Engineering targeted IL-7 delivery to enhance anti-tumor T cell fitness and persistence

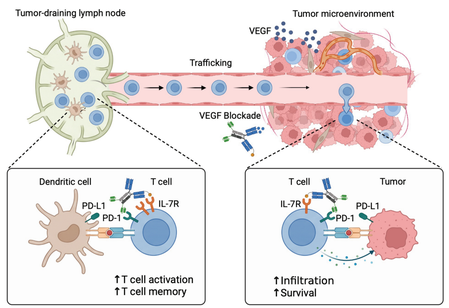
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Abstract #810

Introduction

PD-1 blockade is now the standard of care across multiple cancer indications, however, many patients fail to respond, highlighting the necessity of novel strategies to overcome resistance. Cytokines can complement T cell responses by providing signals that shape anti-tumor immunity. IL-7 is a homeostatic cytokine that sustains persistence of T cell memory and could improve the durability of anti-tumor responses. Despite being well tolerated, clinical use of IL-7 in cancer has been limited by systemic expansion of non-tumor-specific T cells. In order to target IL-7 to tumor-specific T cells, we incorporated IL-7 into a PD-1 x VEGF multispecific antibody for PD-1-mediated targeting, and engineered IL-7 via masking and potency attenuation to limit cytokine activity on bystander T cells.



AI-Driven Design Combined with Automated Workflow Accelerates Development of IL-7 Immunocytokine

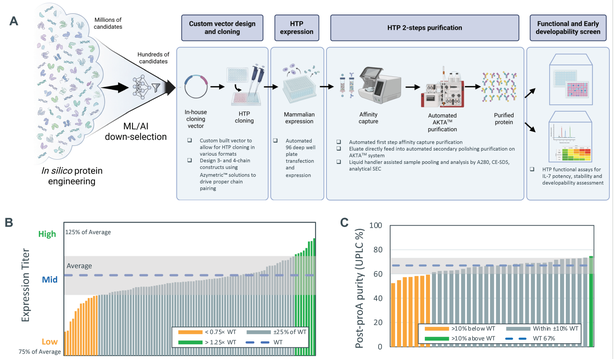
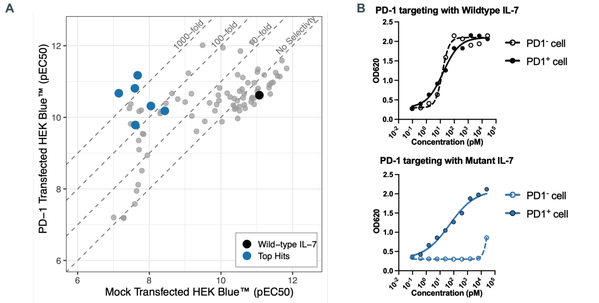


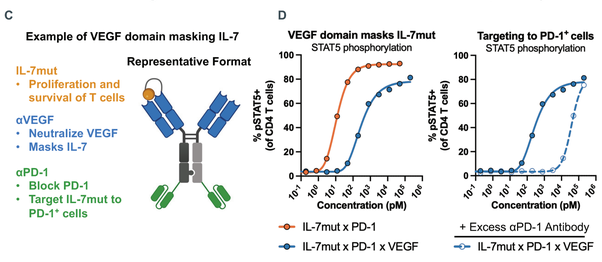
Figure 1. AI-driven high throughput multispecific antibody screening workflow. (A) AI-driven design and automation incorporated into a HTP workflow for the development of IL-7 x PD-1 x VEGF immunocytokine. **(B)** Expression titer of IL-7 x PD-1 x VEGF immunocytokine library. **(C)** Purity of samples post protein A purification.

Attenuation of IL-7 Potency and Selection of Optimal Geometry Enables Targeting to PD-1⁺ T cells

Attenuation of IL-7 potency is required for *cis*-targeting to PD-1⁺ cells



VEGF binding domain acts as mask for IL-7 and attenuates potency



Systematic evaluation of IL-7 placement to enhance PD-1 selectivity window

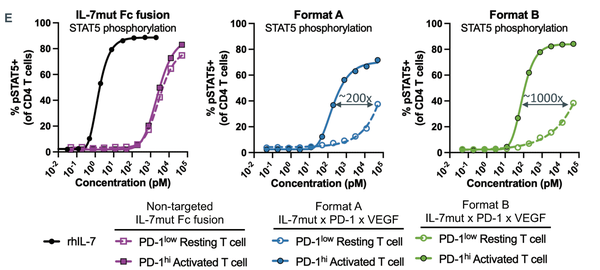


Figure 2. Attenuation of IL-7 potency via mutations and selection of optimal geometry enables targeting to PD-1⁺ T cells. (A) Select IL-7 mutations in an IL-7 x PD-1 x VEGF multispecific format were evaluated for IL-7 activity using mock and PD-1-transfected HEK Blue™ IL-7 reporter cells. **(B)** Example of PD-1 targeted IL-7 with wildtype and mutant cytokine. **(C)** Illustrative example of VEGF binding domain used to mask IL-7. **(D)** IL-7mut x PD-1 bispecific and IL-7mut x PD-1 x VEGF trispecific were evaluated for STAT5 phosphorylation in T cells with or without pre-treatment of excess αPD-1 antibody. **(E)** T cells activated with CD3/CD28 Dynabeads™ to induce high PD-1 expression or resting T cells with low PD-1 expression were treated with test articles and measured for pSTAT5.

Evaluating Combinations of Paratope and Geometry for Optimal PD-1 and VEGF blockade

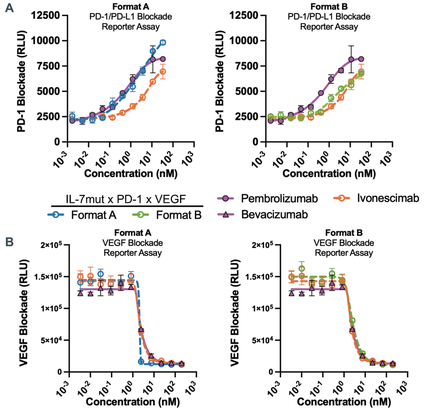


Figure 3. Placement and geometry of IL-7 can impact PD-1 blockade activity. (A) Two different IL-7mut x PD-1 x VEGF multispecific formats and controls were evaluated for PD-1 blockade using PD-1⁺ Jurkat NFAT-RE effector cells co-cultured with PD-L1⁺ CHO-K1 cells.

(B) VEGF blockade was measured in a bio-luminescent reporter assay. Test articles were pre-incubated with 2 nM VEGF-165 before adding to HEK293 KDR/NFAT-RE cells. Luminescence was read after incubation for 5 hours in both assays.

Complexing with VEGF Enhances PD-1 blockade and IL-7 Potency

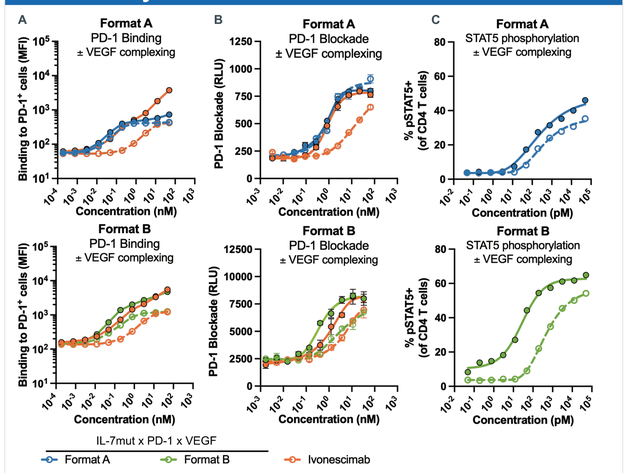


Figure 4. Complexing with VEGF results in enhanced PD-1 blockade and IL-7 potency. (A) Test articles were mixed with VEGF-165 at a 1:2 ratio and binding to PD-1⁺ Jurkat cells were determined by flow cytometry. **(B)** Test articles in the presence of VEGF-165 were used to evaluate PD-1 blockade activity with a bioluminescent reporter assay. **(C)** Primary T cells were activated with CD3/CD28 Dynabeads™ to express PD-1, and test articles incubated with VEGF-165 were used to determine IL-7 signaling potency via pSTAT5 staining by flow cytometry.

IL-7 x PD-1 x VEGF Reinvigorates Dysfunctional T cells while Promoting T cell Memory and Survival

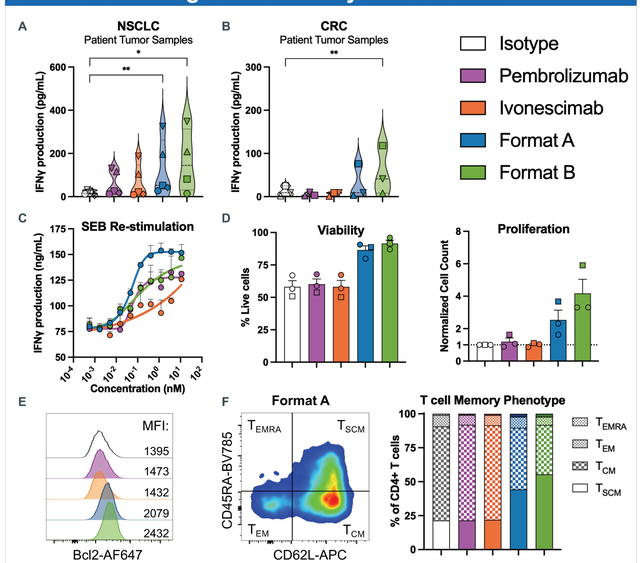


Figure 5. IL-7 x PD-1 x VEGF enhances T cell survival, function and fitness. (A) Dissociated tumor cells from NSCLC and **(B)** CRC patients were purchased from Discovery Life Sciences and treated with 100 nM test articles. After 48 hours, IFNγ production was determined by MSD. Each symbol denotes a different patient. **(C)** Healthy donor PBMCs were re-stimulated with 0.5 ng/mL Staphylococcal enterotoxin B (SEB) in the presence of test articles and IFNγ was quantified. **(D)** Healthy donor T cells were activated with CD3/CD28 agonist TransAct™ in the presence of test articles for 12 days, and viability and cell number was determined by AO/PI. Proliferation was normalized to isotype control **(E)** Bcl-2 and **(F)** T cell memory markers on CD4 T cells was measured by flow cytometry after CD3/CD28 activation in the presence of test articles. Statistical analysis by repeated-measures mixed effects model with Šídák's correction. *P < 0.05, **P < 0.01 (adjusted).

Conclusions

- Combination of selected IL-7 mutation with masking strategies in an IL-7mut x PD-1 x VEGF multispecific enables for approximately 200 to 1000-fold selectivity towards PD-1⁺ T cells
- Format of IL-7mut x PD-1 x VEGF is key for optimal PD-1 blockade and enhanced functional activity when complexing with VEGF
- IL-7mut x PD-1 x VEGF can restore function to exhausted T cells in patient tumor samples

*Ivonescimab, Pembrolizumab and Bevacizumab were produced in-house.

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