Novel IL-2/mAb Complexes Mediate Potent Anti-tumor Immunity which is Augmented with Anti-PD-1 mAb Therapy

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Abstract

Background: Recent success and FDA approval of immune checkpoint inhibitors (CI) in a growing number of cancers are transforming cancer treatment and revitalizing interest in immunotherapies. However, while efficacy is observed in patients with advanced metastatic diseases treated with CI, not all patients respond and most responses are incomplete. Preclinical studies suggest that combinations of additional modalities with CI will provide opportunities to improve patient responses. As both IL-2 and CI therapy can independently augment anti-tumor immunity in patients, likely in mechanistically distinct ways, we hypothesized that we could improve anti-tumor immunity by combining IL-2 and anti-PD-1 mAb therapies.

Methods: To improve IL-2 efficacy and therapeutic index, we generated novel anti-IL-2 mAbs which, when complexed with IL-2 (IL-2/mAb) offer advantages over standard IL-2 therapy(1-3). First, binding to an anti-IL-2 mAb increases IL-2 half-life and biological activity. Second, depending on the epitope at which the mAb binds to IL-2, antibody binding can modulate which IL-2 receptor subunits (alpha, beta, or gamma) are engaged. Antibodies that interfere with binding of IL-2Rα can reduce activation of high-IL-2Rα-expressing cell types, such as suppressive Tregs, and steer activity toward cell types expressing only IL-2Rβ and γ, such as memory $CD8^+$ T cells and NK cells. In this way, these complexes may have more effective anti-tumor activity(1-3). We screened human antibody phage libraries to identify antibodies that shift IL-2 receptor binding and activity differentially on different cell types in vitro and in vivo. Complexes of these antibodies were tested in vivo for their effects on T cell frequency and activation markers, and in a subcutaneous Lewis lung carcinoma model for their ability to mediate anti-tumor immunity, both alone and in combination with anti-PD-1 mAb.

Results: In normal mice, IL-2/mAb complexes potently expanded $CD8^+$ T cells and NK cells with minimal expansion of Tregs. As single agent therapy, IL-2/mAb complexes or anti-PD-1 mAb reduced tumor growth, although most mice succumb to tumor growth eventually. Combination of IL-2/mAb complexes with anti-PD-1 mAb therapy resulted in durable, complete responses in nearly half of the mice.

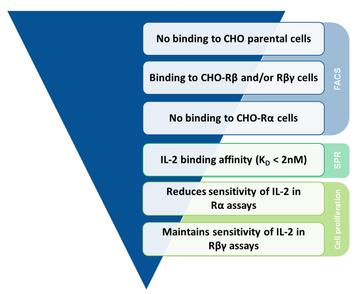
Conclusions: While immune based therapies such as anti-PD-1 mAb can be highly effective in select patients, even in those patients that obtain clinical benefit, disease may recur. Our results suggest that the addition of IL-2/mAb complexes to therapy with anti-PD-1 mAb could broadly increase the percentage of patients deriving benefit from immune-based therapy

1. Sato *et al., Biotherapy* 6 (3):225-31 (1993)
2. Boyman *et al., Science* 311, 1924-27 (2006)
3. Létourneau *et al., PNAS* 107, 2171-2176 (2010)

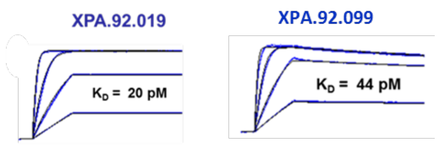
Results

Figure 1. Screening Strategy for Anti-IL-2 Monoclonal Antibody Discovery

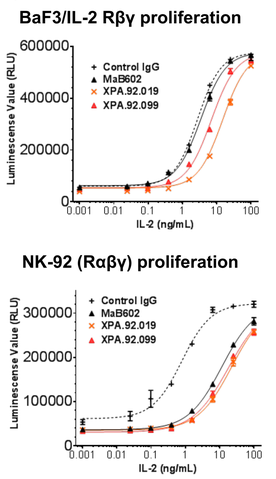
A. Human antibody phage display libraries



B. Screen identified high affinity anti-IL-2 modulating antibodies



C. Antibodies differentially reduce IL-2 sensitivity of cells based on IL-2Rα expression



D. Fold-shift in EC_{50} of IL-2-stimulated proliferation shows greater inhibition of cells expressing IL-2Rα

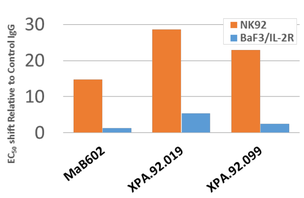


Figure 1. A.) Antibodies were stratified using a flow-cytometry screen based on IL-2-mediated binding to cell lines engineered to express different combinations of human IL-2 receptor chains. **B.)** Affinity of two select mAbs binding to IL-2 as determined by SPR. **C.)** Antibodies of interest were complexed with IL-2 and tested in functional assays using IL-2 dependent cell lines that either express all three receptor chains or only IL-2Rβ and γc. (BaF3 cells were transfected with IL-2Rβ and γ common and weaned from IL-3 to IL-2 dependence). **D.)** The effect of antibody on IL-2-dependent cells' sensitivity to IL-2, as measured by inhibitory shift in EC_{50} of dose-response, is greater for cells expressing IL-2Rα.

Results

Figure 2. Novel IL-2/mAb complexes drive expansion of CD8 memory-phenotype T cells and NK cells

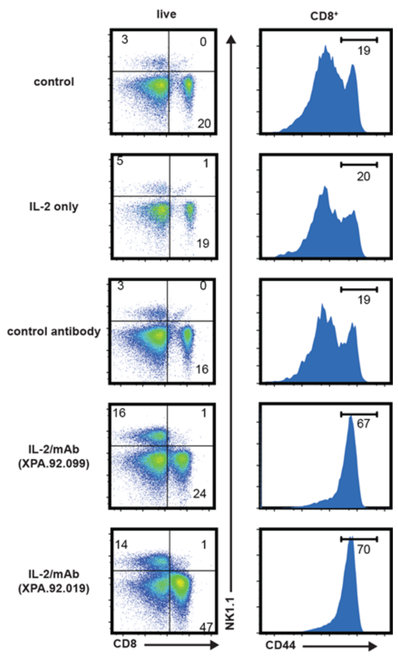


Figure 2. B6 mice were injected (ip) on days 0, 2, and 4 with IL-2 (1 μg), irrelevant antibody (5 μg), or IL-2 (1 μg) pre-associated with the indicated anti-IL-2 mAb clone (5 μg). On day 6, splenocytes were analyzed by flow cytometry. The left column shows total splenocytes with dotplots depicting CD8 and NK1.1 staining. The right column is gated on $CD8^+$ T cells and shows the frequency that are CD44hi (memory-phenotype). IL-2 alone at this relatively low concentration was not sufficient to cause expansion of NK cells or CD8+D44hi T cells, while IL-2 complexed with either antibody led to strong expansion of both cell types.

Figure 3. IL-2/mAb complexes augment the expansion of $CD8^+$ T cell and NK cell effector cell populations during tumor growth

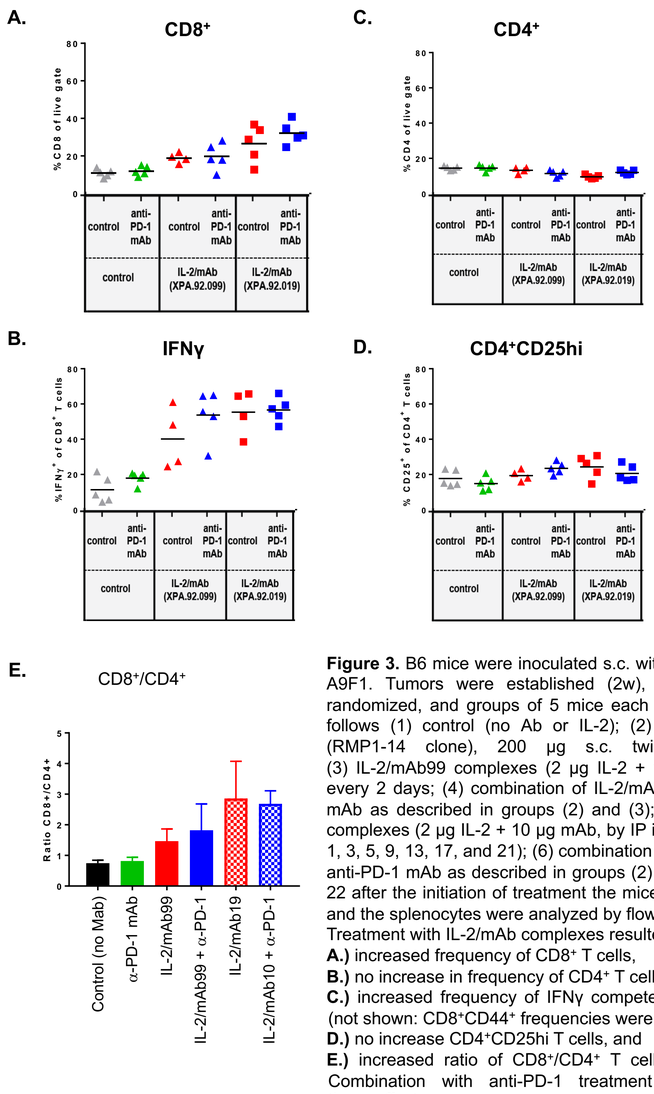
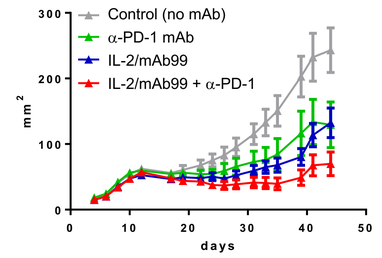


Figure 3. B6 mice were inoculated s.c. with 10^6 cells LLC-A9F1. Tumors were established (2w), the mice were randomized, and groups of 5 mice each were treated as follows (1) control (no Ab or IL-2); (2) anti-PD-1 mAb (RMP1-14 clone), 200 μg s.c. twice per week; (3) IL-2/mAb99 complexes (2 μg IL-2 + 10 μg mAb) i.p. every 2 days; (4) combination of IL-2/mAb99 + anti-PD-1 mAb as described in groups (2) and (3); (5) IL-2/mAb19 complexes (2 μg IL-2 + 10 μg mAb, by IP injection on days 1, 3, 5, 9, 13, 17, and 21); (6) combination of IL-2/mAb19 + anti-PD-1 mAb as described in groups (2) and (5). On day 22 after the initiation of treatment the mice were sacrificed and the splenocytes were analyzed by flow cytometry. Treatment with IL-2/mAb complexes resulted in
A.) increased frequency of $CD8^+$ T cells,
B.) no increase in frequency of $CD4^+$ T cells,
C.) increased frequency of IFNγ competent $CD8^+$ T cells (not shown: $CD8^+CD44^+$ frequencies were similar.)
D.) no increase $CD4^+CD25$hi T cells, and
E.) increased ratio of $CD8^+/CD4^+$ T cells in circulation. Combination with anti-PD-1 treatment did not alter these effects

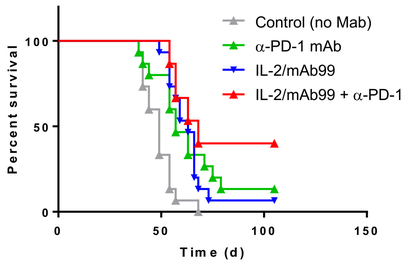
Figure 4. Timeline for treatment of tumor-bearing mice with IL-2/mAb complexes and anti-PD-1 mAb.

Figure 5. Combination of IL-2/mAb complexes and anti-PD-1 mAb therapy leads to superior anti-tumor immunity

A. Treatments reduced tumor growth



B. Combination increased survival



C. Combination increased complete responses

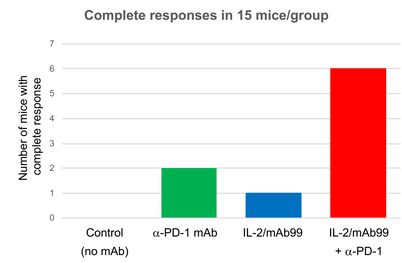


Figure 5. Mice were inoculated with LLC tumor cells as in Figure 3. Four groups of 15 mice per group were treated as follows: (1) vehicle only control (PBS); (2) anti-PD-1 mAb (RMP1-14 clone) given 2x per week, 200 μg sc; (3) IL-2/mAb99 complexes (every 48 hours, IP injection, 2 μg IL-2 + 10 μg mAb); (4) combination of IL-2/mAb99 + anti-PD-1 mAb as described in groups (2) and (3). Tumors measurements (length and width) were performed blinded, and area was described in mm^2.
A.) Average tumor volume +/- SEM. While IL-2/anti-IL-2 complex and anti-PD-1 mono-therapies significantly reduced tumor growth compared to control–treated mice, the combined effect of IL-2/anti-IL-2 complex with either anti-PD-1 was significantly greater than that of either reagent alone.
B.) Survival plot shows increase with combination treatment.
C.) No mice in the control group had a complete response (no tumor detectable), while such a complete response was achieved by one mouse in the IL-2/mAb99 alone group and two in the anti-PD-1 mAb alone group. Combination of IL-2/mAb99 + anti-PD-1 mAb yielded complete response in nearly half (6/15) of the treated mice.

Conclusions

- Novel IL-2 monoclonal antibodies (mAbs) from XOMA's human antibody phage display libraries differentially modulate activity on IL-2-responsive cells based on their pattern of expression of IL-2 receptors (Rα, Rβ, and γ common).
- Antibodies augment and modulate biological activity of low dose IL-2, resulting in superior expansion and activation of $CD8^+$ T cells and NK cells compared with $CD4^+$ T cells. These and other published results are consistent with an improved therapeutic index of IL-2/mAb complexes versus free IL-2.
- IL-2/mAb complexes mediate single agent antitumor efficacy, reducing growth of LLC.
- IL-2/mAb complexes combined with anti-PD-1 mAb demonstrate anti-tumor efficacy superior to either agent alone, inducing complete responses in nearly half of the tumor-bearing mice.