A large, detailed 3D model of an antibody molecule is positioned on the left side of the slide. It has a Y-shaped structure with a textured, bumpy surface, rendered in shades of green and black. The background is a dark, textured green with a subtle pattern of small white dots.

# Multispecific and Multivalent Antibodies as OX40 Agonists

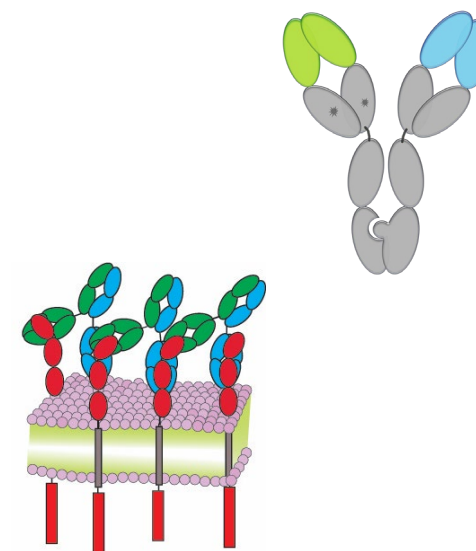
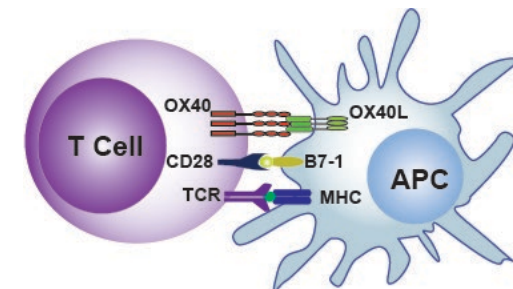
PEGS Europe

November 16, 2018

Bryan Glaser

Vice President of Research, Invenra

- Introduction to Invenra
- ARCHER™ Design Strategy for Soluble Agonists
- Discovery Process
- Characterization of a Soluble OX40 Agonist
- Next Steps
- Acknowledgements



# About Us



Location: Madison, WI  
[www.invenra.com](http://www.invenra.com)

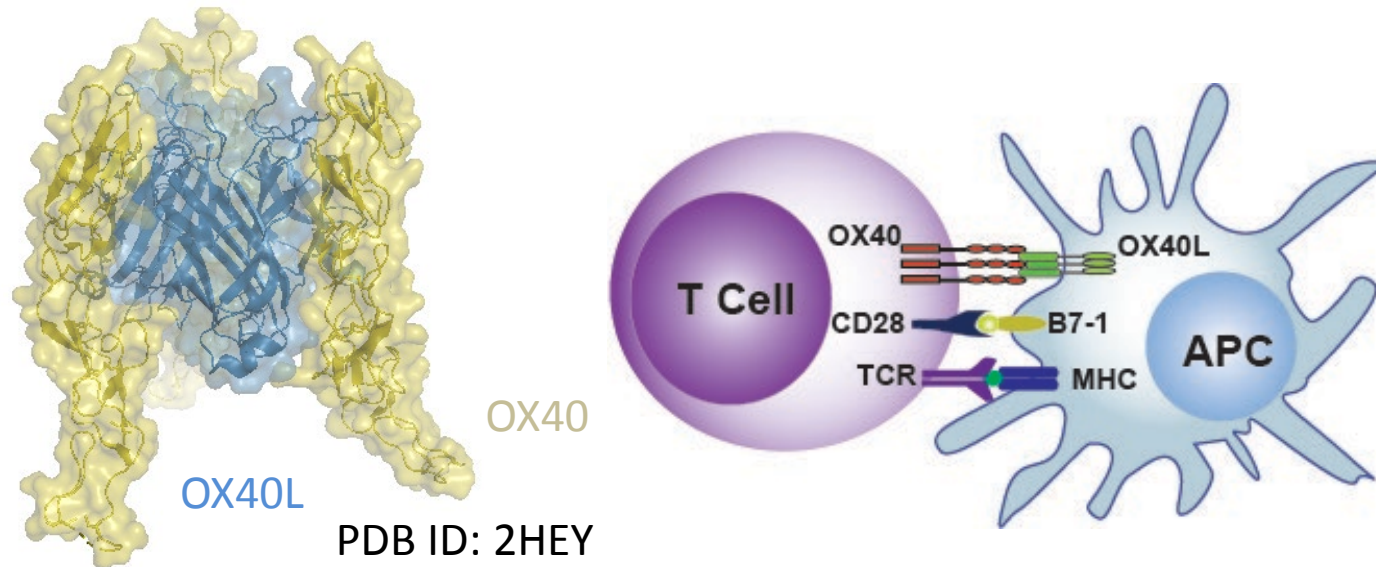
Next generation of multispecific antibodies for immuno-oncology

Proprietary B-Body™ platform has significant competitive advantages in discovery and manufacturing

Exploiting the platform to generate first-in-class therapeutics and fast-follower molecules, for our internal pipeline and strategic partners



# OX40 Biology and The Disconnect



- OX40 is a member of the TNFR superfamily and a co-stimulatory receptor
- OX40 requires high density clustering for activation
- Preclinical data demonstrates significant therapeutic potential of OX40 agonist in immuno-oncology

# First Generation OX40 Agonists

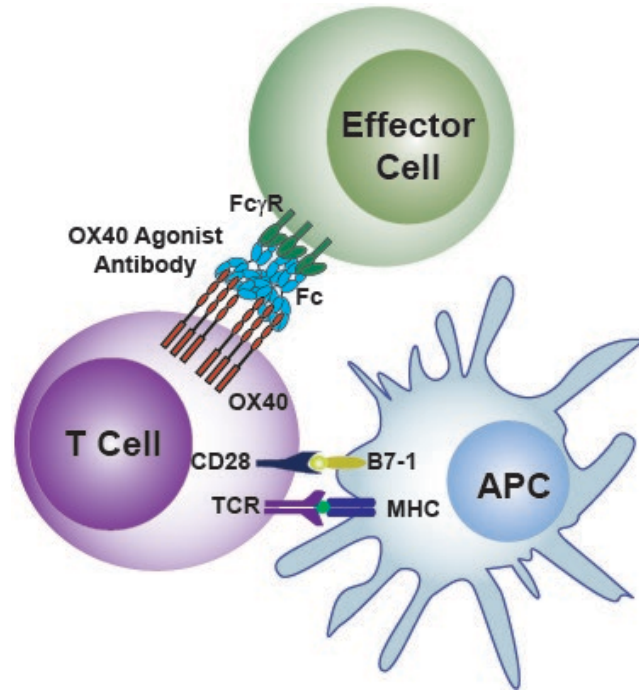
Company	Lead Name	Lead Type	Development Stage	Status of Program	Clinical Results
MedImmune	MEDI-6469	Murine IgG1	Phase I/II	Active	12/30 Patients had tumor shrinkage
	Tavolixizumab	Humanized IgG1	Phase I	Active	5/27 Patients had tumor shrinkage
	MEDI-6383	OX40L-IgG4 Fusion	Phase I	Discontinued	
GSK	GSK3174998	Humanized IgG1	Phase I/II	Active	Not disclosed
Pfizer	PF-04518600	Human IgG2	Phase II	Active	4/25 had tumor shrinkage
Genentech	Pogalizumab	Humanized IgG1	Phase II	Active	Not disclosed
BMS	BMS-986178		Phase I/II	Active	Not disclosed
Incyte	ICAGN-1949	Human IgG1	Phase II	Active	Not disclosed
7-8 Others*	NA	NA	Discovery	Active	Not disclosed

Source: Thompson Reuters Integrity

## First Generation Agonists:

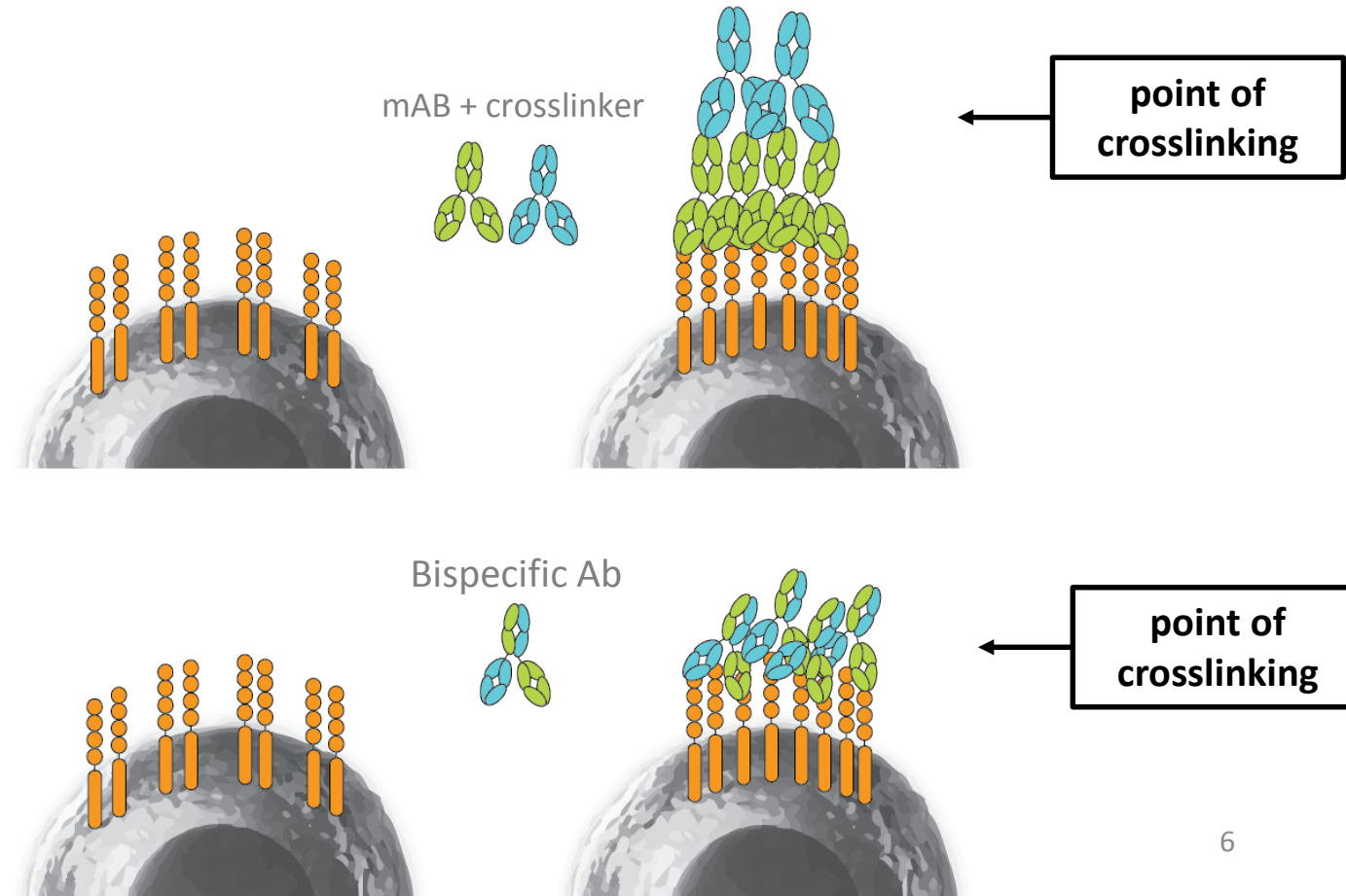
- Current molecules in clinical trials all require Fc engagement for agonist activity
- Secondary cross-linking can come from effector cells binding the Fc or from anti-drug antibodies
- If effector cells are not present at sufficient levels, the antibodies have limited to no efficacy which might explain the lack of efficacy in humans

# Hypothesis and Aim



- OX40 activation requires cross-linking or immobilization of agonist *in vitro*
- *In vivo* cross-linking may come from effector cells binding the Fc, which is variable in tumor microenvironment.

## Targeted Mechanism Of Activation

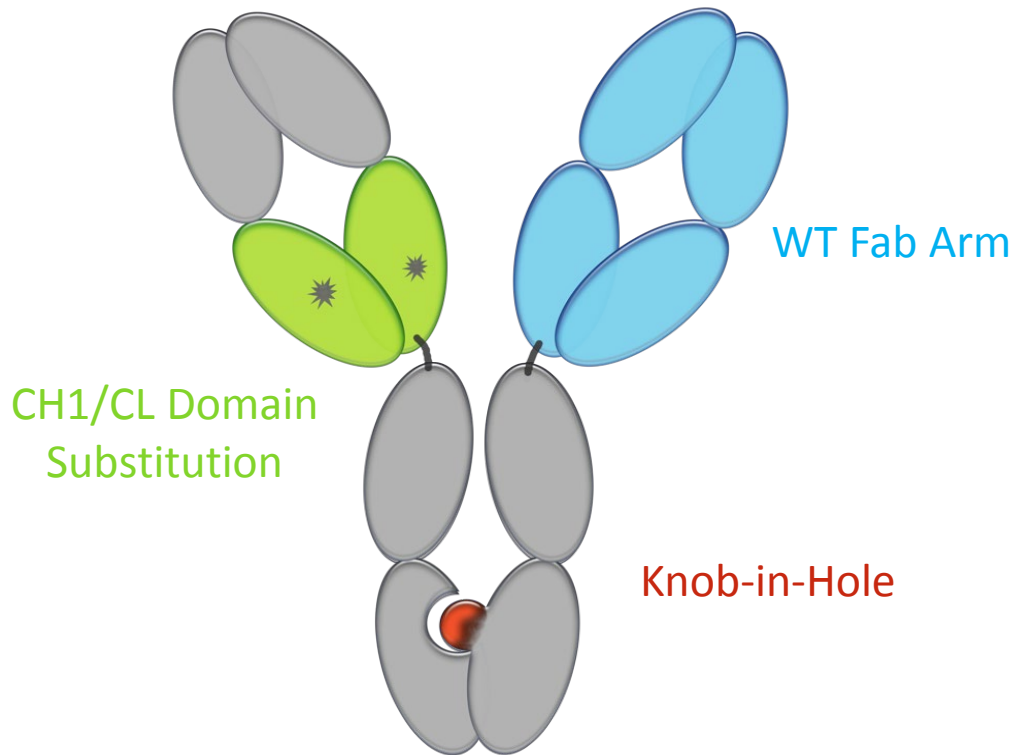


## Agonism in Absence of Cross-linker

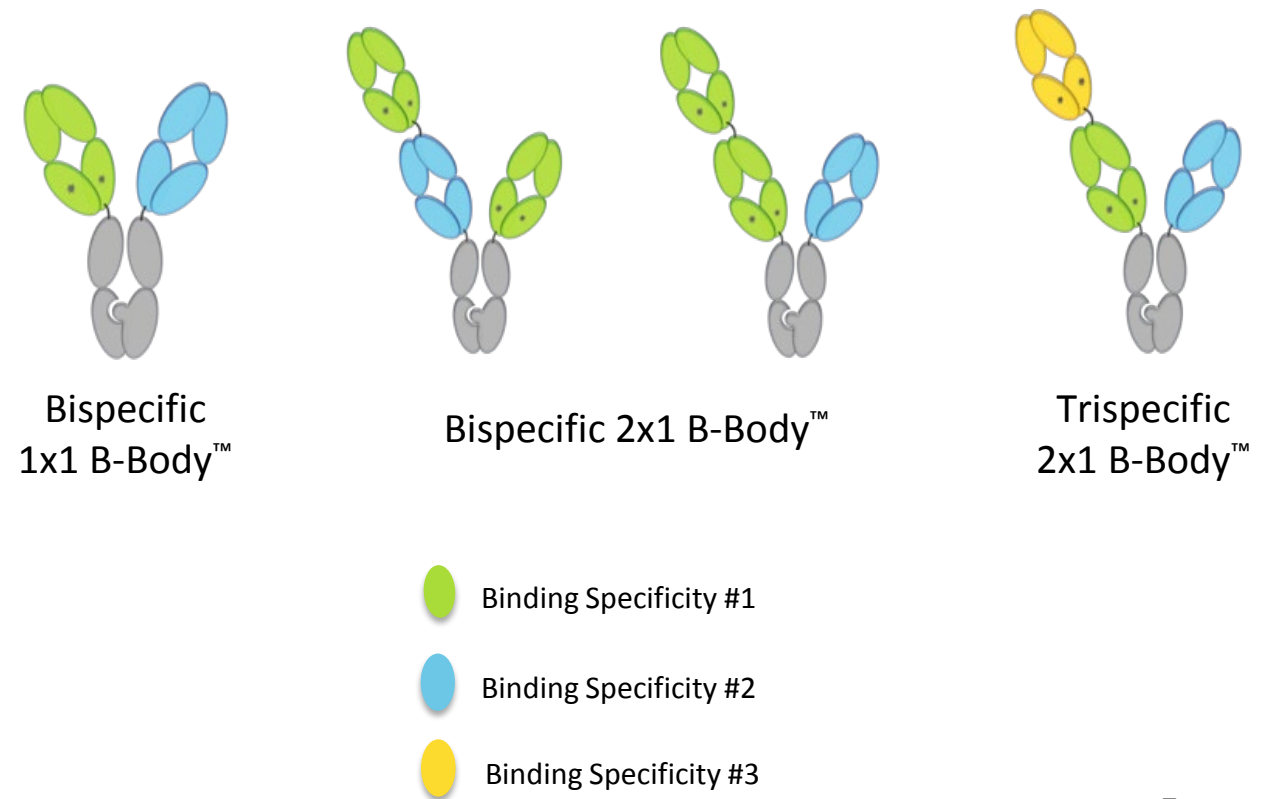
# B-Body™ Platform Design



## Plug-N-Play Variable Domains



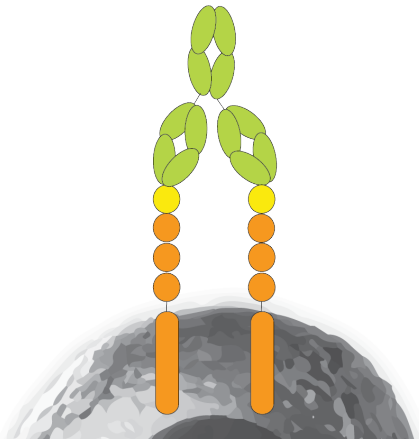
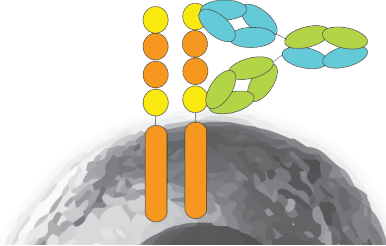
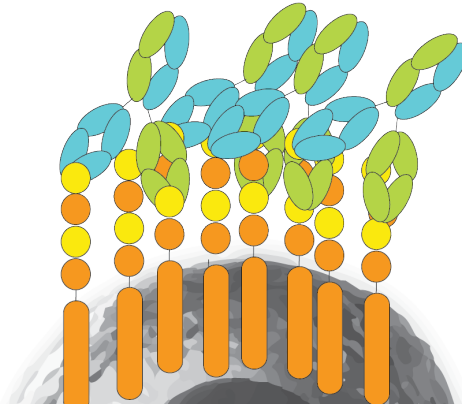
## Invenra's B-Body™ Family



Maximizes Performance in Discovery and Manufacturing

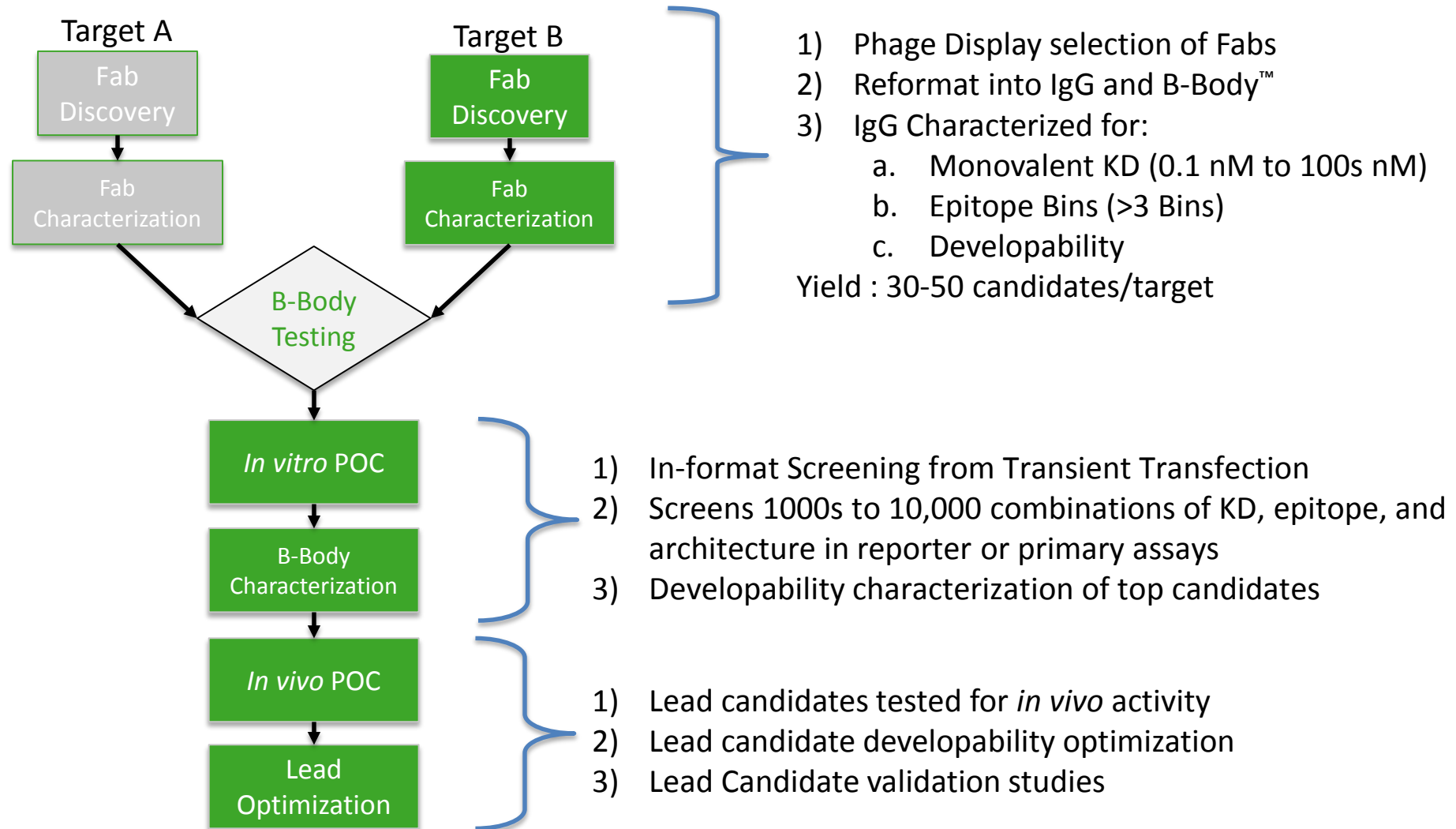
# ARCHER™ Soluble Agonist Design



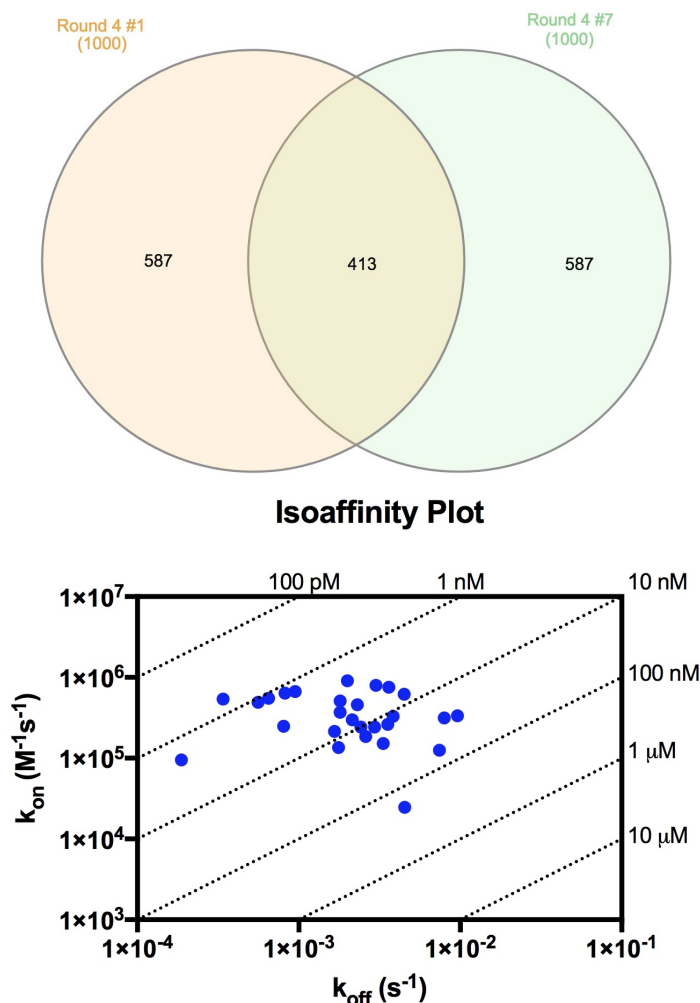
			
Format	Monoclonal Antibody	Biparatopic Antibody	Biparatopic Antibody
Binding	Single Epitope	Two Non-overlapping Epitopes	Two Non-overlapping, constrained Epitopes
Clustering Potential	Binds 2 Receptors	Weak clustering of Receptors	High Density clustering of Receptors



# High-Throughput B-Body™ Discovery



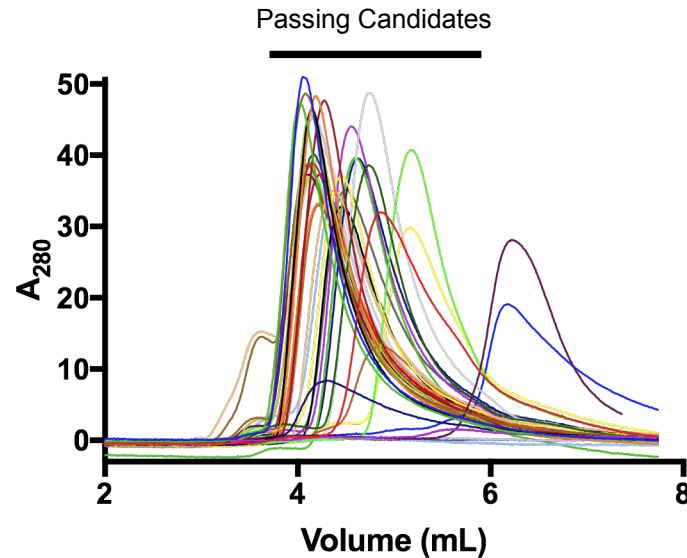
# Invenra's Antibody Library Rapidly Identifies Diverse Panels of Candidates



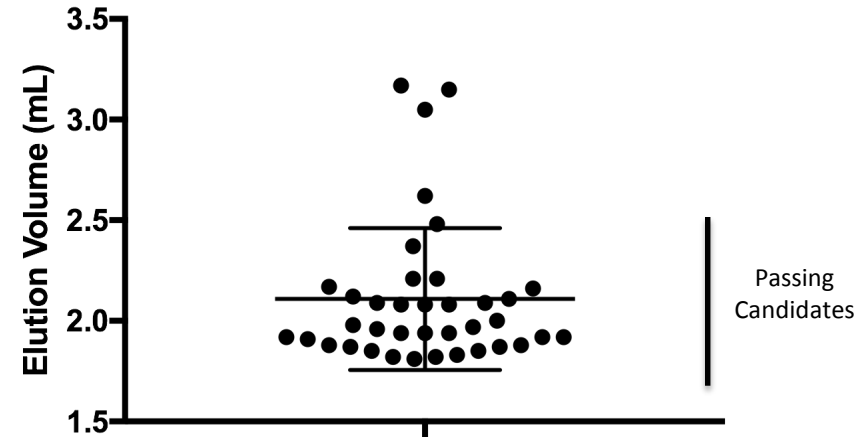
- Phage libraries were used to isolate OX40-specific antibodies
- NGS indicates 100's of variants enriched during panning
- 40 variants were reformatted for characterization
  - Broad range of affinities
  - Stringent developability gate

# Developability of Parent IgGs

Zenix Interaction Chromatography



Cross-Interaction Chromatography

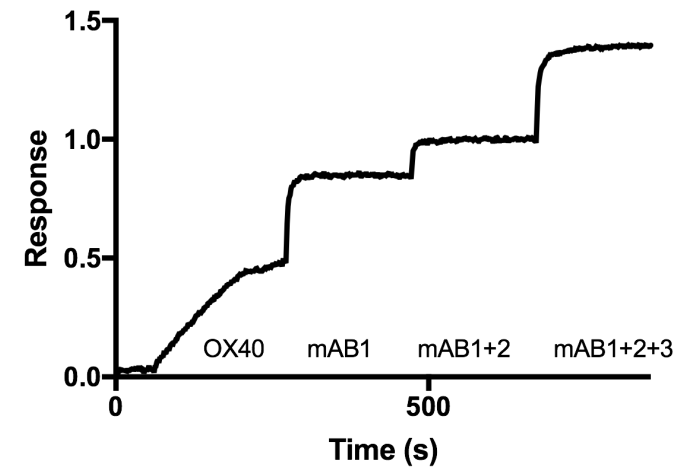


- Parental IgG screened for developability before use in the B-Body<sup>™</sup> In-format functional screen.

# $\alpha$ -OX40 mAbs Recognize Diverse Set of Epitopes

	Mixed IgG																																												
1st IgG	OX1	OX2	OX3	OX4	OX5	OX6	OX8	OX9	OX10	OX11	OX12	OX13	OX16	OX19	OX20	OX21	OX22	OX23	OX24	OX25	OX33	OX35	OX36	OX37	OX38	OX39	OX40																		
1		-2	0	40	10	-17	6	10	18	1	5	5	-4	1	4	11	-11	102	83	72	-5	72	7	80	11	88	81																		
2	6		5	104	4	-8	94	8	18	9	58	3	-4	12	6	63	-7	10	17	-4	-5	50	21	54	16	50	86																		
3	0	-2		99	0	-10	88	4	18	6	63	8	-4	9	0	54	-9	0	17	2	-5	50	21	54	11	69	76																		
4	-3	104	86		80	-17	-16	0	46	9	25	7	-4	9	43	-6	-18	90	81	72	-5	58	61	83	32	79	76																		
5	2	-2	0	88		-17	84	0	19	4	61	7	-4	9	0	40	-14	-6	29	4	-11	31	8	57	17	79	70																		
6	16	11	25	42	12		19	16	19	21	6	22	8	24	15	21	0	96	59	74	0	72	8	73	11	98	81																		
8	-6	111	114	21	100	-17		-4	32	1	19	3	-4	60	64	0	-14	90	77	72	-11	58	45	75	30	79	65																		
9	3	11	11	44	10	0	14		13	16	6	17	0	15	15	14	-5	90	75	64	-11	58	0	72	12	74	59																		
10	58	43	30	65	34	23	44	36		42	0	50	30	47	43	49	59	94	100	79	48	72	-22	73	0	71	65																		
11	2	-6	-5	35	0	-8	3	0	28		5	3	-8	1	6	3	-11	90	95	72	-11	72	13	81	20	98	89																		
12	10	89	100	49	90	-2	17	18	19	18		20	8	16	62	7	0	86	76	64	0	58	8	73	11	79	65																		
13	-2	0	-5	28	0	-19	2	2	20	3	6		-8	4	0	0	-16	90	100	85	-11	75	17	89	18	98	86																		
16	5	9	0	42	10	-2	14	12	17	15	5	15		16	0	11	-7	90	74	74	-7	69	27	66	10	86	76																		
19	-2	-6	-5	42	0	-25	63	-2	38	-1	13	0	-6		0	-1	-18	82	129	74	-11	72	20	113	27	93	81																		
20	5	0	0	76	4	-15	78	2	19	9	39	7	-4	12		46	-9	-2	35	60	-11	72	0	57	11	55	70																		
21	-2	79	91	28	60	-15	0	-2	41	3	21	7	-4	1	87		-14	88	78	2	-11	58	26	80	25	79	76																		
22	10	15	5	42	10	-4	22	12	20	16	41	20	2	16	6	9		98	75	8	0	78	25	66	6	71	76																		
23	94	15	14	125	14	100	100	108	82	99	11	103	94	91	9	84	95		17	62	98	3	14	49	63	-7	3																		
24	100	43	34	125	36	65	88	102	85	101	0	110	96	94	25	80	118	24		8	75	0	10	36	60	10	11																		
25	84	43	41	118	36		100	102	56	96	45	103	94	90	34	77	86	50	-10		84	31	0	5	45	10	16																		
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35	79	81	95	111	74	88	89	80	11	90	-5	97	80	79	91	74	84	20	30	11	80		0	100	0	5	5																		
36	6	15	7	118	4	-8	72	4	45	12	27	13	-2	12	4	59	-9	0	14	15	-11	0		47	23	7	65																		
37	82	68	75	118	64	96	106	88	117	93	13	102	84	85	57	81	-23	56	-14	0	89	33		25	17	16																			
38	10	4	7	44	10	-4	13	12	23	16	0	20	0	16	6	14	0	100	71	72	-11	72	-10	74		90	76																		
39	85	89	93	114	100	96	86	92	50	91	44	103	86	88	62	71	80	12	0	0	93	3	-9	46	56		0																		
40	74	121	105	111	100	96	84	88	93	99	53	107	84	85	87	80	91	34	19	15	84	11	25	43	53	7																			

- Comprehensive epitope binning was performed
- >100 non-overlapping antibody pairs
- Multiple epitopes from naïve selection



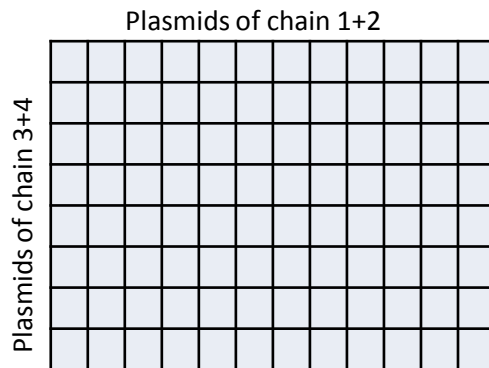
OX40 Candidates Bind Multiple Non-Overlapping Epitopes



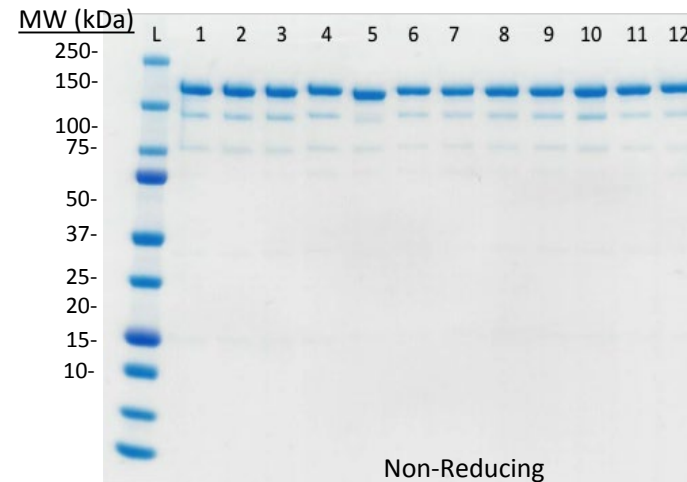
# HTP B-Body™ Purification



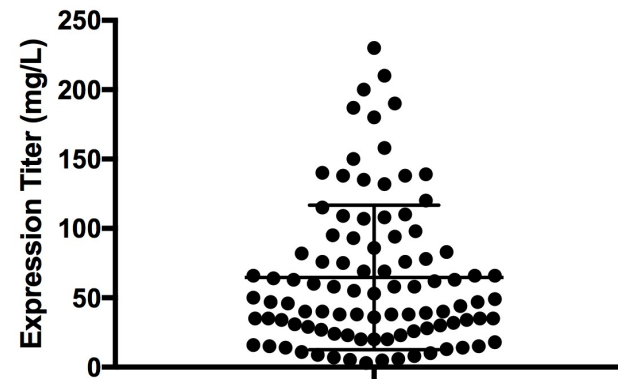
4 Plasmid Mixing  
Transient Transfection  
Protein Expression and Purification



Representative B-Body™ Purification  
A1-A12 of 96-Well Plate



96-well Plate Expression



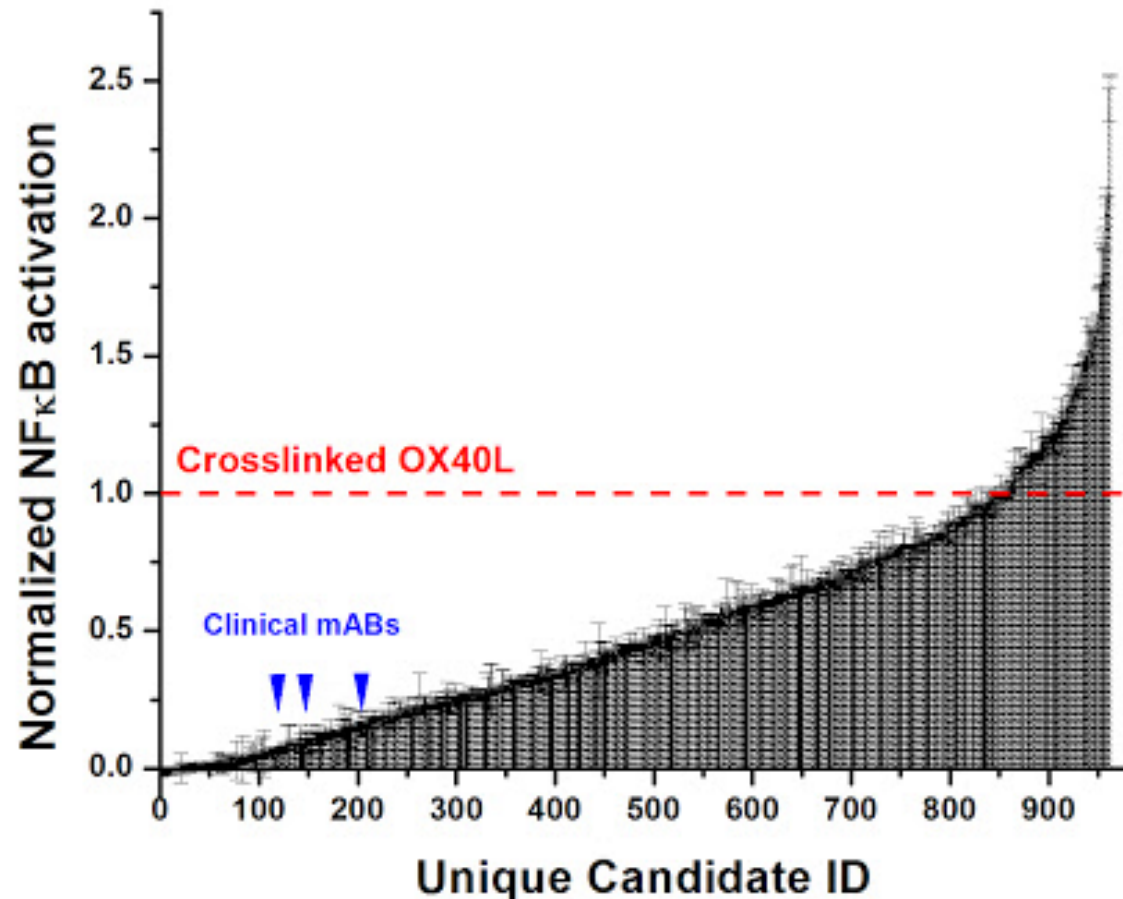
- Small-scale transient expression and one-step purification resulted in B-Body™ of sufficient yield and purity for downstream assays

One-Step Purification Yields High Quality Material for Screening

# High-Throughput Cell Assay Screening

## OX40 Agonist Discovery Panel

Model cell line: HEK293/NF $\kappa$ B/GFP-Luc:OX40

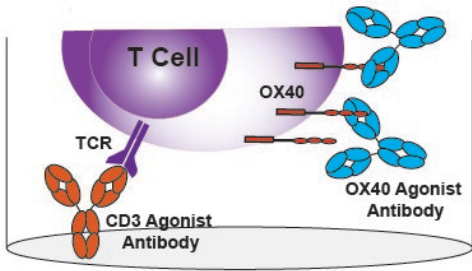


- > 900 B-Body<sup>™</sup> agonists generated in a single experiment → both 1x1 and 2x1 formats
- A range of activities were observed in reference to crosslinked OX40L and clinical mABs (cMAbs)

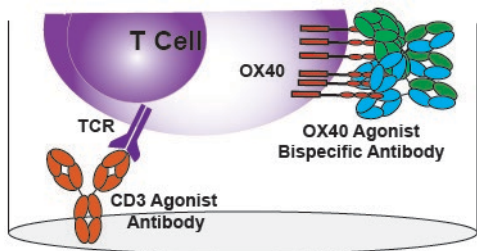
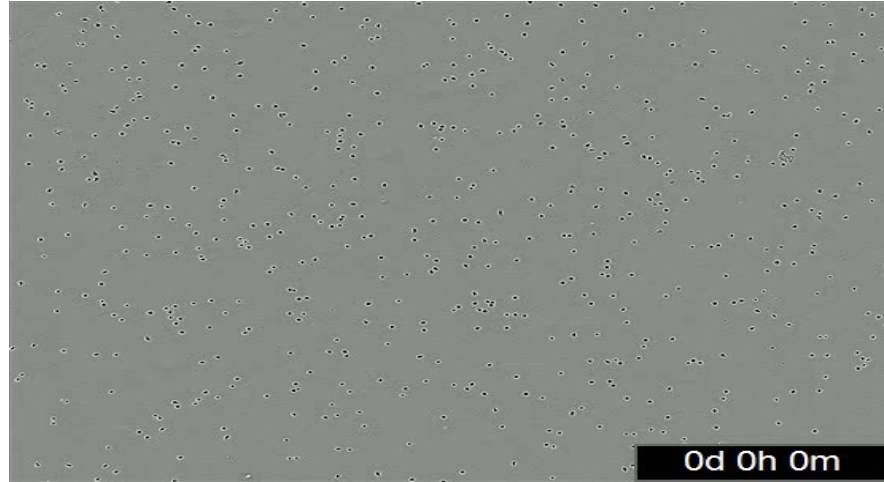
# Primary T-cell Activation by Soluble Agonist

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

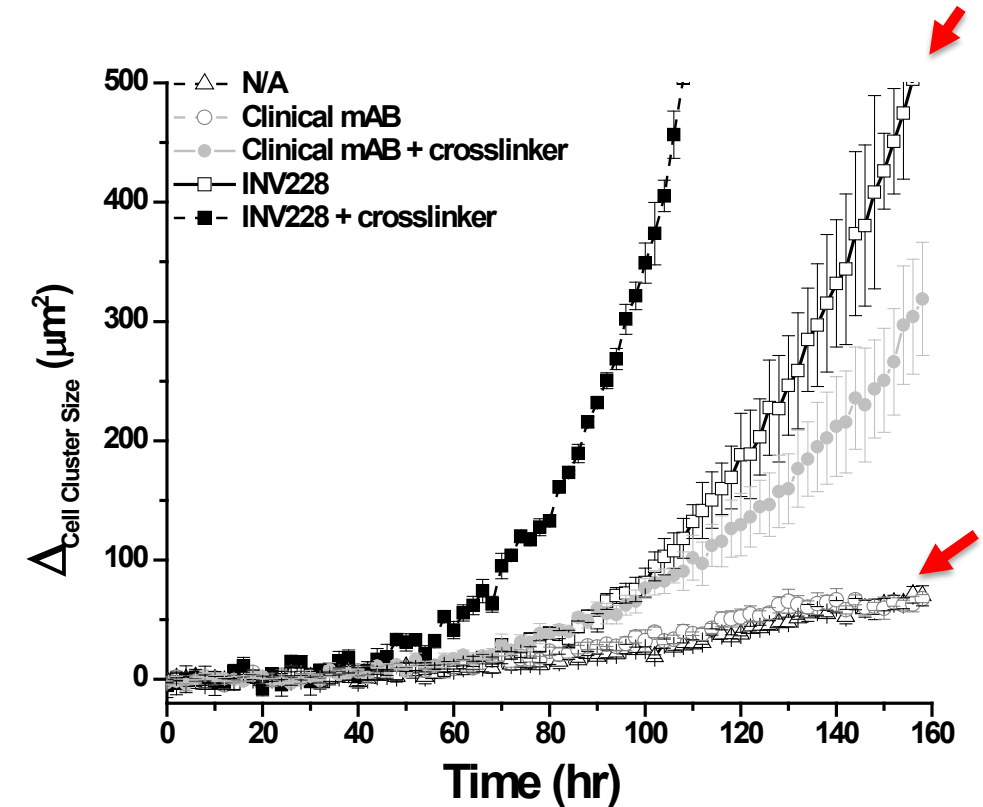
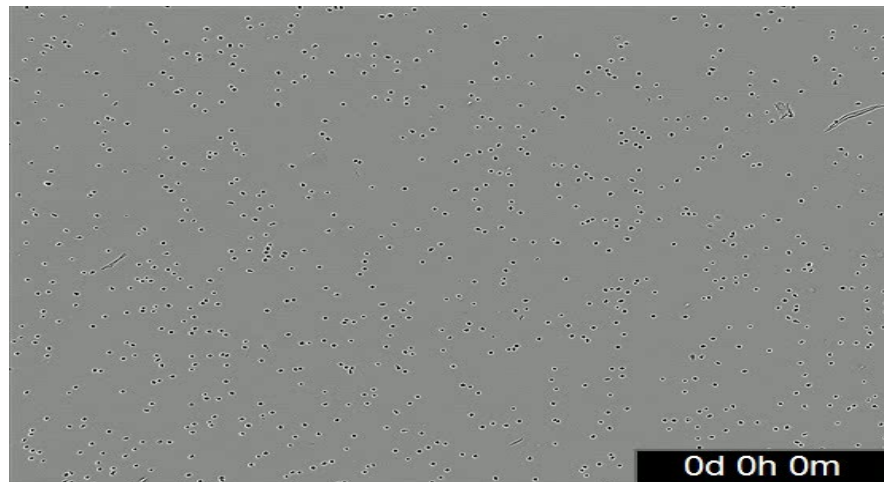
The Kinetics



Soluble Clinical mAB

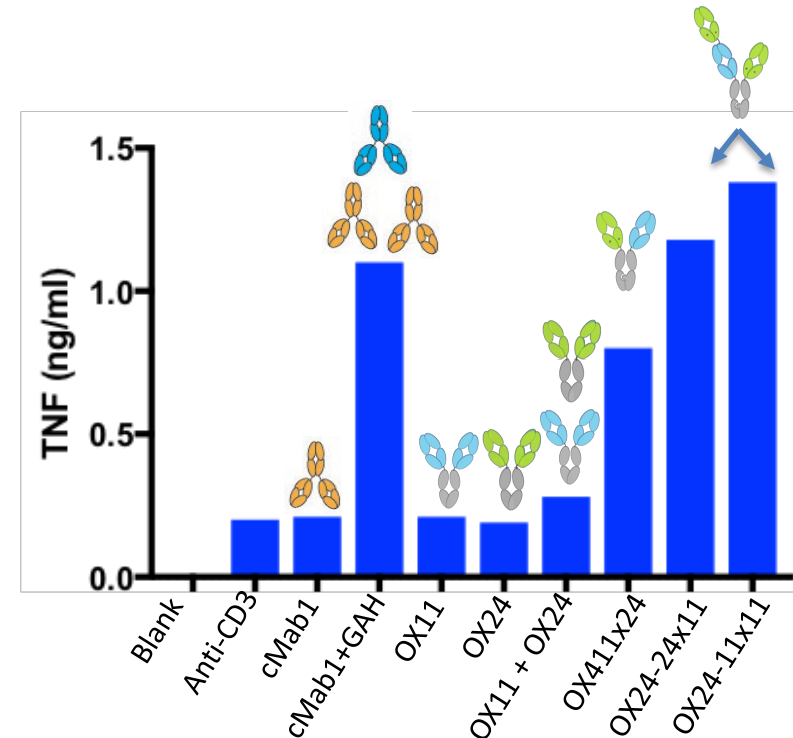
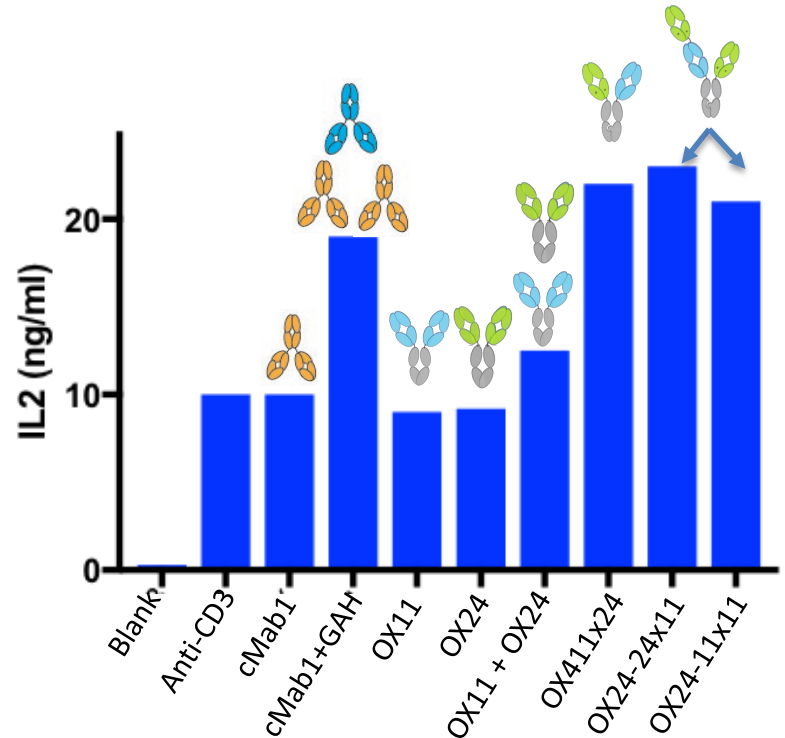


Soluble Bispecific



Bispecific OX40 Agonist is Potent in Soluble Format on Primary Cells

# Lead Agonist Selection

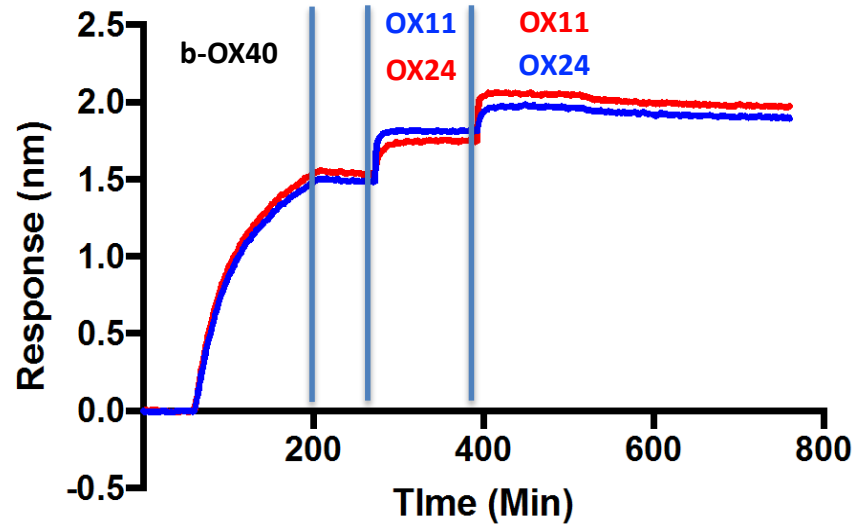


- ARCHER™ bispecifics out perform cross-linked clinical candidates and combinations of parent IgG in primary T-cell activation assays.
- Soluble IgG have no activity without secondary cross-linking

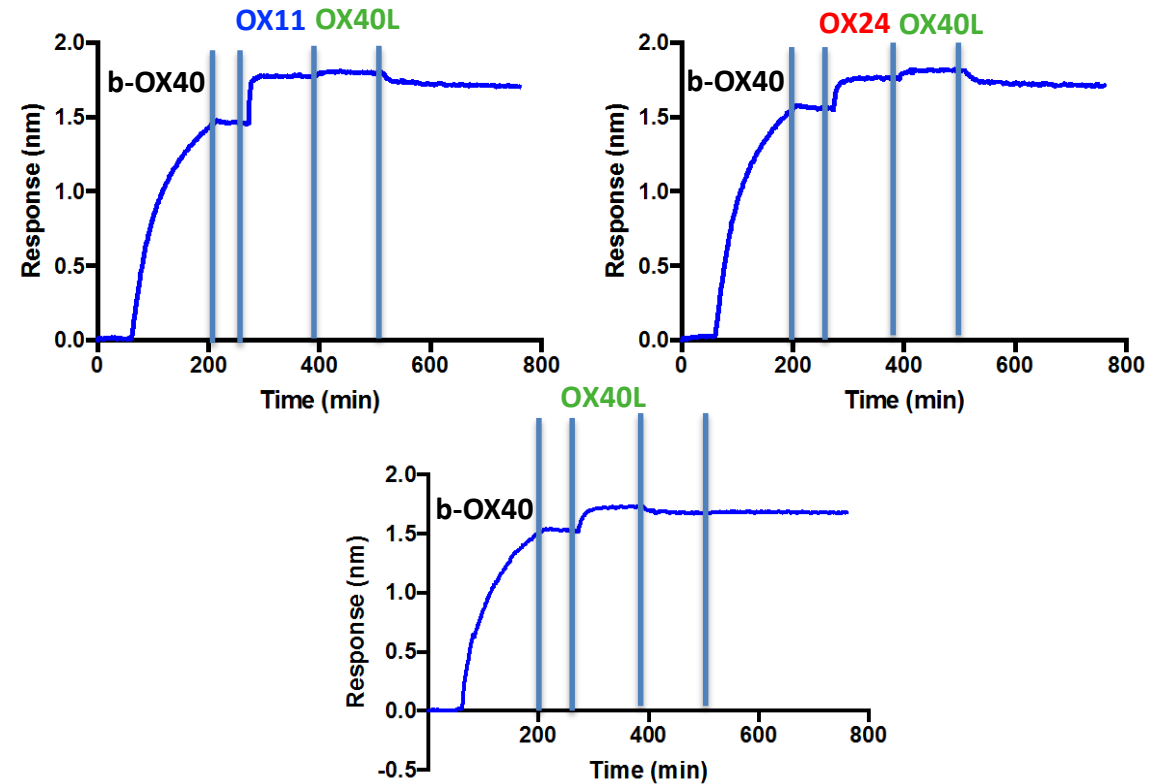


# Lead Binding Characterization

Epitope Binning



OX40-L Competition Binding

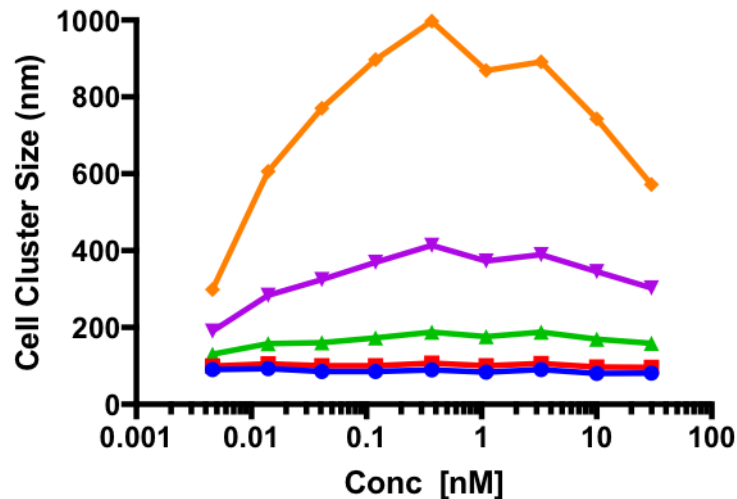


- OX24 and OX11 IgG can simultaneously bind OX40
- Both OX24 and OX11 prevent OX40L binding to OX40

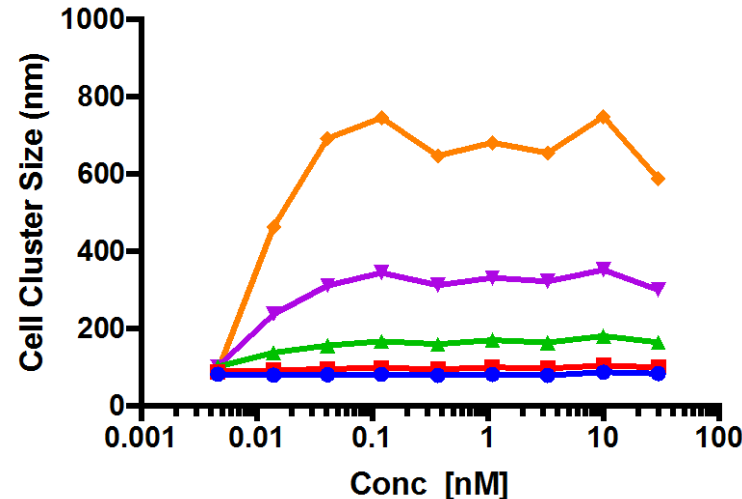
Lead Candidate Achieved Original Binding Design Specs

# Proliferation of CD4+ T-cells

**T-cell Proliferation  
OX24-11x11**



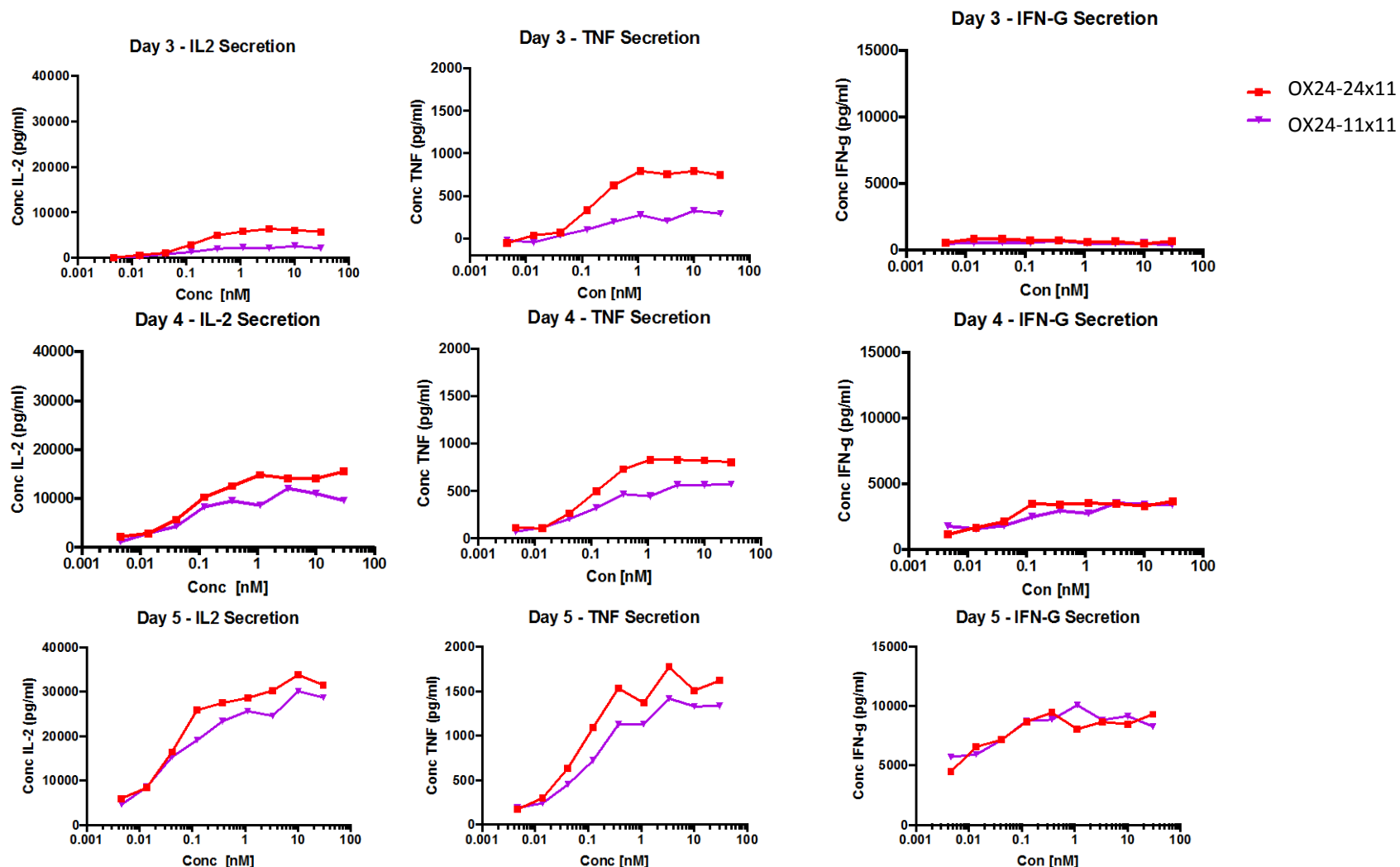
**T-cell Proliferation  
OX24-24x11**



- Day 1
- Day 2
- Day 3
- Day 4
- Day 5

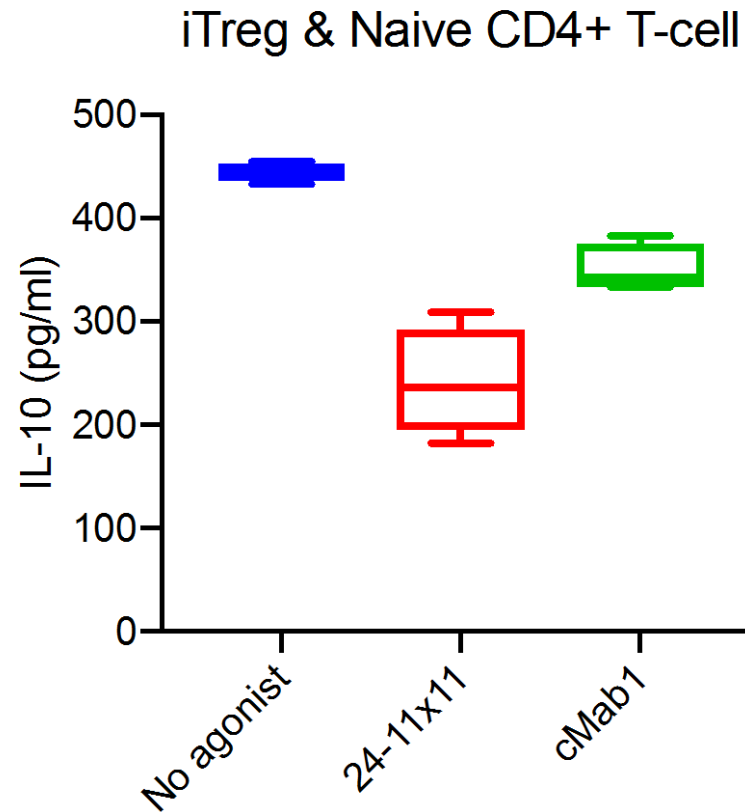
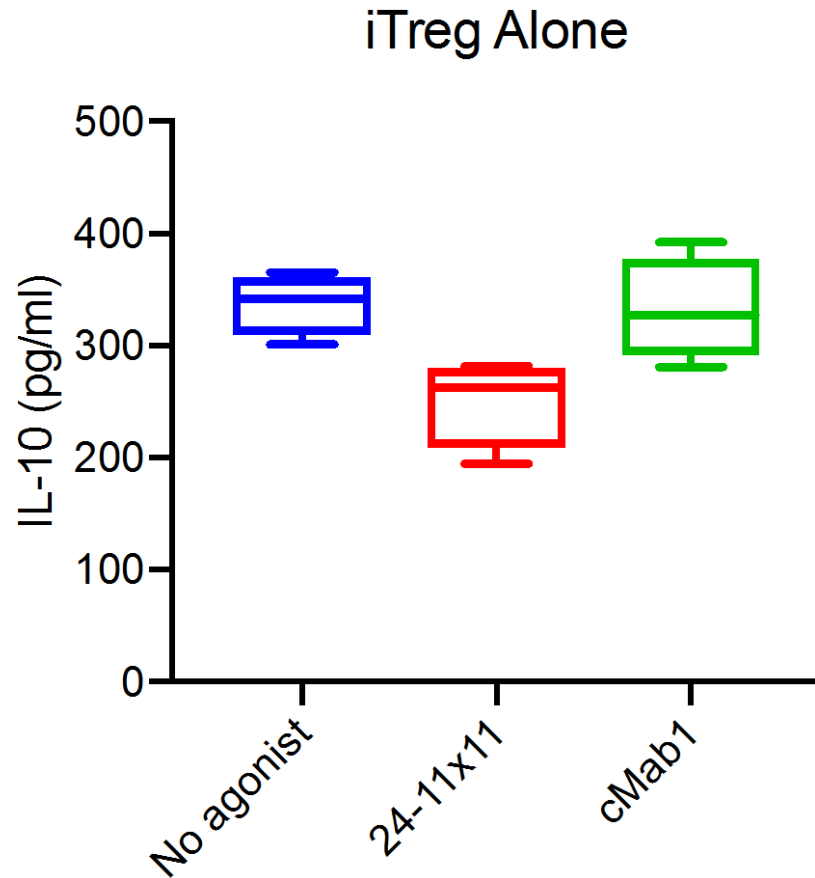
- Soluble OX40 agonists maximize proliferation between ~ 0.1 to 0.4 nM
- Proliferation of CD4+ T-cells initiates ~ 3 days post treatment

# Kinetics of Cytokine Expression



- Soluble OX40 agonists maximize cytokine expressions at ~ 1 nM
- Cytokine expression from CD4+ T-cells initiates ~ 3 days post treatment

# Suppression of iTregs

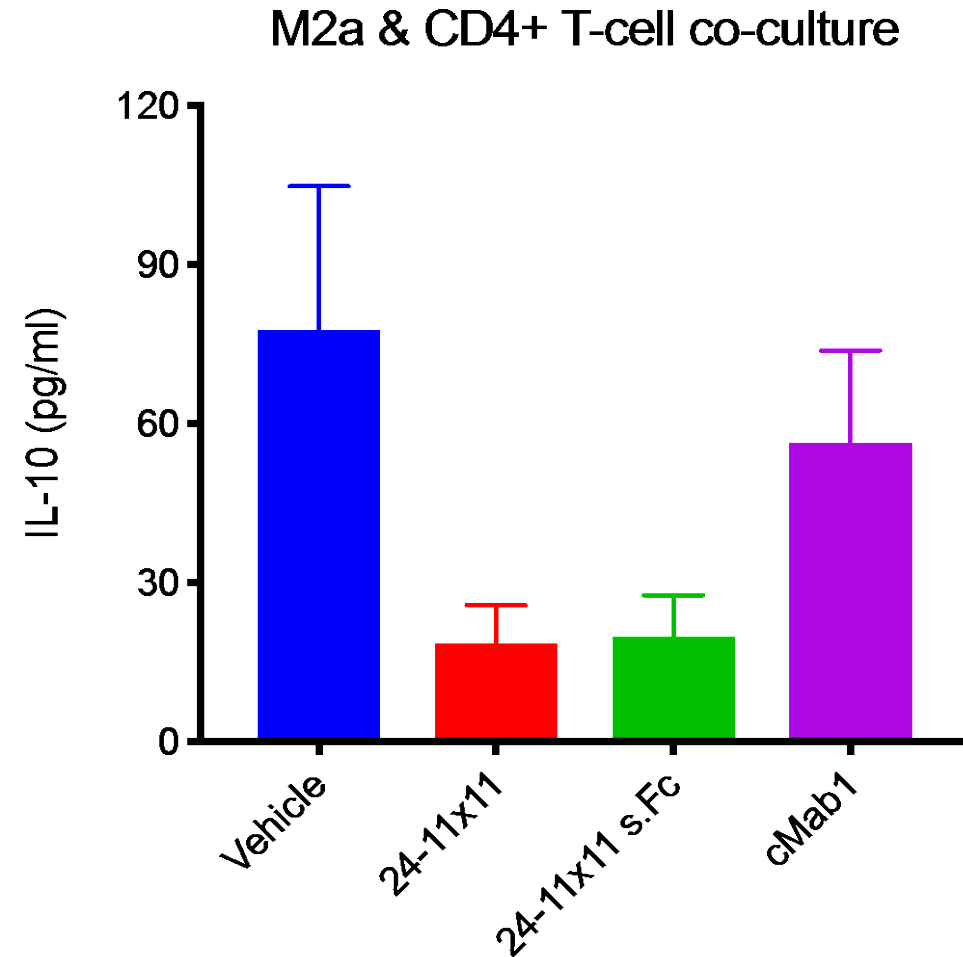


- Agonist added to CD4+ T-cells co-cultured with iTregs or iTregs alone
- Soluble OX40 treatment inhibits IL-10 secretion from induced Tregs
- Soluble OX40 agonists outperform the suppression level of clinical candidate



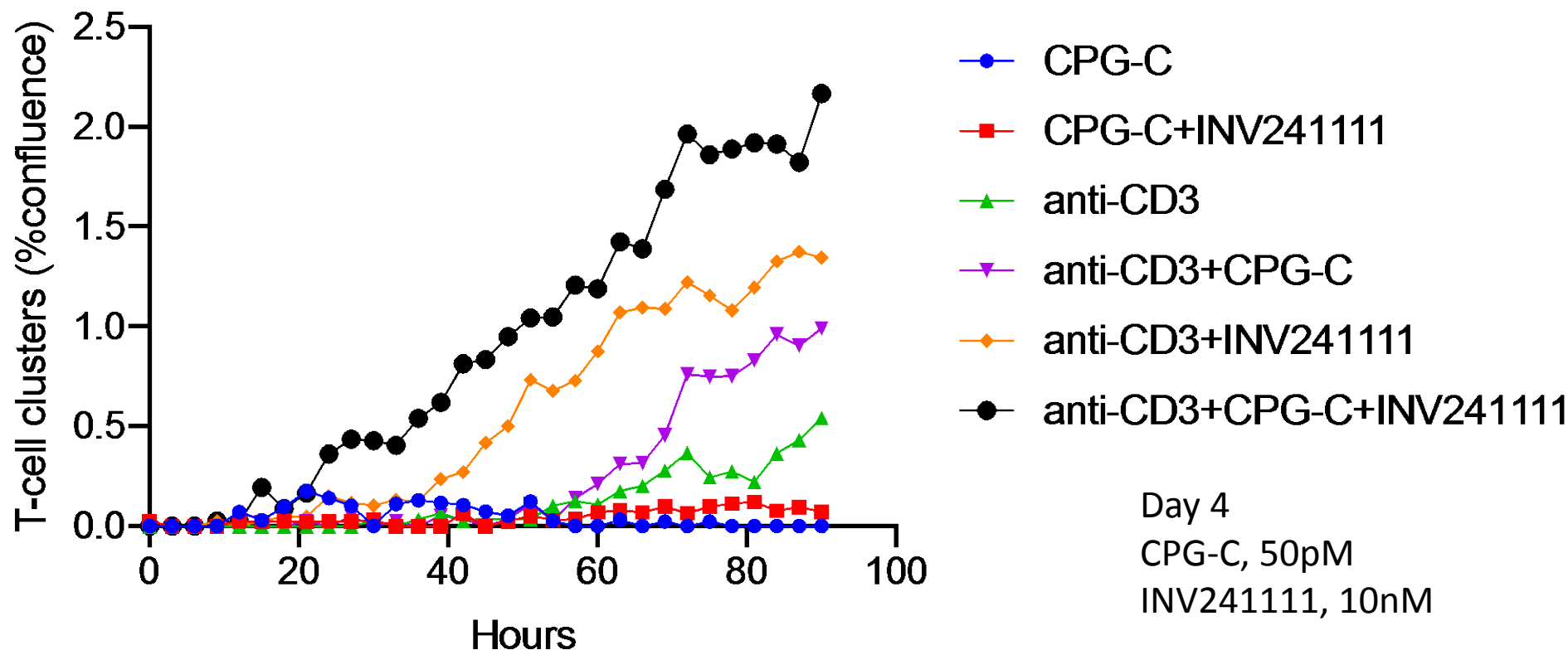
# OX40 agonist suppresses TAM function

- Tumor associated macrophages (TAMs) are known to secrete immunosuppressive cytokines such as IL-10, TGF-beta, that suppress T-cell function.
- CD4+ T-cells were co-cultured with IL-10 secreting monocyte derived M2a macrophages, in presence or absence of OX-40 agonist (100pM)



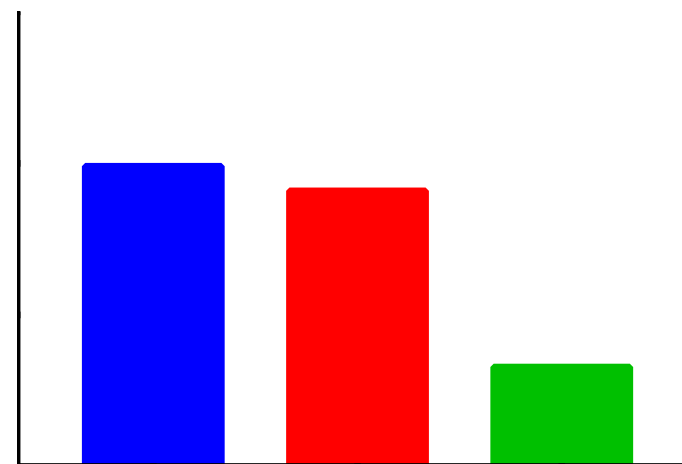
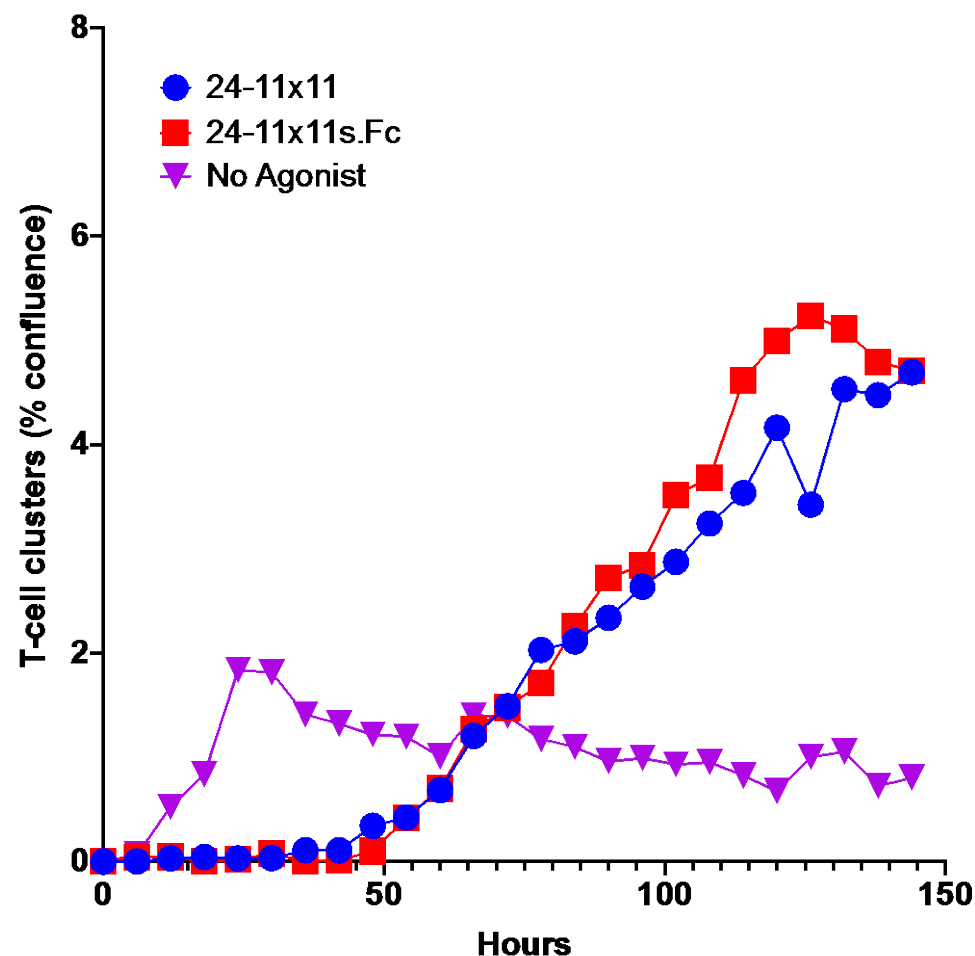
# CPG-C and INV24-11x11 combination studies

## PBMC Proliferation Assay - Day 4



# OX40 Agonist in Rhesus T-cell Model

Rhesus PBMC Proliferation



- Human OX40 agonist is active in the Rhesus T-cell activation model

Soluble OX40 Agonist is Active in Rhesus Models

- *In vivo* efficacy models
  - NSG GVHD model to monitor T-cell activation
  - Single-dose Rhesus efficacy/PK study
  - Human tumor efficacy model
- Preclinical Development
  - Cell line development (underway)

- The B-Body™ is a robust, versatile platform capable with multiple formats
- High-fidelity assembly and favorable biophysical properties enable HT in-format bispecific discovery
- OX24-11x11 is able to enhance CD4 function (proliferation and cytokines) while reducing immunosuppressive (IL10 from Tregs and Macrophages) function in soluble format
- The concept of bispecific agonist is generally applicable to receptors where clustering is a critical component for the mechanism of action

# Acknowledgements



## **Discovery Group**

Lauren Lehmann

## **Cell Biology Group**

Bonnie Hammer

Mandar Bawadekar

Matthew Bissen

## **Analytics Group**

John Painter

## **Past Members**

Lucas Bailey

Francis Qufei Li

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