Abstract 563: A Biparatopic Agonist Antibody for OX40 That Exhibits Superior Activity Without Secondary Crosslinking

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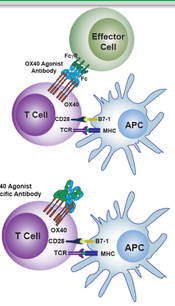
Abstract

The development of agonistic antibodies that activate T-cell co-stimulatory pathways represents a therapeutic strategy with significant clinical potential. However, challenges remain for the translation from *in vitro* efficacy to clinical success. OX40 and other tumor necrosis factor receptor (TNFR) superfamily members are notorious for requiring high-order receptor clustering in order to achieve full activity. For monoclonal antibodies, this high-order clustering is generally achieved through secondary cross-linking strategies. *In vivo*, this secondary cross-linking is often supplied through internal immune effector cells via Fc engagement. Bispecific and biparatopic antibodies represent an emerging class of drug molecules that enable unique mechanisms of action relative to their monoclonal counterparts. Here, we describe the use of our bispecific platform for the generation of large panels of biparatopic antibodies which enabled high-throughput screening for the discovery of an array of OX40 agonistic molecules. We have optimized these multivalent antibodies that exceed the potency of the OX40 ligand in NFkB activation without the need for secondary cross-linking. These agonist antibodies have additionally been characterized using primary T cell assays to monitor the kinetics of growth proliferation and cytokine secretion, outperforming cross-linked antibodies currently being tested in clinical trials. In co-culture systems, these agonist antibodies were effective in inhibiting immuno-suppressive properties of Tregs. Our lead OX40 agonist antibody has been optimized for activity and developability and has entered stable cell line development to further support pre-clinical activities.

Background and Challenges

First-generation of OX40 antibodies are monoclonal

- Higher order cross-linking dependent upon local tumor environment, Fc engagement
- Good activity in preclinical animal studies
- Limited activity in clinical studies



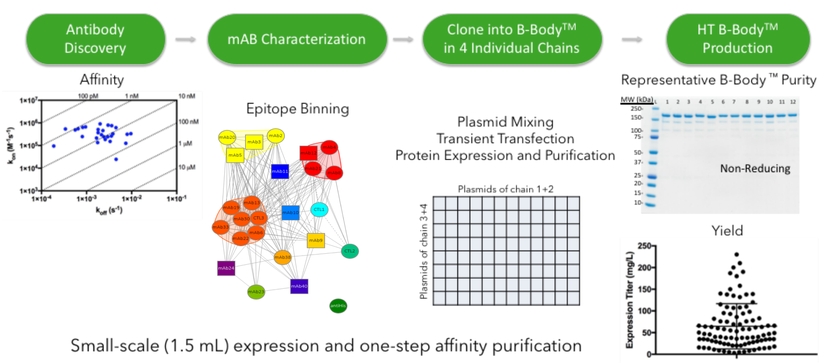
Second-generation agonists exploit alternative strategies for higher order clustering

- OX40 x Tumor Antigen
- Fc Oligomerization variants
- Biparatopic Antibodies

B-Body™ Platform Design and Screening Strategy

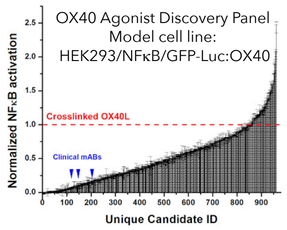
Invenra's B-Body™ Family

While the conventional mAB is monospecific bivalent, we constructed anti-OX40 antibodies into four distinct formats: bispecific bivalent, bispecific trivalent, trispecific trivalent and monospecific trivalent.



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

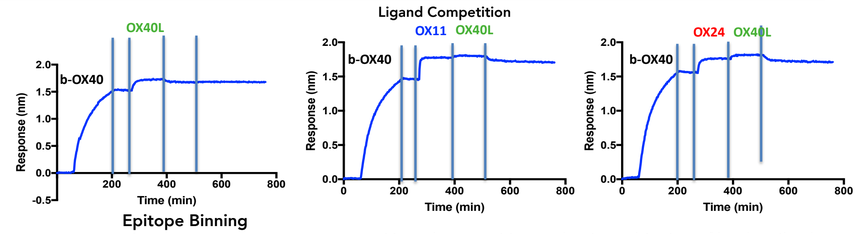
Antibody Discovery and Characterization



High throughput NFkB activation screening for multispecific multivalent antibodies. The activity is normalized with blank cell (negative control, value 0) and crosslinked natural ligand OX40L (positive control, value 1). Three clinical mABs are included in the same assay.

- > 900 B-Body™ 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).

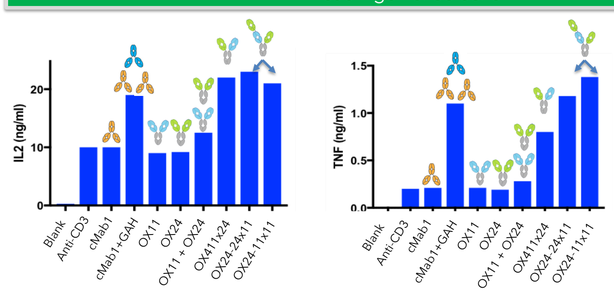
Lead Agonist Binding Characterization



Ligand competition and epitope binning of lead antibody candidates was carried out on the Octet.

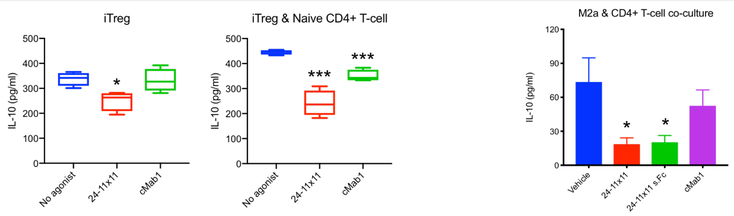
- Both antibodies block OX40 ligand binding
- The antibodies have non-overlapping epitopes

Lead Agonist Selection



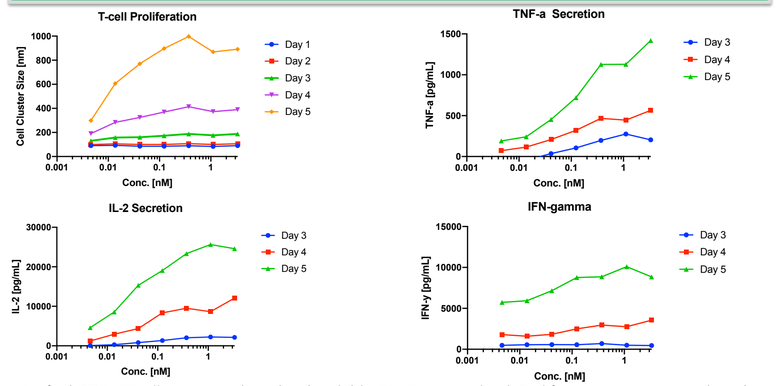
- ARCHER™ bispecifics out perform cross-linked clinical candidates and combinations of parent IgG in primary T-cell activation assays.
- Soluble IgG have no activity without secondary cross-linking.

Suppression of iTreg and M2a Macrophage function



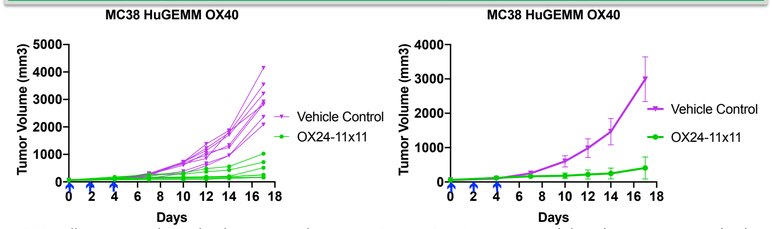
Soluble OX40 agonist was added to CD4+ T-cells co-cultured with iTregs or M2a macrophages or iTregs/M2a macrophages alone. Soluble OX40 treatment inhibits IL-10 secretion from iTregs and M2a macrophages. Soluble OX40 agonists exceed the suppression level of a clinical candidate.

Kinetics of Proliferation of CD4+ T cells and Cytokine Production



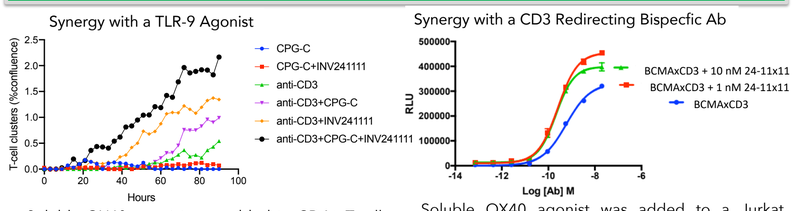
Purified CD4+ T-cells were incubated with soluble OX40 agonist lead. Proliferation was measured on the Incuctye. Cytokine secretion in the supernatants was measured on day 5 by using BD OptEIA ELISA.

In Vivo Efficacy



MC38 cells were implanted subcutaneously in a HuGEMM OX40 mouse model. When tumors reached 50-100 mm3, mice were randomized into groups (N=8) and one group was treated with 5 mg/kg of OX40 Agonist Ab (INV241111) while the other group received a vehicle control on Days 0, 2, and 4 (blue arrows) .

Combination Potential



Soluble OX40 agonist was added to CD4+ T-cells alone or in combination with the TLR-9 agonist, CPG-C. A synergistic response in T-cell proliferation as measured on the Incucyte was observed.

Soluble OX40 agonist was added to a Jurkat reporter gene co-culture assay in which OX40 expressing Jurkat cells, RPMI-8226 cells, and a BCMAxCD3 bispecific antibody are incubated together. The soluble OX40 agonist enhanced the activity of the redirecting T cell antibody.

Summary

- The B-Body™ platform is a robust, versatile multispecific antibody platform.
- High-fidelity assembly and favorable biophysical properties enable HT in-format bispecific discovery.
- OX24-11x11 is able to enhance CD4 function (proliferation and cytokine secretion) while reducing immunosuppressive (IL-10 from Tregs and Macrophages) function in soluble format.
- OX24-11x11 is able to significantly reduce tumor size in an OX40 HuGEMM model.
- OX24-11x11 is able to enhance proliferation of TLR9 agonist stimulated CD4 cells and enhance the signal in a redirecting T cell antibody reporter gene assay.
- The concept of biparatopic agonist antibodies is generally applicable to receptors where clustering is a critical component for the mechanism of action.

Next Steps:

- OX24-11x11 is currently in stable cell line production.