



OX40 Agonist

OX40

MARKET LANDSCAPE

OX40 (a.k.a. CD134) is a costimulatory molecule belonging to the TNF receptor family expressed primarily on activated effector T (T eff) cells and naive regulatory T cells. Ligation of OX40, primarily on CD4⁺ T cells, activates NF- κ B pathway and up-regulates antiapoptotic molecules of the Bcl-2 family, leading to T cell clonal expansion, activation, memory, and cytokine production. OX40 engagement on CD4⁺ FoxP3⁺ Treg cells leads to expansion, deactivation, or cell death depending on the local milieu. Given that OX40 triggering can potently stimulate T cells and potentially block/eliminate regulatory T cells, OX40 agonists have been investigated in multiple preclinical tumor models and anti-human OX40 monoclonal antibodies are currently being evaluated in clinical trials.

¹PLOS ONE; <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0089350>

CURRENT MARKET PARTICIPANTS

Agent	Target	Structure	Phase	Company
Tavolixizumab (MEDI0562*)	OX40	Humanized anti-OX40 mAb	Phase 1	AstraZeneca
Pogalizumab (MOXR0916/ RG7888)	OX40	Humanized anti-OX40 mAb	Phase 1	Genentech/Roche
PF-04518600	OX40	Fully Human anti-OX40 mAb	Phase 1	Pfizer
GSK3174998	OX40	Humanized anti-OX40 mAb	Phase I	GSK

*MEDI0562 replaced murine MEDI6469 whose trials were recently halted
MEDI6383 is an OX40L-Fc fusion protein also in trials

OX40 mAb LEAD DISCOVERY

PROJECT PLAN



1 Immunization and Serum Assay

2 B-Cell Harvest and IgM Depletion

3 High-Speed, High-Content Single B-Cell Sorting

4 Antibody Amplification & Expression

5 High-Throughput Screening

6 Scale-Up and Cellular Assay Validation

B-cell repertoire harvested from AbeoMouse™

Purify B-cells surface expressing via FACS
(For identification of putative agonists)

PCR amplify and clone heavy and light variable regions into human IgG expression vectors

Transfection and screening of 1000's of recombinant antibodies

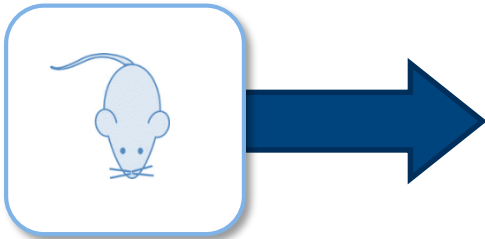
Scale-up highest affinity mAbs to 100ug scale and screen for mAbs which function in OX40 Activation Assay

OX40 mAb LEAD DISCOVERY

PROJECT STATUS

1

Immunization and Serum Assay



- 10 mice immunized subcutaneously with the extracellular domain of human OX40 in a proprietary adjuvant
- Mouse serum is assayed for its ability to bind human OX40 extracellular domain at varying dilutions

Mouse 359 | Mouse 360

Pre Post1 Post2 Pre Post1 Post2

1:500

1:2K

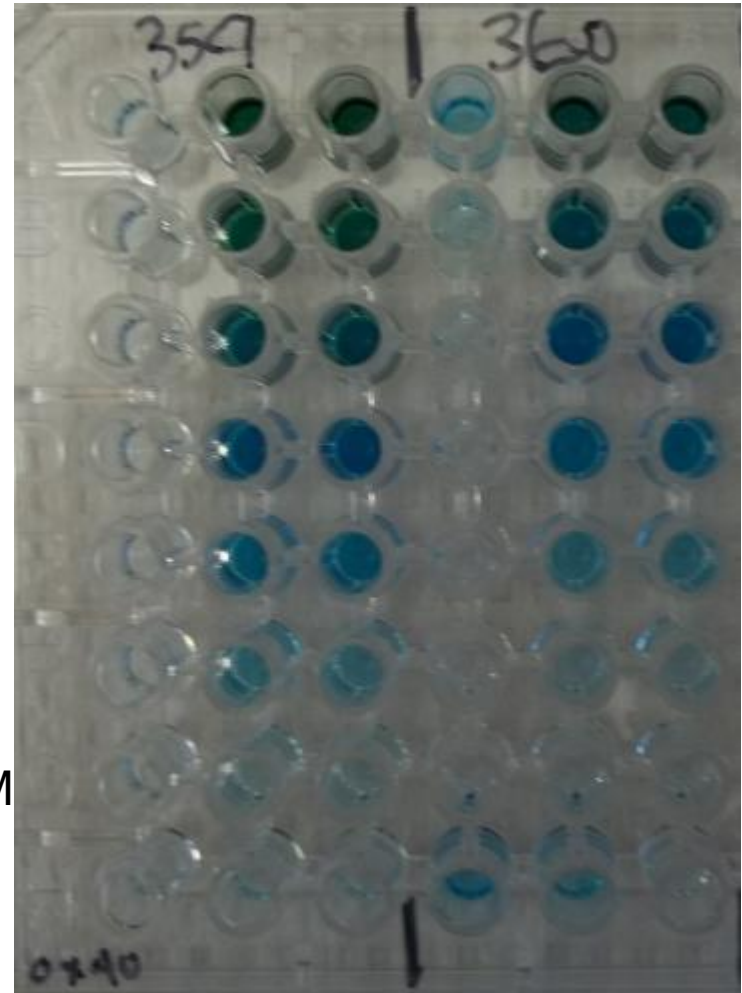
1:8K

1:32K

1:128K

1:512K

1:2.048M

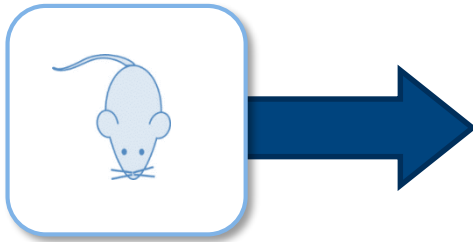


OX40 mAb LEAD DISCOVERY

PROJECT STATUS

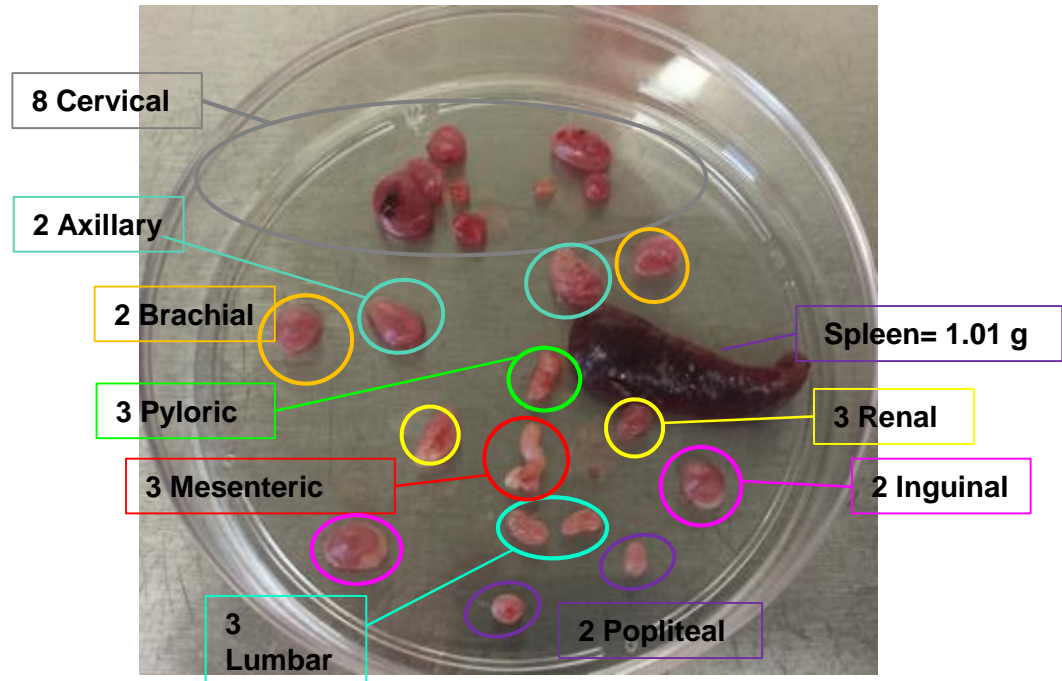
2

B-Cell Harvest



2×10^8 lymphocytes, spleen mass
1.01g harvested from a single
AbeoMouse

5.6×10^7 cells remained after IgM
depletion



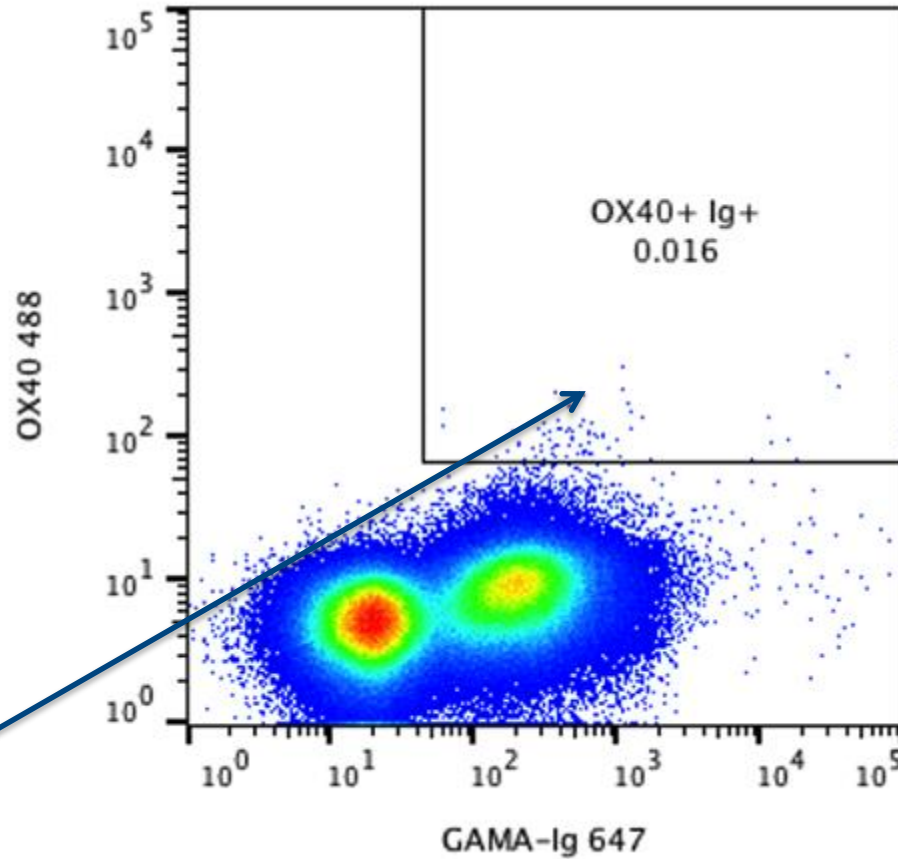
Spleen | Bone Marrow | Lymph Nodes

OX40 mAb LEAD DISCOVERY

PROJECT STATUS

3

High-Speed,
High-Content
Single B-Cell
Sorting



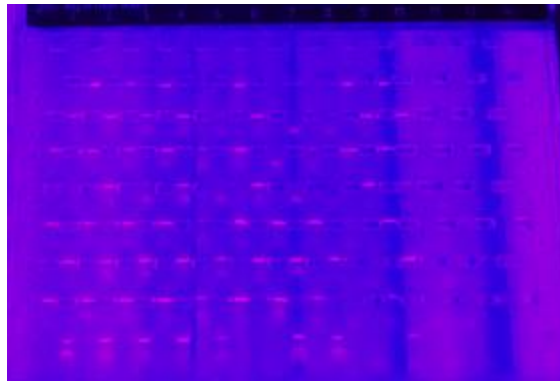
- 0.016% of B cells from the first AbeoMouse™ immunized with OX40 surface-express an antibody binding to OX40
- 1632 Ig+OX40+ cells were single-cell deposited for amplification and expression

OX40 mAb LEAD DISCOVERY

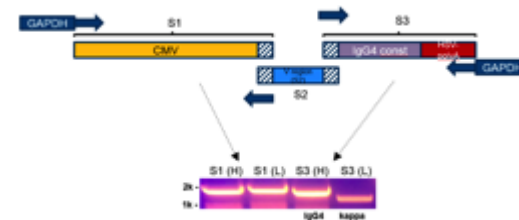
PROJECT STATUS

4

Antibody
Amplification &
Expression



96-Well Gel of Variable
Region PCR Products



Fully Synthetic (Plasmid-Free)
Gene Expression Cassettes

Proprietary Single-Cell
RT-PCR Process



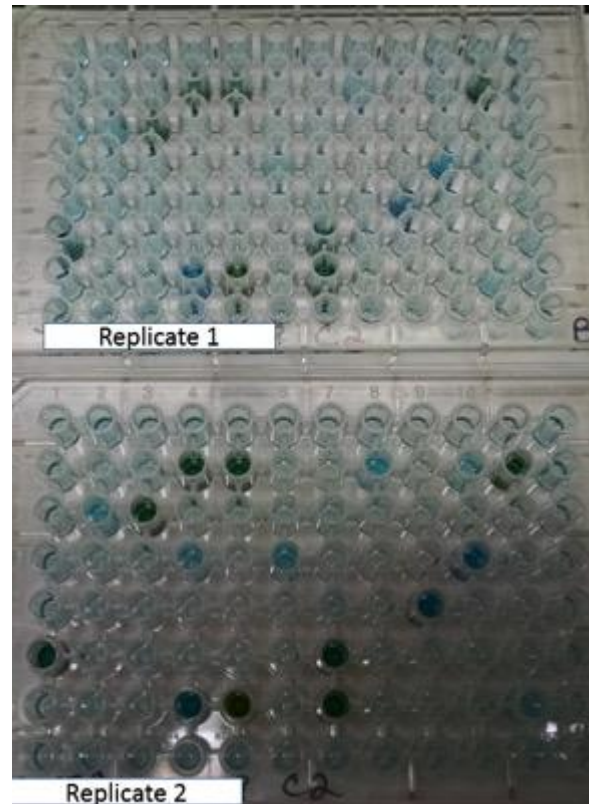
Proprietary Expression
Vector Systems

OX40 mAb LEAD DISCOVERY

PROJECT STATUS

5

High-throughput
Screening



Rapid Expression
And Assay Systems Test
1000's of mAbs for binding
strength per day

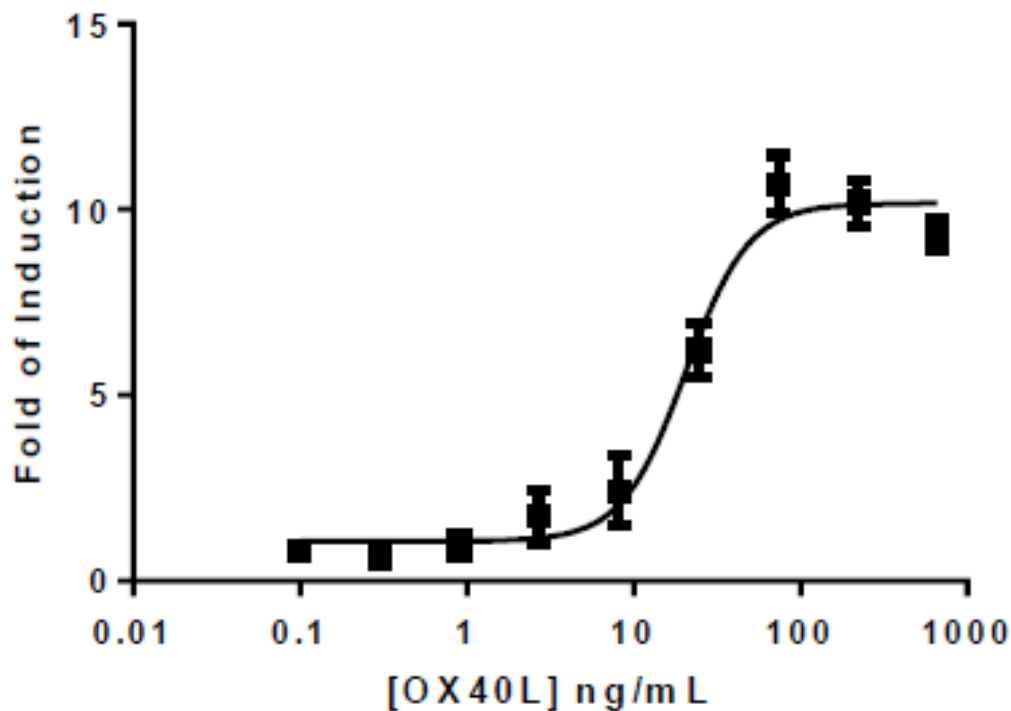
OX40 mAb LEAD DISCOVERY

ASSAY VALIDATION

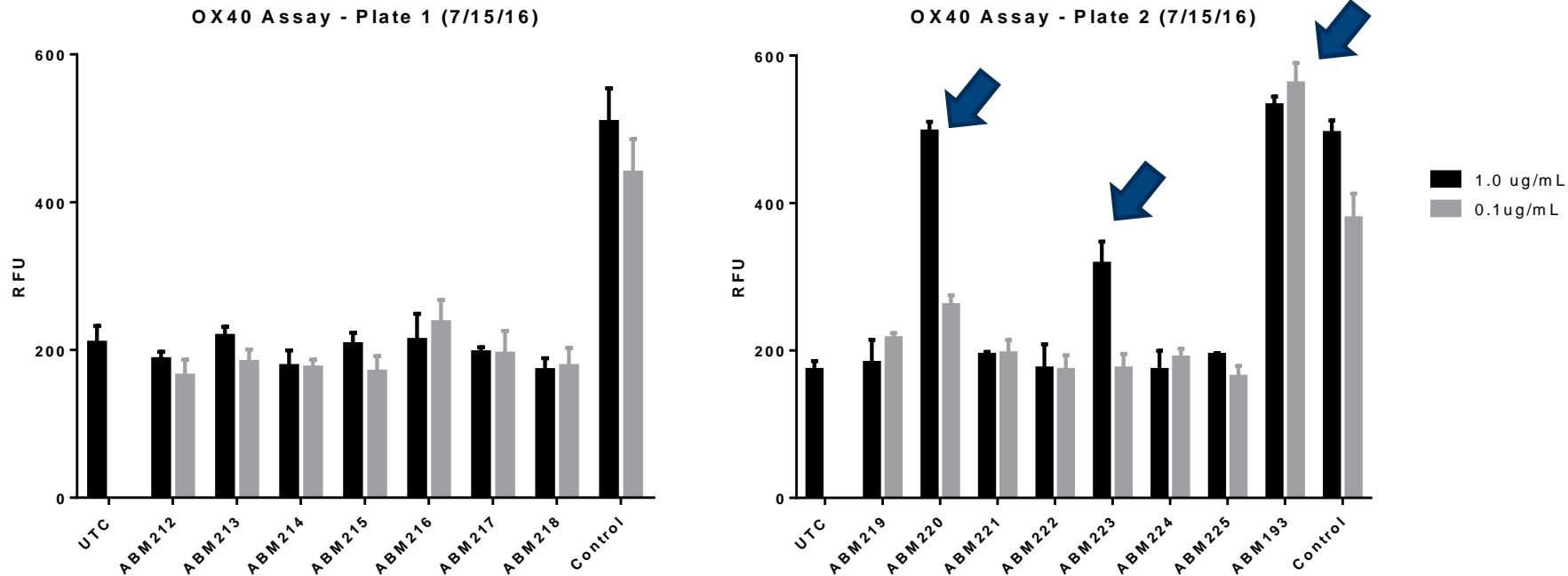
6

Scale-up and Cellular Assay Validation

- Abeome has an in-house human Jurkat cell line engineered with a NFkappaB luciferase reporter which is activated and detected upon OX40 activation
- Abeome has synthesized, expressed and purified a reference antibody PF-04518600 (Pfizer clinical control)



Functional Screen: Activation of OX40 Reporter Line

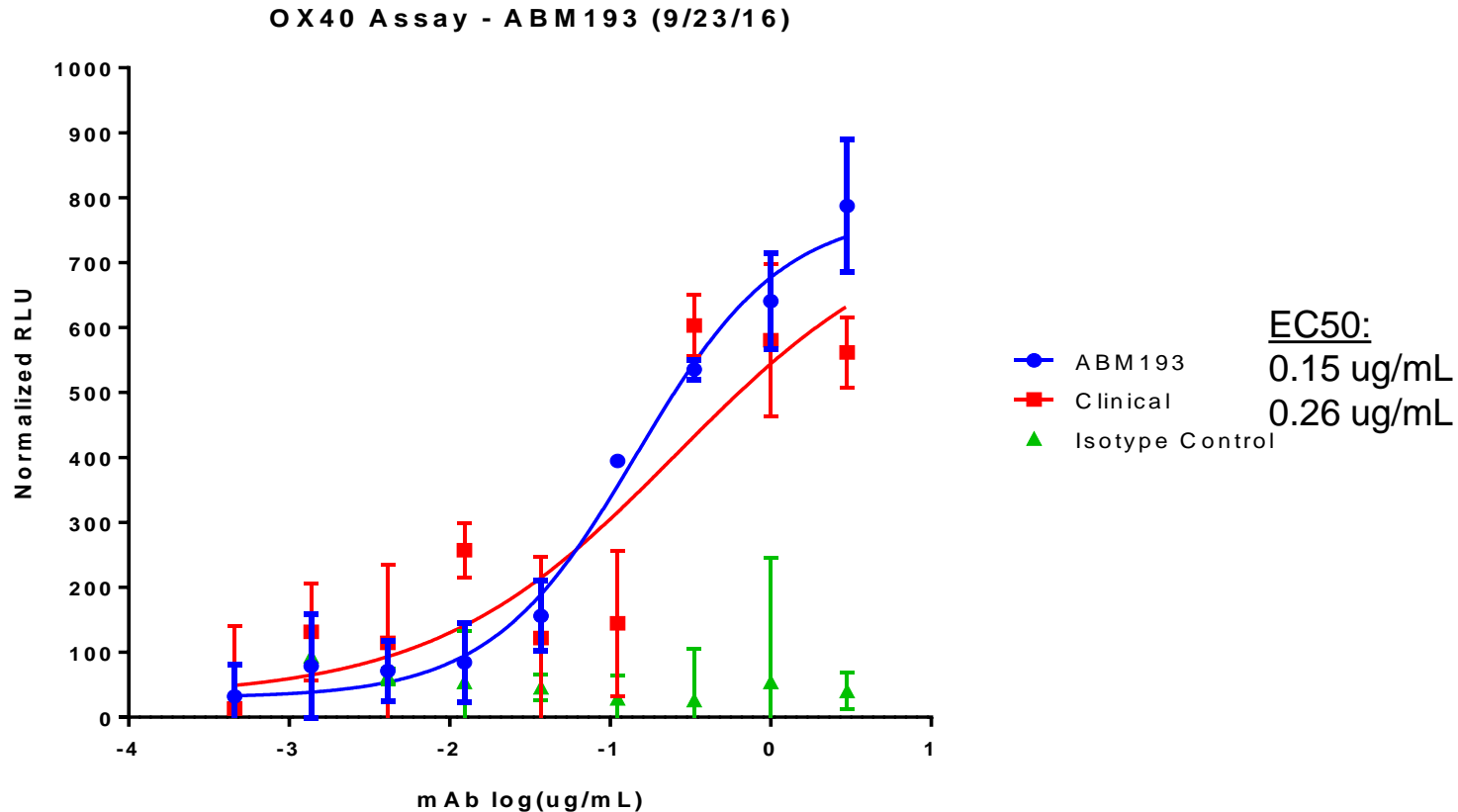


*note: All treatments are in presence of anti-human F(ab')₂ to cross-link

Analysis of 40+ antibodies in cellular assay revealed 3 functional agonists

Titration of mAbs in OX40 Reporter Jurkat Cell Line

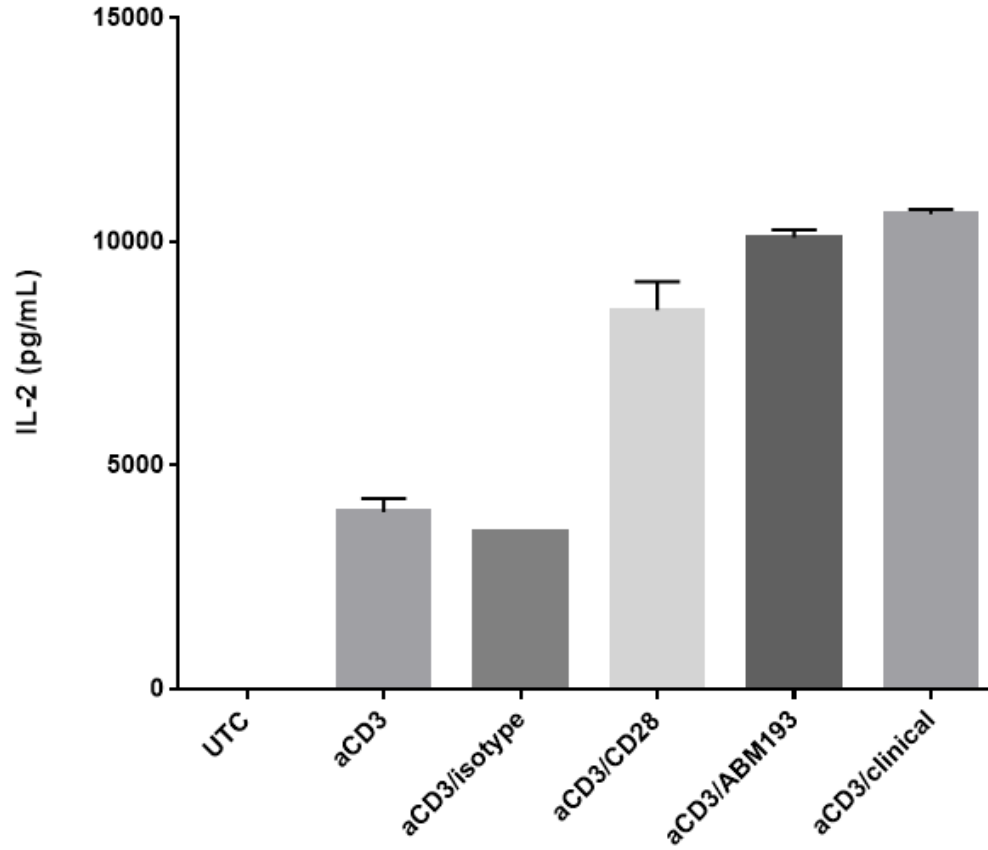
ABM193, Clinical Control and Isotype Control



- ABM193 demonstrates more total activation of T cells
AND
- Better potency than Pfizer's PF-04518600

Activation of Primary T Cells

ABM193, Clinical Control and Isotype Control

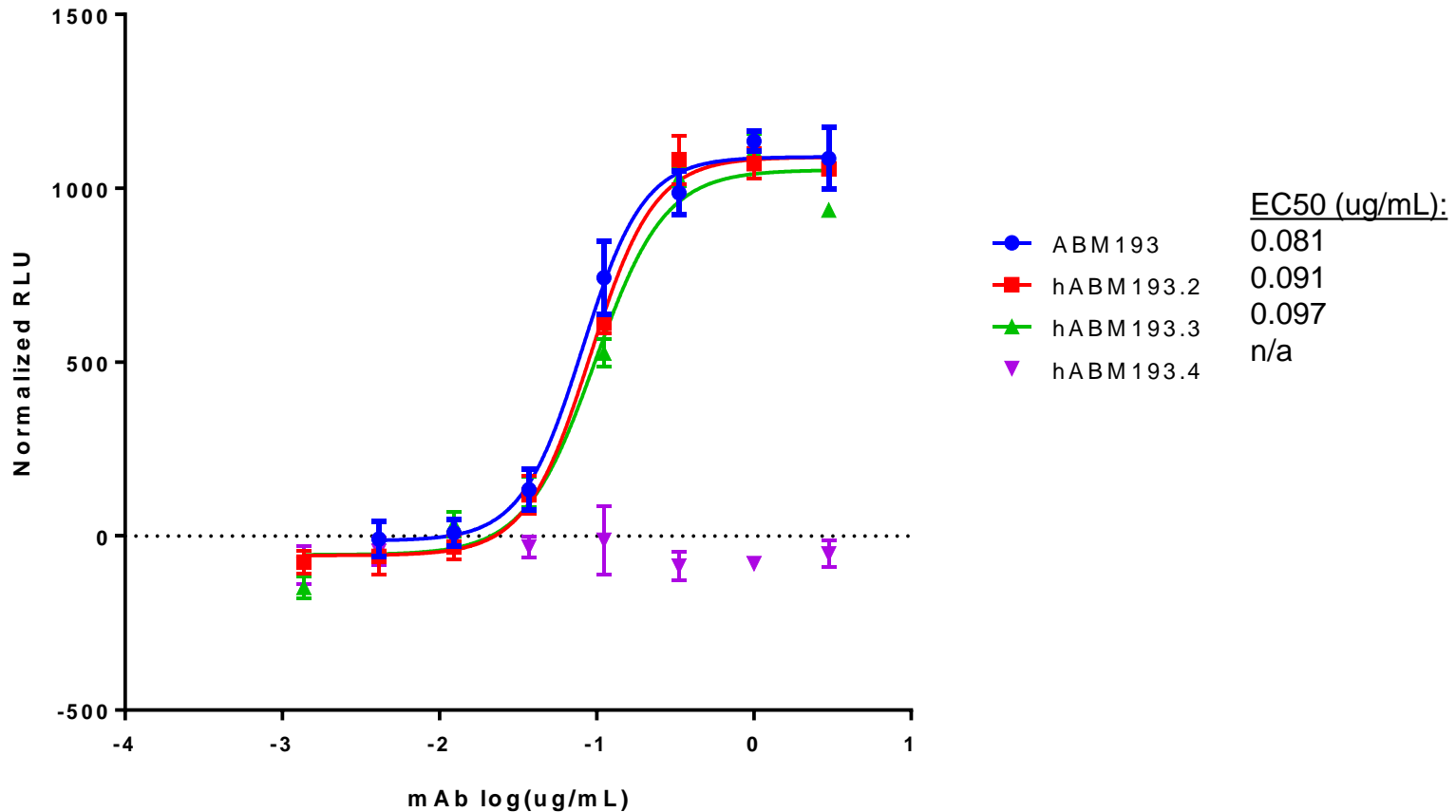


➤ ABM193 Activates Primary T Cells

Titration of Humanized Antibodies

hABM193.2, hABM193.3 and hABM193.4

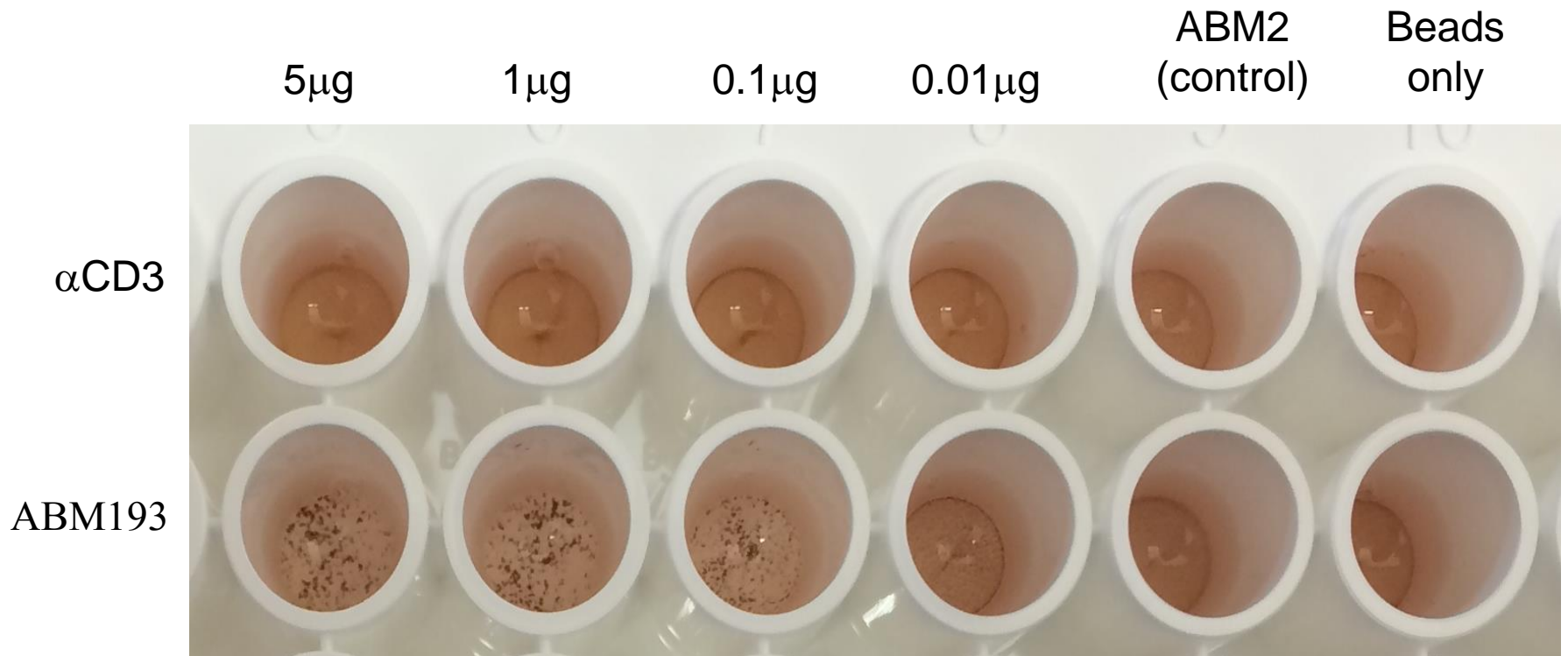
OX40 Assay - Humanized ABM193 (10/19/16)



*anti-human Fab cross-linker added to top dose at 2.5ug/mL

- hABM193.2 and hABM193.3 demonstrate equivalent potency to chimeric parent ABM193

Protein G Dynabead Antibody Capture



Visual Clustering of T Cells with Bead-Bound anti-OX40 mAb ABM193

OX40 Agonist mAb Summary:

- ABM193 shows superior *in vitro* activity compared to Pfizer's PF-04518600 in 2-cell assay and equivalent in primary T cells
- ABM193 has been successfully humanized without significant loss of potency
- Mouse model of human allogeneic tumors in combination with PD-1 or PDL-1 would be a logical next step on the development path
 - NSGTM and NSGTM-SGM3 mice
- Abeome is seeking a partner to move this lead forward and possibly to generate further leads