Augmenting Anti-tumor Immunity with a Novel IL-2 Antibody Immunotherapy
Interleukin-2 Has Durable Clinical Activity

- Aldesleukin (Proleukin®), recombinant human IL-2, was the first cancer immunotherapy approved by the FDA in 1992 for metastatic renal cell carcinoma (mRCC).

- IL-2 has demonstrated durable long-term responses in ~10% of patients for metastatic melanoma and renal cancer\(^1\).

- Approximately 70% of patients with complete responses have been cured, maintaining complete regression for more than 25 years\(^1\).

- Commercial use limited due to cytokine release syndrome/vascular toxicity/ICU care.

- Anti-tumor efficacy is limited by expansion of the immunosuppressive Treg pool.

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\(^1\) Rosenberg SA. Raising the bar: the curative potential of human cancer immunotherapy. Sci Transl Med 2012;4:127ps8;
SUMMARY: XOMA’s mAb19 Next Generation IL-2 Approach

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔ NK &amp; CD8 T cell expansion without cytokine release and vascular leak</td>
<td>Increased therapeutic window</td>
</tr>
<tr>
<td>✔ Limited expansion of Tregs (i.e. limit IL-2Rα)</td>
<td>Allows full cytotoxic activity</td>
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<tr>
<td>✔ Long half-life - estimated ~ q2wk dosing</td>
<td>Ease meets current strategies</td>
</tr>
<tr>
<td>✔ Potential for stand-alone activity</td>
<td>Augments anti-tumor efficacy</td>
</tr>
<tr>
<td>Able to combine with current immunotherapies</td>
<td></td>
</tr>
<tr>
<td>✔ No issues with immunogenicity</td>
<td>Fully human molecules</td>
</tr>
<tr>
<td>✔ Ease of manufacturing</td>
<td>Established mAb CMC</td>
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</table>
IL-2 Normally Acts at Both the High-Affinity IL-2Rαβγ on Treg Cells AND the Intermediate Affinity IL-2Rβγ on Effector T and NK Cells.

<table>
<thead>
<tr>
<th>High Affinity (K_d ~10 pM)</th>
<th>Intermediate Affinity (K_d ~1 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treg</td>
<td>T_{eff} &amp; NK</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>Anti-tumor immunity enhanced</td>
</tr>
<tr>
<td>Vascular Leak</td>
<td></td>
</tr>
</tbody>
</table>
XOMA’s mAb19 Disrupts IL-2 Interaction with IL-2Rαβγ but Not IL-2Rβγ, Thereby Promoting T_{eff} and NK Anti-tumor Immunity without Treg Immunosuppression

- mAb19 binds to the IL-2Rα-binding domain of IL-2
- IL-2 activity directed to IL-2Rβγ-bearing anti-tumor effector cells and not Tregs

mAb19 administered with very low dose Proleukin

T_{reg} Immunosuppression Vascular Leak
T_{eff} & NK anti-tumor immunity enhanced
Screening Strategy Flowchart for XOMA’s Fully Human anti-human IL-2 mAbs

FACS

- No binding to CHO parental cells
- Binding to CHO-Rβ and/or Rβγ cells
- No binding to CHO-Rα cells

SPR

- IL-2 binding affinity ($K_D < 2nM$)
- Reduces sensitivity of IL-2 in Rα assays
- Maintains sensitivity of IL-2 in Rβγ assays

Cell proliferation

TWO CANDIDATES IDENTIFIED
Lead Anti-IL-2 Modulating mAbs are Potent Binders to Human IL-2

- mAb19 is the most potent binder to human IL-2

*Analysis by surface plasmon resonance*
XOMA Lead mAbs Especially Disrupt IL-2 Activity at the IL-2R\(\alpha\beta\gamma\) Complex (i.e. \(T_{\text{reg}}\)) vs \(\beta\gamma\) (desired effector cells)

**BaF3/IL-2 R\(\beta\gamma\) cell line is a surrogate for CD8\(^+\) T cells and NKS**

**NK92 cell line is a surrogate for \(T\) regs**

<table>
<thead>
<tr>
<th>EC(_{50}) fold-shift from control Ig</th>
<th>Test Antibody</th>
<th>BaF3 cells (IL-2R (\beta\gamma))</th>
<th>NK-92 cells (IL-2R (\alpha\beta\gamma))</th>
<th>EC(_{50}) IL2R(\beta\gamma) mAb19/IL2=0.65nM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mAb19 anti-IL-2</td>
<td>4.5</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mAb99 anti-IL-2</td>
<td>2.5</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>
Study Design: FACS Analysis for Immune Cell Subpopulation Shifts in Naïve B6 Mice

- Mice treated i.p. with XOMA mAb + low-dose human IL-2 or standard high-dose IL-2
  - mAb’s are not cross-reactive with mouse IL-2

- N = 4 animals per group

- Doses:
  - HD IL-2 = 100 µg/mouse (standard requirement for mouse anti-tumor efficacy)
  - LD IL-2 = 1.5 µg/mouse
  - mAb19 = 7.5 µg/mouse (<1 mg/kg as precomplexed with IL-2)

- Dosing interval:
  - LD IL-2 & mAb 19: M, W, F (less frequent dosing given longer duration of action)
  - HD IL-2: M, T, W, T, F

- SPLC isolation and FACS analysis on Day 7
And Low Dose IL-2 + mAb19 Induces Stronger Ratios of CD8 & NK to Treg Ratios vs Standard IL-2 in B6 Mice

Splenocyte Subpopulation Ratios

CD8/Treg

NK/Treg

Control HD IL-2 LD IL-2 mAb19 LD IL-2 + mAb19

Control HD IL-2 LD IL-2 mAb19 LD IL-2 + mAb19
Study Design for Focus Study of Lewis Lung Carcinoma Tumor Response by IL-2 + mAb and/or anti-PD-1 mAb

• **Timeline**
  - 1x10^6 LLC (LLC-A9F1) injected s.c.
  - Treatment begins (day 17, median tumor size ~50mm^2)
  - Treatment continued x 3 weeks

• **Conditions (n=15/group; all injections i.p.):**
  - control (vehicle)
  - Human LD IL-2 + mAb99 only
  - anti-PD-1 mAb only
  - Human LD IL-2 + mAb99 + anti-PD-1 mAb

• **Dosing**
  - IL-2 + mAb99
    - 2ug IL-2 + 10ug antibody, pre-complexed, i.p. 3x/week
  - anti-PD-1 mAb (clone RMP1-14)
    - 200ug/injection, i.p. 2x/week
Low-Dose IL-2 + mAb99 is Effective Alone and Efficacy is Increased Upon Combination with anti-PD-1
Long-term Anti-tumor Memory With Combination LD IL-2 / mAb99 + Anti-PD-1

LLC Tumor Rechallenge Study

- **Objective**: test induction of memory by rechallenging complete responders from initial experiment with LLC tumor, injected subcutaneously
- Rechallenge conducted approximately five months after start of initial experiment and approximately four months after final treatment (1E6 cells initially, 1.5E6 in rechallenge)
- **Day 22 Post-Rechallenge Results**
  - Synergy demonstrated between mAb / IL-2 complex and checkpoint blockade
    - In mAb / LD IL-2 complex + αPD-1 mAb group, 100% (6/6) of rechallenged mice remained tumor free

<table>
<thead>
<tr>
<th>Previous Treatment</th>
<th>CRs</th>
<th># mice challenged</th>
<th># tumor free</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control naïve mice)</td>
<td>0/15</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>mAb99 / LD IL2 complex</td>
<td>1/15</td>
<td>1</td>
<td>(1)</td>
<td>markedly delayed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tumor growth</td>
</tr>
<tr>
<td>αPD-1 mAb</td>
<td>2/15</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>mAb99 / LD IL-2 complex + αPD-1 mAb</td>
<td>6/15</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
Mouse Colon Carcinoma CT26 Tumor Model Study Design

- **Balb/c mice**

- **N:** 6-12 animals per group

- **Doses:** HD IL-2 = 50 µg, 100 µg per mouse (2.5, 5 mg/kg)
  
  LD IL-2 = 1.5 ug/mouse (0.075 mg/kg)

  mAb19 = 15 µg/mouse (0.75 mg/kg)

- **Dosing interval:**
  
  i.p.
  
  HD IL-2 = once daily for five days with two days off followed by another five day regimen;
  
  mAb 19 15 µg + IL-2 1.5 µg: i.p. q3-4d
Proof-of-Concept and Initial Dose-Ranging of mAb19 + low-dose IL-2 in the CT26 Colon Carcinoma Model

Q3d dosing i.p. BALB/c mice

Preferred dose and molar ratio
mAb19+IL2 Dosing Favors CT26 Tumor Infiltrating NK Expansion without Tregs
The mAb19 Immunotherapy Approach Engages Multiple Anti-tumor Mechanisms

- Enhanced $T_{\text{effectors}}$ (including CD8 memory)
- Enhanced innate immunity (e.g. NKs)
- Potentially increases PD-1, PD-L1, MHC cl, cold to hot tumor
- Suitability for combination therapy – e.g. checkpoint inhibitors, tumor-targeted ADCC agents, others