ANV700 is a novel proximity-activated IL-21 receptor agonist designed to selectively reinvigorate tumor-reactive PD-1 expressing T cells

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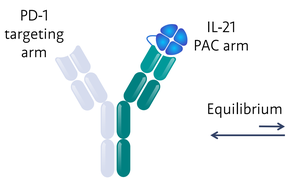
Background

Selective delivery of IL-21 to PD-1⁺ cells through a Proximity-Activated Cytokine (PAC)

IL-21 has the potential to increase the cytotoxicity and maintain survival of tumor reactive CD8⁺ T cells in chronic antigen exposure settings, such as cancer. Clinical studies with recombinant IL-21 showed promising therapeutic benefit, which was limited by dose-limiting toxicity and immunosuppressive effects on antigen-presenting cells. ANV700 addresses these limitations by targeting a novel proximity-activated IL-21 specifically to PD-1-expressing cells. This approach aims to reinvigorate tumor-specific T cells while minimizing off-target effects.

Cytokine inactive

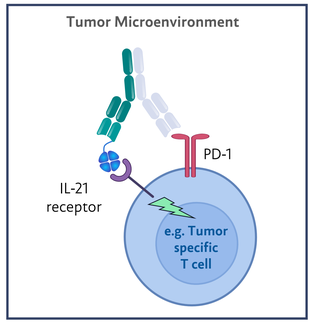


In the **bound state** the antibody prevents cytokine binding to its receptor and keeps it in an **inactive state**

Cytokine active

In the **free state** the cytokine can bind to the receptor and elicit an activating **cytokine signal**

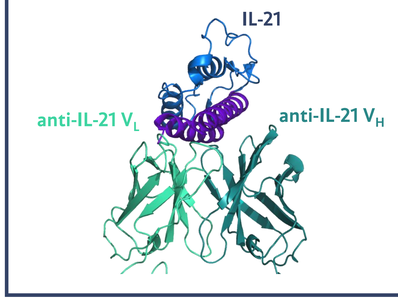
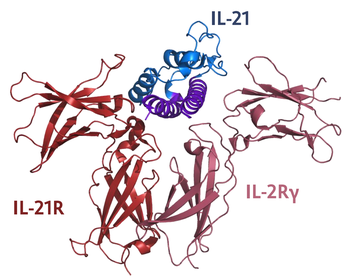
PAC signalling



Methods

Generation of the IL-21 PAC fusion protein

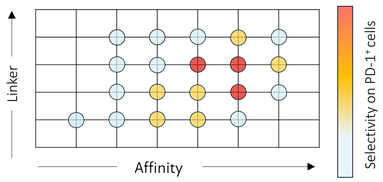
An anti-IL-21 antibody blocking the interaction of IL-21 and its receptor complex (IL-21R and IL-2Rγ) was selected. The crystal structure of the IL-21/anti-IL-21 Fab revealed that the antibody binds primarily to helices A and D (purple) of IL-21, which are key regions involved in IL-21R binding.

ANV700 anti-IL-21 mAb / IL-21 complex	IL-21 / IL-21R complex
	

Crystal structure of recombinant IL-21 and ANV700 αIL-21 Fab solved at 2.25 Å. IL-21 signaling complex structure from PDB 8ENT.

PAC optimization by engineering of anti-IL-21 mAb binding affinity and linker

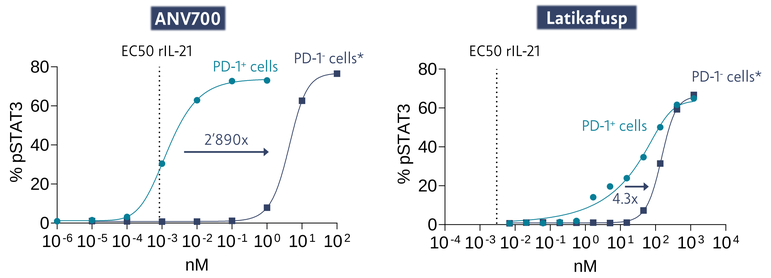
The anti-IL-21 antibody was fused to the IL-21 polypeptide by an amino acid linker. The linker composition and anti-IL-21 antibody affinity to IL-21 were engineered to obtain the optimal PAC compound with high selectivity for PD-1 expressing cells and favorable developability characteristics.



Results

High signaling selectivity of ANV700 for PD-1⁺ cells

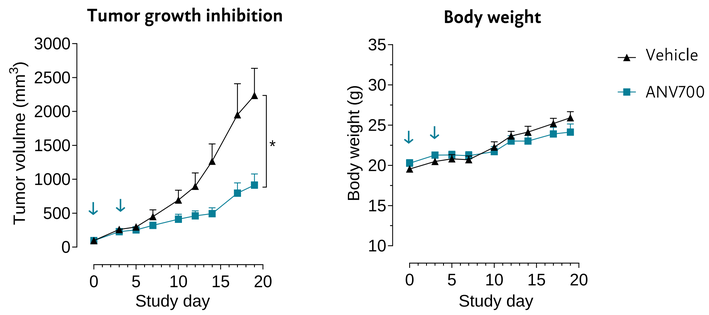
The PAC design of ANV700 strongly attenuates STAT3 phosphorylation on PD-1⁻ Jurkat cells, while maintaining potent signaling on PD-1⁺ Jurkat cells at a level comparable to recombinant IL-21. In contrast, the clinical stage PD-1 targeted IL-21 mutein (Latikafusp) shows strong attenuation of signaling on both PD-1⁺ and PD-1⁻ cells.



**PD-1 targeting prevented with parental anti-PD-1 mAb*

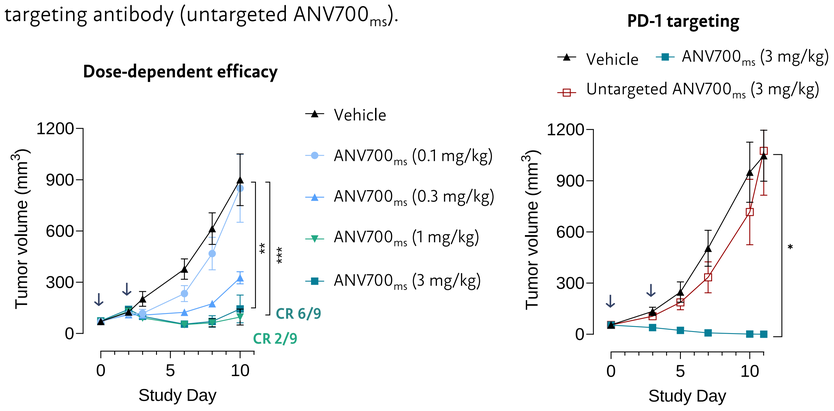
ANV700 monotherapy induces tumor growth inhibition *in vivo*

In human IL-21R and human PD-1 transgenic mice, ANV700 (↓) induces strong tumor growth retardation in the MC38 subcutaneous (s.c.) tumor model compared to treatment with vehicle control. ANV700 treatment is well tolerated at 3mg/kg, indicating that systemic toxicity is minimized.



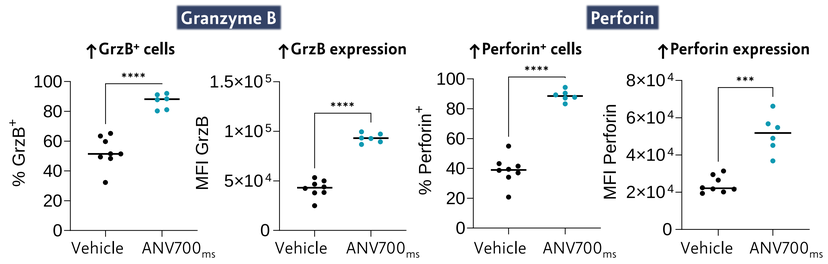
Anti-tumor efficacy of $ANV700_{ms}$ is dose-dependent and relies on PD-1 targeting

The ANV700 mouse surrogate compound ($ANV700_{ms}$) exhibits dose-dependent tumor growth inhibition in the MC38 s.c. model, achieving complete tumor responses at doses of greater than 1 mg/kg. The efficacy of $ANV700_{ms}$ is lost when using an irrelevant targeting antibody (untargeted $ANV700_{ms}$).



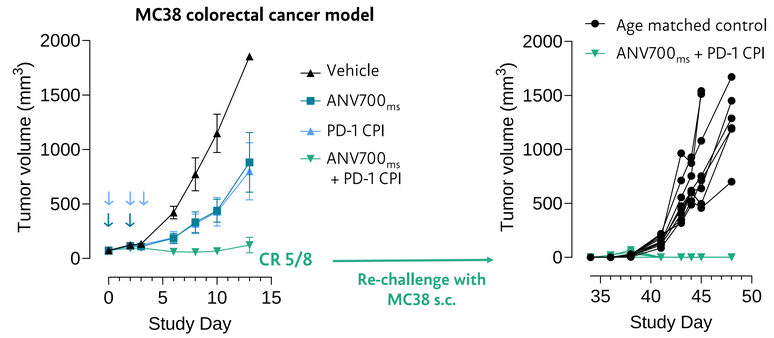
Enhanced cytotoxic effector function of CD8⁺ TILs in response to $ANV700_{ms}$

$ANV700_{ms}$ treatment of s.c. MC38 tumor bearing mice (1mg/kg) increases the number of Granzyme B (GrzB)⁺ and Perforin⁺ cytotoxic TILs (CD8⁺PD-1⁺TCF-1⁻CX3CR1⁺) as well as their expression levels of GrzB and Perforin.



$ANV700_{ms}$ synergizes with PD-1 checkpoint inhibitors

Single agent treatment with a suboptimal dose of $ANV700_{ms}$ (0.3mg/kg ↓) or PD-1 checkpoint inhibitor (CPI, 10mg/kg ↓) moderately delay tumor growth. Combination treatment induces cures (5 out of 8 mice) and protective memory immune responses.



Conclusions

- ANV700 is a novel Proximity-Activated antibody-cytokine fusion protein which delivers IL-21 to PD-1⁺ T cells re-invigorating tumor-specific T cells while minimizing off-target activation.
- The PAC design of ANV700 markedly attenuates IL-21 potency on PD-1⁻ cells and maintains close to native IL-21 potency on PD-1⁺ cells.
- By selectively enhancing effector T cell function of CD8⁺ PD-1⁺ cytotoxic exhausted T cells in MC38 s.c. tumors, $ANV700_{ms}$ treatment leads to marked tumor growth inhibition with no signs of systemic toxicity.
- Combination of $ANV700_{ms}$ with PD-1 inhibition results in synergistic efficacy, leading to complete tumor regression and durable anti-tumor memory.
- These promising preclinical findings support the exploration of ANV700 in clinical settings, both as a monotherapy or in combination with PD-1 inhibitors.