OX40 Agonist
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-κB 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

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Structure</th>
<th>Phase</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tavolixizumab (MEDI0562*)</td>
<td>OX40</td>
<td>Humanized anti-OX40 mAb</td>
<td>Phase 1</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td>Pogalizumab (MOXR0916/ RG7888)</td>
<td>OX40</td>
<td>Humanized anti-OX40 mAb</td>
<td>Phase 1</td>
<td>Genentech/Roche</td>
</tr>
<tr>
<td>PF-04518600</td>
<td>OX40</td>
<td>Fully Human anti-OX40 mAb</td>
<td>Phase 1</td>
<td>Pfizer</td>
</tr>
<tr>
<td>GSK3174998</td>
<td>OX40</td>
<td>Humanized anti-OX40 mAb</td>
<td>Phase 1</td>
<td>GSK</td>
</tr>
</tbody>
</table>

*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
• 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
2x10^8 lymphocytes, spleen mass 1.01g harvested from a single AbeoMouse

5.6x10^7 cells remained after IgM depletion

Spleen | Bone Marrow | Lymph Nodes
• 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
Antibody Amplification & Expression

OX40 mAb LEAD DISCOVERY
PROJECT STATUS

Proprietary Single-Cell RT-PCR Process → Proprietary Expression Vector Systems

96-Well Gel of Variable Region PCR Products

Fully Synthetic (Plasmid-Free) Gene Expression Cassettes
OX40 mAb LEAD DISCOVERY
PROJECT STATUS

High-throughput Screening

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

Greater 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

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

EC50 (ug/mL):
- hABM193.2: 0.081
- hABM193.3: 0.097
- hABM193.4: n/a

*anti-human Fab cross-linker added to top dose at 2.5ug/mL
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
  - NSG™ and NSG™-SGM3 mice
- Abeome is seeking a partner to move this lead forward and possibly to generate further leads