AU-007, a computationally designed human antibody to the CD25-interacting domain of IL-2, demonstrates strong activity in murine syngeneic tumors in combination with IL-2 and PD-1 or PD-L1 antibodies

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Background

IL-2 binds to two forms of the IL-2 receptor (IL-2R). The low affinity IL-2R dimeric receptor expressed on CD8 T effector cells, memory T cells, NK cells and NKT cells is composed of CD122 and CD132. The high affinity IL-2R trimeric receptor expressed on Tregs and vascular endothelium is composed of CD25, CD122 and CD132. The trimeric receptor has 100-fold greater affinity for IL-2 over the dimeric receptor, making Tregs far more sensitive to IL-2 than the NK, effector CD8 and naïve T-cells, and creates an IL-2 sink. Thus, IL-2 must be given at high doses (HD IL-2) to overcome the trimeric receptor sink and activate dimeric receptors to drive expansion of the effector arm of the immune system against cancer cells. Pulmonary vascular leak associated with HD IL-2 is the primary dose-limiting toxicity, which is mediated by signaling through CD25+ vascular endothelial and smooth muscle cells around the vasculature. This greatly reduces the therapeutic index of HD IL-2 and has limited its use in clinical practice. However, HD IL-2 can produce durable complete responses in some melanoma and renal cell cancer patients, and this potential has led to many efforts to attempt to improve upon IL-2's therapeutic index.

To address these challenges of existing treatments, Biolojic Design used its artificial intelligence (AI) platform to computationally design AU-007, an antibody that specifically inhibits human IL-2 from binding to CD25 in trimeric receptors while preserving IL-2's binding to dimeric CD122/CD132. Thus, AU-007 promotes immune effector activation by IL-2 while preventing the Treg expansion and vascular endothelial activation driven by IL-2 or the autoinhibitory loop caused by endogenous IL-2 secreted from activated CD4 T helper cells and CD8 T effectors. AU-007 is an IgG1 antibody that has a human VDJ sequence and LALA mutations in the Fc domain to silence effector function, binds human IL-2 (hIL-2) with picomolar affinity and demonstrates excellent biophysical properties with low potential for anti-drug immunogenicity. AU-007 has been found to be safe and well tolerated in primates (data not shown), does not cross-react with human tissues, and is likely to be the first computationally designed human antibody to enter clinical development. Here we report on the generation of AU-007 using a unique AI platform, and on its anti-cancer activity in combination regimens including checkpoint inhibitors.

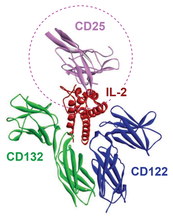
Biolojic Design's AI Platform

Biolojic Design's artificial intelligence platform uses machine learning and big data to mimic the way the human immune system designs antibodies. This allows the design of fully human antibodies that bind pre-selected epitopes with surgical precision and lead to the desired biological outcome.

How we applied our artificial intelligence platform to design AU-007
The challenge: design an antibody that (i) captures IL-2, (ii) binds it in a way that antagonizes Tregs but agonizes Teffs, NKs and NKTs, (iii) is fully human, non immunogenic and developable.

Computational design challenge

*Antibody that binds endogenous human IL-2, **prevents Treg expansion, encourages Teff expansion***



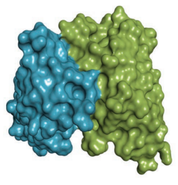
Human IL-2 trimeric receptor complex

To succeed, we need to design an antibody that binds IL-2 (red) with ultrahigh affinity, fully blocks the binding of IL-2 to CD25 but does not hinder the binding of IL-2 to CD122 and CD132.

Given the small size of IL-2 and the large size of human antibodies, such surgical binding is hard to achieve.

Step 1

In silico identification of a template Ab



Template: Does not bind human IL-2 but predicted to be complementary

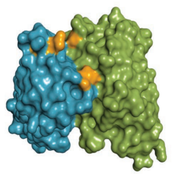
How the immune system does it:
When facing a new epitope, the immune system always looks for an existing antibody (germline Ab or memory Ab) that could serve as a template. This Ab may not detectably bind the target.

How the Biolojic platform does it:
Machine learning algorithms trained on known Ab-Ag pairs screen existing antibodies to find one that is predicted to be a good template.

How it worked in this case:
We found a template Ab that does not bind human IL-2 but the platform predicted it has a good complementarity to the desired surface on IL-2.

Step 2

In silico design



Positions predicted to enhance affinity to the desired epitopes

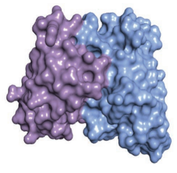
How the immune system does it:
Once the template is selected, somatic hypermutations are introduced to enhance the affinity of the template Ab to the target epitope.

How the Biolojic platform does it:
Machine learning algorithms trained on billions of human somatic hypermutations predict which positions should be mutated in the template Ab, and what they may be changed to, in order to precisely bind the target epitope with high affinity. We screen a library with these suggestions and train a model to predict which sequence will do the job.

How it worked in this case:
These algorithms suggested variations in three positions in the template Ab (marked in yellow).

Step 3

In vitro evolution and computational monitoring



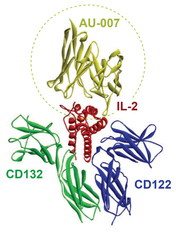
AU-007: High affinity human sequence that binds precisely where desired

Just three mutations in the selected positions sufficed to turn the non-binding template Ab into a tight binder of IL-2 that binds exactly where we wanted.

We incorporated the paratope into a fully human VDJ to guarantee the humanness of the Ab, its stability and developability.

Developable human antibody

Developable human antibody with desired effect



AU-007 blocking access of IL-2 to CD25

The resulting Ab has low pM affinity to IL-2, completely blocks the binding to CD25 but does not hinder the binding to CD122 and CD132. It is extremely stable and has high yield of >5g/L.

Template selection tool:

Input: Sequence of an existing Ab; Desired epitope on the target

Machine Learning models trained on:
- Known Ab-Ag interfaces, Ab-Ag pairs and residue-residue contacts between known paratopes and epitopes

Output: Interface between the Ab and epitope does not look like native interfaces / Interface between the Ab and epitope is similar to native, cognate interfaces

Humanness and developability tool:

Input: Ab sequence

Machine Learning models trained on:
- Millions of human Abs
- Experimental data on the effect of point mutations on stability and expression

Output: Human sequence, with good developability

Somatic hypermutations tool:

Input: Epitope-paratope pair

Machine Learning models trained on:
- Billions of human SHMs in different Abs
- Experimental data on the effect of point mutations on binding

Output: 1. Which positions in the input Ab should be altered to enhance affinity of Ab to epitope? 2. What may they be changed to?

AU-007 demonstrates strong anti-cancer activity in MC38 colorectal model

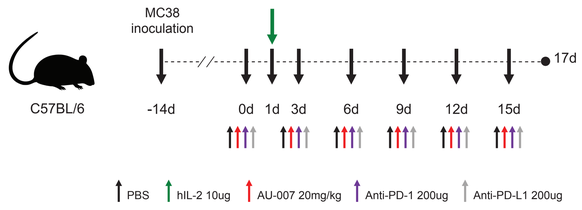
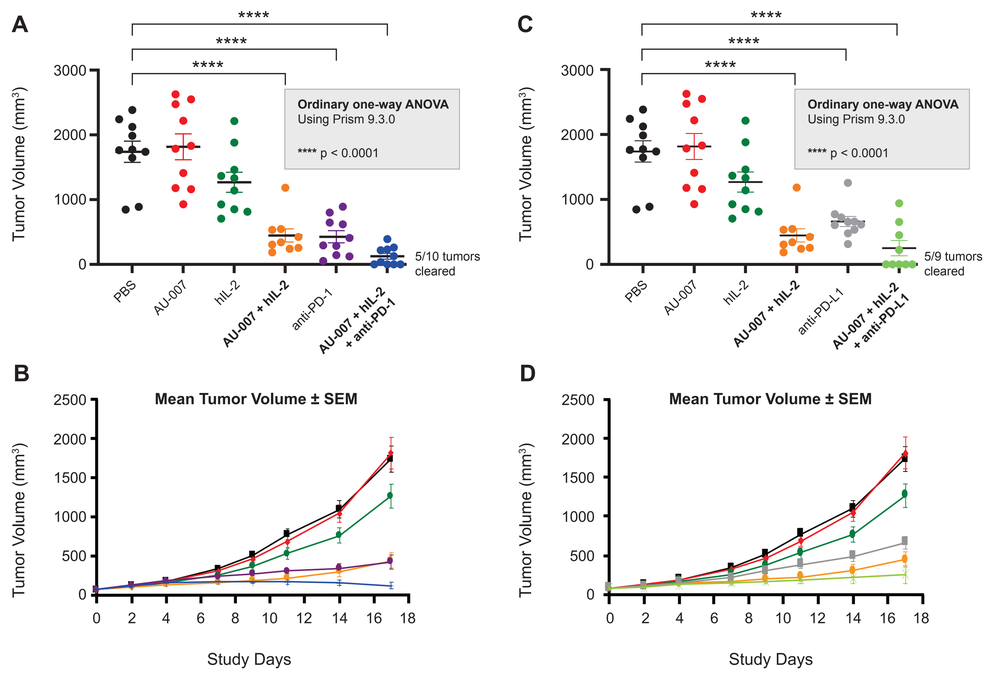


Figure 1: Treatment groups
- **Group 01**, PBS
- **Group 02**, AU-007
- **Group 03**, hIL-2
- **Group 04**, ***AU-007 + hIL-2***
- **Group 05**, anti-PD-1
- **Group 06**, ***AU-007 + hIL-2 + anti-PD-1***
- **Group 07**, anti-PD-L1
- **Group 08**, ***AU-007 + hIL-2 + anti-PD-L1***

Methods: C57BL/6 mice were inoculated with 1x10^6 MC38 mouse colorectal tumor cells and allowed to grow for 2 weeks. On study day 0, mice were randomized to groups of 10 and began antibody treatment and were dosed every 3 days. Human IL-2 was administered on day 1 only. **Note: As AU-007 does NOT bind mouse IL-2, human IL-2 is required to be given in these studies.** Treatment groups are in Fig. 1.
Tumor volumes were measured on days 0, 2, 4, 7, 9, 11 ,14, and at termination. Study was terminated at day 17 as the tumor volumes in the hIL-2 GEM mice control arms obtained ethical limits.

Figure 2: Efficacy of AU-007/IL-2 alone or in combination with immune checkpoint treatment of MC38 in C57BL/6 mice. **A:** terminal tumor volumes in anti-PD-1 cohorts **B:** growth curves for anti-PD-1 cohorts **C:** terminal tumor volumes in anti-PD-L1 cohorts **D:** growth curves for anti-PD-L1 cohorts. Note that the same control arms are in A/B as in C/D. Color coding matches color coding in methods above.



Conclusion: The results of this study clearly demonstrate AU-007+ a single boost of IL-2 can induce tumor growth inhibition in the MC38 colorectal model. This was not observed with IL-2 alone. In addition, in combination with anti-PD-1 or anti-PD-L1, tumors were cleared from mice (i.e., no measurable tumors in 5/10 in combination with anti-PD-1 and 5/9 in combination with anti-PD-L1). This was not observed with anti-PD-1 or anti-PD-L1 alone, though both single agents did have anti-tumor effect with 3/10 beginning to regress with anti-PD-1. No regressions or tumor clearance were observed with anti-PD-L1 alone.

Conclusion: AU-007 plus a single loading dose of hIL-2 (added because AU-007 does not cross-react with mouse IL-2) significantly inhibited MC38 colorectal tumor growth relative to controls, including single dose hIL-2. Additionally, strong anti-tumor activity was demonstrated with AU-007 plus a single loading dose of hIL-2 plus a checkpoint inhibitor (PD-1 antibody or PD-L1 antibody). The majority of mice that received AU-007 plus a loading dose of hIL-2 plus a checkpoint inhibitor demonstrated tumor regressions, and several complete responses with full tumor elimination were achieved. These data strongly support AU-007's unique mechanism of action as an antibody that can redirect IL-2 toward effector cells that exert anti-cancer activity, and away from immunosuppressive Tregs and vascular endothelium. Preparations are underway to advance AU-007, likely the first computationally designed human antibody to enter clinical development, into a Phase 1/2 trial in solid tumor indications.