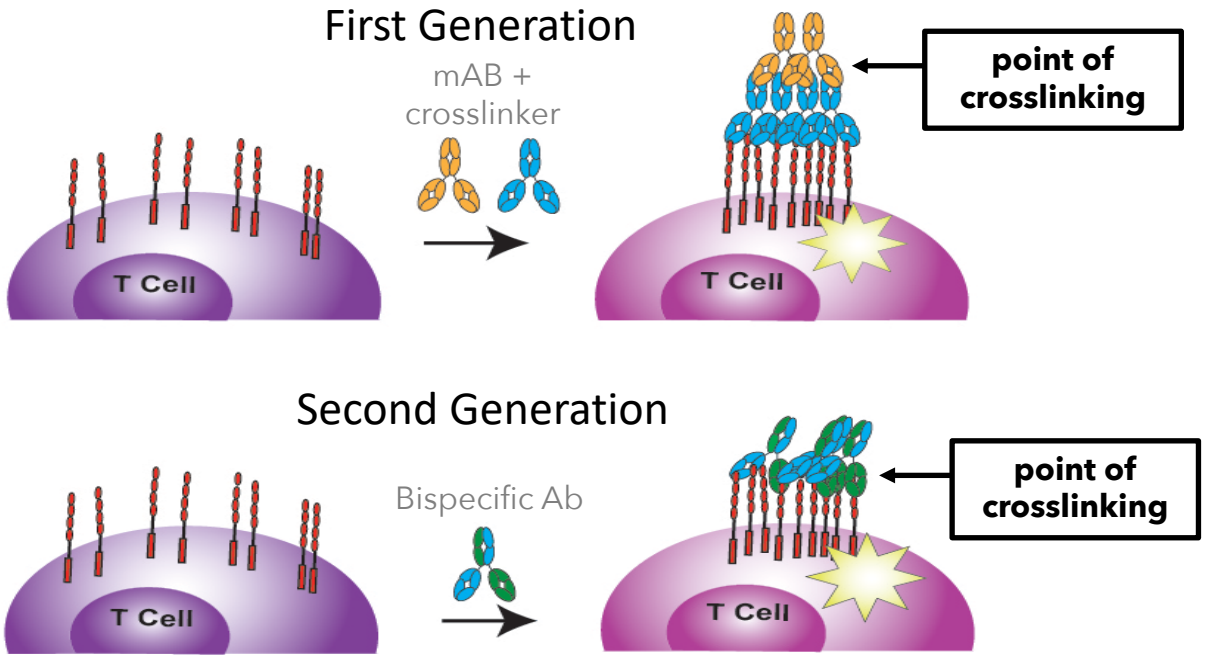




- First-generation of OX40 antibodies are monoclonal
  - Higher order cross-linking dependent upon local tumor environment, Fc engagement
  - Very good activity in preclinical animal studies
  - Have had limited activity in clinical studies
- Second-generation agonists exploit alternative strategies for higher order clustering
  - OX40 x Tumor Antigen
  - Fc Oligomerization variants
  - Bispecific Antibodies



**Current OX40 Agonists Have Not Reached Expected Potential**

# Screening for Novel Mechanisms




- Strategy: Exploit in-format, HTP screen of bispecific to directly cross-link and activate OX40
- All vs. all matrix of α-OX40 variants with diverse
  - Epitope
  - Affinity
  - Geometries

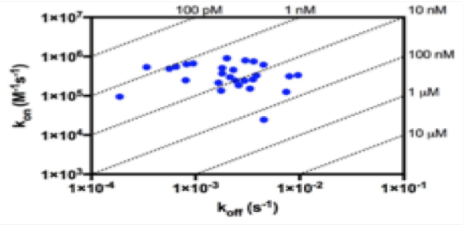


**Bispecifics Enable Receptor Clustering w/o 2º Crosslinking**

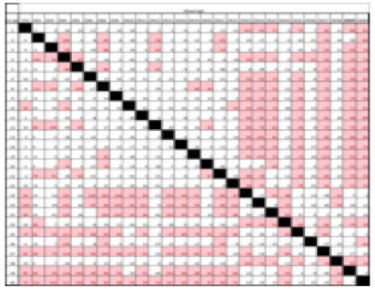
# High Throughput B-Body™ Discovery

Antibody Discovery → mAB Characterization → Clone into B-Body™ in 4 Individual Chains → HT B-Body™ Production

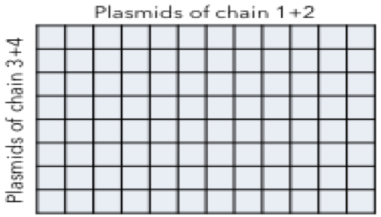
Affinity




Epitope Binning

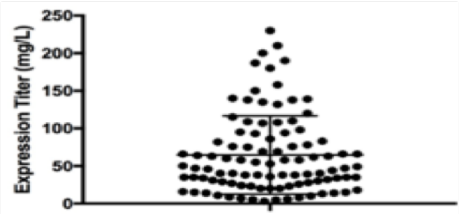


Plasmid Mixing
Transient Transfection
Protein Expression and Purification




Representative B-Body ™ Purity


Yield

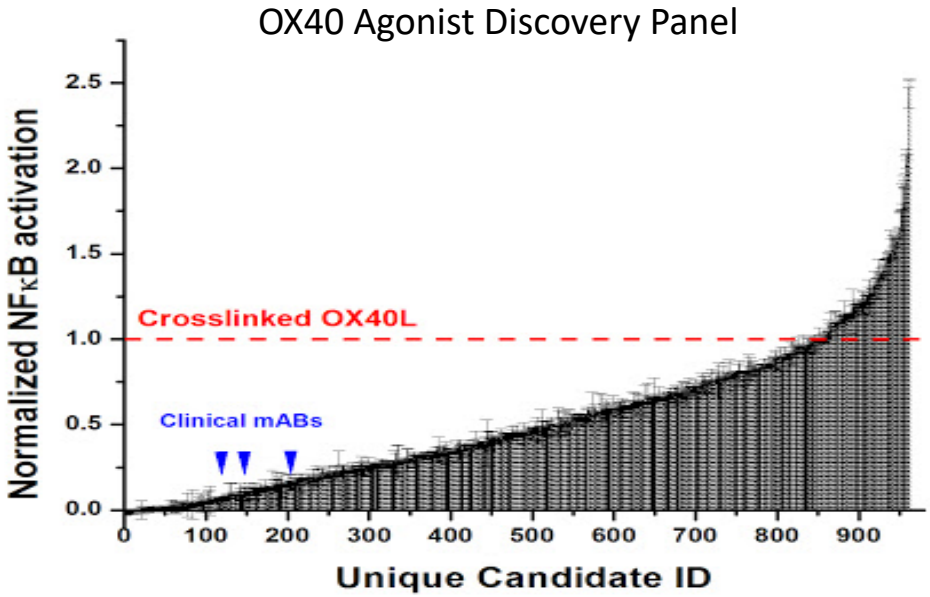



Small-scale (1.5 mL) expression and one-step affinity purification



**B-Body™ Platform Enables High Throughput Functional Discovery**

# B-Body™ Enables High-Throughput Discovery

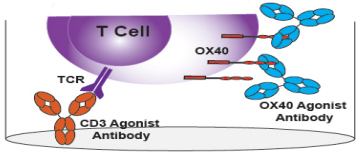



- > 900 B-Body™ agonists generated in a single experiment → both 1x1 and 2x1 formats
- A range of activities were observed
- A number of candidates were superior to the cross-linked OX40L

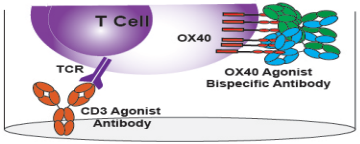


B-Body™ Platform Enables Functional Candidate Discovery

# Primary T-cell Activation by a Bispecific OX40 Agonist Antibody

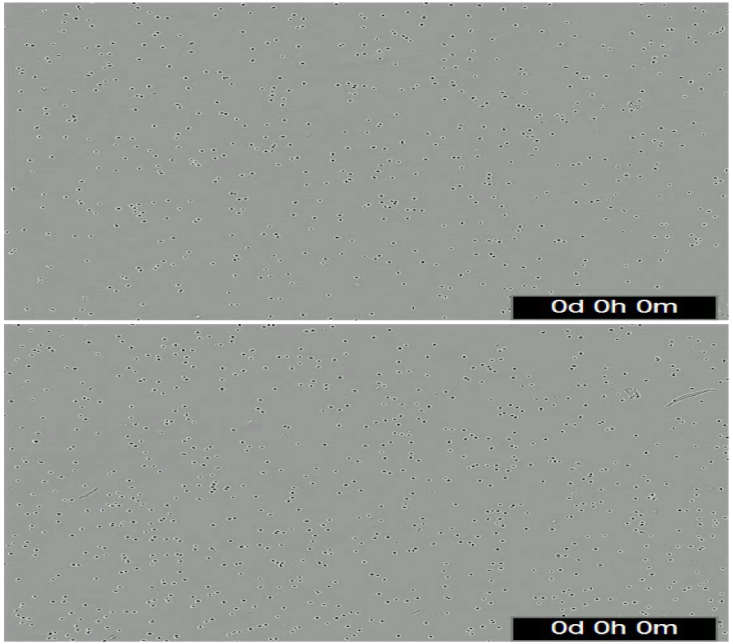



Soluble Clinical mAB

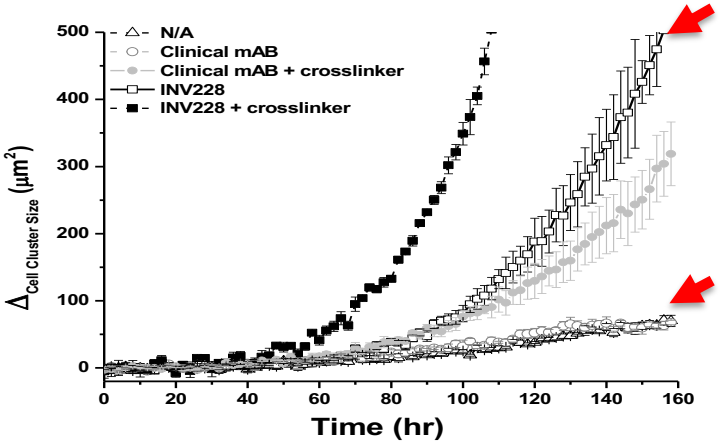



Soluble Bispecific

Real Time CD4+/CD45RA+/CD25- T-cell Activation




The Kinetics

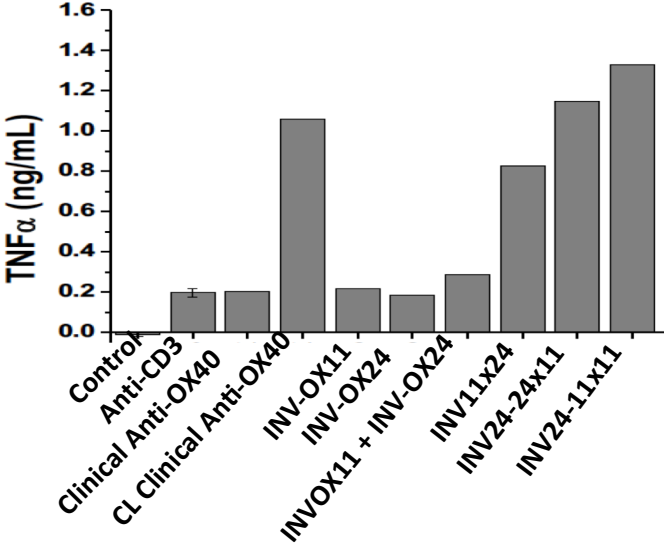


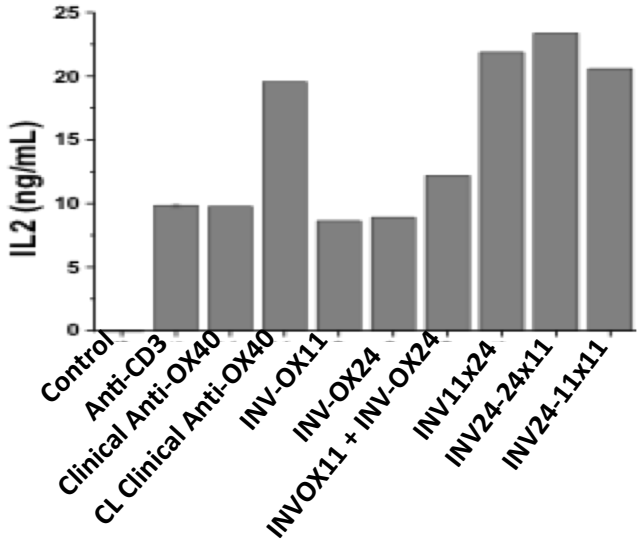

- Critical combinations of epitope, affinity, and architecture are essential
- In-format discovery was required to find soluble agonists



**Bispecific Engagement Enables Discovery of Soluble OX40 Agonist**

# Lead Characterization & Selection




- Efficacy on primary cell was confirmed by diverse activation markers: proliferation, Ki-67 staining and cytokine secretions (IL2, TNFα, and IFNγ).

- Lead candidates ranked top in efficacy, assembly efficiency and developability assays.



Identification of Leads with Maximum Efficacy and Developability

# Next Steps

- PK Studies
  - Scaling up lead for PK Studies
- Cyno Studies
  - In discussions with CROs for a custom Cyno study to monitor toxicity and efficacy
- Preclinical Studies
  - Finalizing work plans and contracts for CMC

# Summary

- Bispecific/Multispecific antibodies open up new avenues for IO and beyond
  - Redirection of immune cells
  - Better specificity of targeting of cells
  - Novel mechanisms of action

- New bispecific/multispecific platforms enable HT functional discovery of antibodies to allow for empirical testing of various epitopes, affinities, and geometries in the final format

# Acknowledgements

## Invenra Team

Roland Green, CEO
Bryan Glaser, VP Research
Dileep Pulukkunat
Daniel Gerhardt
Matthew Bissen
Sara Counter
Lauren Lehmann
John Painter
Jennifer Schmitz
Allyson Skoien
Justin Wetter



BD Opportunites contact Mark Kubik: mkubik@invenra.com