T Cell Agonists

Andrew Weinberg, PhD
Providence Cancer Institute
Portland, Oregon

• Targets: **OX40**, 4-1BB, CD27, GITR, and ICOS
• Cells that express these targets
• Molecular mechanism(s) of action
• How to go from Preclinical studies to a Clinical Trial
• Agonist Abs in Clinical trials
• Outstanding questions in the field
IMMUNOLOGICAL PARADIGM

The major function of the immune system is to recognize and eliminate harmful entities within the body without destroying “self” tissue

Cancer is “harmful” – Immune Recognition of tumor Ags

Theoretically, leading to existing immunity in every cancer patient
Tumor-Immune Microenvironment

Lung Cancer

TGF-β

TGF-β

OX40

Tumor-Specific T Cell

TLR agonist

Tumor

Immune Inhibitory Effect

Immune Stimulatory Effect

IL-10

PD-L1

CD80 or CD86

CD28

MHC

TNF-α

IL-12

OX40 Agonist

DC

Teff

Treg

TCR

PD-1

TGF-β

Lung Cancer
Multiple co-stimulatory and inhibitory interactions regulate T cell responses

T Cell Agonist Expression

**TNF-Receptors**

1) **OX40** – CD4s, Tregs, CD8s, and NK T cells
2) **4-1BB** – CD8s, Tregs, CD4s, DCs, B cells, NK, granulocytes, and blood vessel walls

1) **GITR** – Tregs, CD4s, CD8s, NK, B cells, and myeloid cells
2) **CD27** – CD8s, CD4s, Tregs, B cells, and NK cells

**Ig-Super Family Member**

1) **ICOS** - CD4, Tregs, and CD8s
Biochemical Structure of the TNF/TNF-receptor Family Members
Overview of TNF-R Signaling

Croft M., Nature Reviews Immunology, 2003, 3:609
Costimulation of ICOS Pathway/Signaling

- TCR
- CD28
- CD80/86
- MHC
- ICOS
- ICOSL
- PI3K
- AKT
- p50α
- IL-4
- IL-5
- IL-6
- IL-10
- IL-21
- TNF-α
- ACTIVATED T_{EFF} CELLS
- T_{FH} CELLS
- T_{REG} CELLS
- Costimulation
- T-cell response enhancement
- GC formation
- Antibody response
- Tumor immune escape, immunosuppression

Survival
Proliferation
Cytokine Production

Anti-Tumoral
Proc. Tumoral
In vitro costimulation anti-OX40 Costimulation Assay
(Effector CD4 T cell proliferation)

- Recombinant OX40L:Ig
- Anti-OX40 antibody
- Constant anti-T cell Receptor (2ng/ml)

Graph:
- H$_3$-Thymidine incorporation
- OX40 agonist ng/ml
- Recombinant OX40L:Ig
- Anti-OX40 antibody

Data points represent different concentrations of OX40 agonist (ng/ml) and the corresponding H$_3$-Thymidine incorporation.
Mouse Model to Assess Agonist Ab Activity
In Vivo

Solid Tumor Administered s.c.

Days 3 and 7 after tumor injection

- Control
- Sol. mu OX40L
- anti-OX-40
OX40L:Ig Treatment of MCA 303

% Survival vs Days After Tumor Injections

- Control
- 100 ug DR3:Ig
- 100 ug muOX40L:Ig
Tumor Models Successfully Treated with OX40 Engagement

- Breast (4T1, SM1, EMT-6)
- Sarcoma (MCA 303, 205, 203)
- Colon (CT-26)
- Glioma (GL261)
- Melanoma (B16/F10)
- Prostate (TRAMP-C1)
- Lung (Lewis Lung)
CD4 and CD8 T cells Roles in anti-OX40 Enhanced Tumor Immunity (Glioma Model)
Fc-Receptor Importance for Therapeutic Effects of Agonist Abs
(OX40 agonists performed in Fc-Receptor ko mice)

Survival OX86
- FcKO OX86
- FcKO Rat IgG
- Ctrl OX86
- Ctrl Rat IgG

***p=.0002

Survival OX40L
- FcKO OX40L
- FcKO Rat IgG
- Ctrl OX40L
- Ctrl Rat IgG

**p=.0028

FcKO 4/18 = 22% Cure Rate
WT 10/18 = 56% Cure Rate

FcKO 2/18 = 11% Cure Rate
WT 7/18 = 39% Cure Rate
First OX40 Agonist Trial in Cancer Patients

- Phase I: Three doses delivered in a one week span
- Anti-OX40 was well-tolerated
- No CRs or PRs; however,
  - 12 patients had regression of at least one tumor nodule
  - 17/30 had SD by RECIST criteria for 56 days

Patient #14  
CD8⁺CD95⁺ T cell (Blood)

Day 0 | Day 5 | Day 8 | Day 15
--- | --- | --- | ---
0.57% 1.39% 1.13% 1.59% 8.32% 15.46%
35.86% 62.18% 50.50% 47.55% 47.41% 48.90% 32.62% 43.61%

Day 29 | Day 36 | Day 43 | Day 57
--- | --- | --- | ---
1.86% 7.91% 0.59% 0.58% 1.98% 0.66% 1.49%
30.71% 59.52% 35.83% 60.80% 42.17% 55.26% 37.25% 60.60%
Anti-OX40 induces robust proliferation in peripheral blood
Do increases in PBL-Ki-67 predict clinical outcome?

![Graph showing percent change in tumor volume across different cohorts.]

**Progressors**
- Cohort 1 (0.1 mg/kg)
- Cohort 2 (0.4 mg/kg)
- Cohort 3 (2 mg/kg)

**Non-Progressors**

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**Figures a-d**

- **Figure a** (CD4⁺ Foxp3⁻ T cells)
- **Figure b** (CD8⁺ T cells)
- **Figure c** (CD4⁺ Foxp3⁺ T cells)
- **Figure d** (CD3⁺ cells)
PRE-OP ANTI-OX40: 3-ARM SURGICAL WINDOW STUDY IN H&N CANCER

Pre-treatment immune assessment

- INCISIONAL TUMOR BIOPSY
- PERIPHERAL WHOLE BLOOD

Post-treatment immune assessment

- RESECTED TUMOR
- DRAINING NODES (NORMAL AND METASTATIC)
- PERIPHERAL WHOLE BLOOD

Day 8
- Surgery

Expansion cohort

Day 12
- Surgery

Day 19
- Surgery

OX40 6469

0.4 mg/kg
Multi-Plex Immune Fluorescence FFPE:HOX04
2 week post-therapy

PRE
Ki67 = orange
CD8 = green

POST
PD-L1 = red
CD3 = purple
Survival Data in Immune Responders
OX40 Pre-Op Study

DFS - survival probability (%)

n=4

n=13

Follow-up (months)

log-rank test, p=0.17
## Costimulatory Agonist Antibodies in Development

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<th>Drug</th>
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Combinations with Agonist Abs for Future Trials

Combining Agonist Abs
1) anti-OX40 with anti-4-1BB (several publications showing additive/synergistic effects). OX40 more CD4 dominant and 4-1BB more CD8 dominant.
2) anti-OX40 combined with anti-ICOS (publication showing additive/synergy)
3) GITRL:Ig fusion protein with anti-OX40 (publication showing additive/synergy)

Combining Agonist Abs with Checkpoint Blockade
1) anti-4-1BB with PD-1 or CTLA-4 blockade (publications showing additive/synergistic effects)
2) anti-OX40 combined with PD-1 or CTLA-4 blockade (publications showing additive/synergy)
3) GITRL:Ig fusion protein with anti-PD-1 (publication showing additive/synergy)
4) Anti-CD27 with anti-PD-1 (publication showing additive/synergy)

Combining Agonist Abs with Vaccines
1) anti-CD27 with DC vaccine in prostate cancer (publications showing additive effects)
2) anti-OX40 combined with cell-based or peptide vaccines (publications show additive effects)
3) Anti-GITR with cell-based and Listeria vaccine (publications show additive effects)
4) Anti-ICOS with cell-based vaccine (publication showing additive/synergy)
Outstanding Questions for Agonist Abs

1) Why has the efficacy in the clinic been underwhelming as single agent or combination?

2) Dosing and schedule different than checkpoint blockade, although to date all trials have been dosed identical to checkpoint blockade.

3) How should combination therapies be delivered? With checkpoint blockade delivered at the same time as agonist Abs? Publications have indicated that is probably not optimal.

4) What about blocking negative signals delivered in tumor microenvironment in combo with agonist Abs? Blocking TGF-β signaling in combo with anti-OX40 shown dramatic effects.

5) Bi-specifics? Agonist Ab:Checkpoint blockade Ab or Agonist Ab:Agonist Ab?

6) Are there new costimulatory pathways to be exploited?
Timing of PD-1 Blockade Is Critical to Effective Combination Immunotherapy with Anti-OX40

David J. Messenheimer, Shawn M. Jensen, Michael E. Afentoulis, Keith W. Wegmann, Zipei Feng, David J. Friedman, Michael J. Gough, Walter J. Urba, and Bernard A. Fox

Clin Cancer Res; 23(20); 6165–77.

Concurrent PD-1 Blockade Negates the Effects of OX40 Agonist Antibody in Combination Immunotherapy through Inducing T-cell Apoptosis


Cancer Immunol Res; 5(9); 755–66.