ANV600 is a novel PD-1 targeted IL-2Rβ/γ agonist that is combinable with therapeutic PD-1 inhibitors

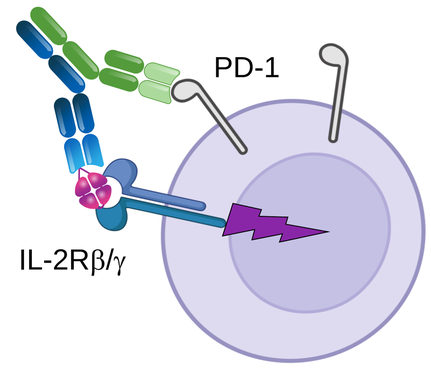
Patrizia Murer, Laetitia Petersen, Nicole Egli, Pia Neubert, Christian Stocker, Alexander Rau, Ulisse Salazar, Kirsten Richter, Andreas Katopodis and Christoph Huber
ANAVEON AG, Basel, Switzerland

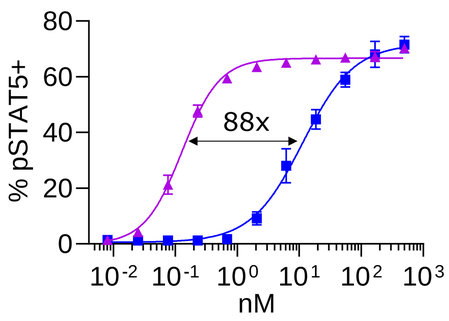
Background

ANV600 combines a unique non-blocking PD-1 targeting approach with an IL-2Rβ/γ selective agonistic principle The first arm of the bispecific antibody features an αIL-2/IL-2 fusion protein, which effectively prevents IL-2Rα from binding to the cytokine and therefore selectively activates IL-2Rβ/γ. The second arm consists of a high affinity αPD-1 antibody to selectively deliver the IL-2Rβ/γ agonist to tumor antigen experienced PD-1$^+$ T cells. The αPD-1 arm binds to a unique epitope on PD-1 that enables combination of ANV600 with PD-1 checkpoint inhibitors.

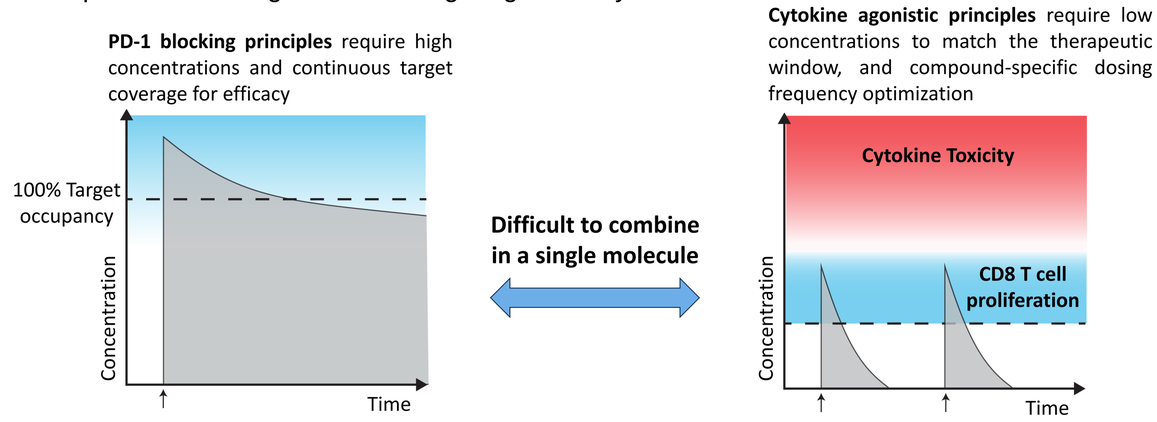
ANV600 anchoring to PD-1 increases IL-2R signaling potency on PD-1+ cells Potency measurements of STAT5 phosphorylation in PD-1+ Jurkat T cells demonstrate a strong PD-1 targeting effect of ANV600. Compared to a non-targeted IL-2Rβ/γ agonist control molecule, ANV600 has an 88-fold increased IL-2R signaling potency on PD-1 expressing cells.

ANV600

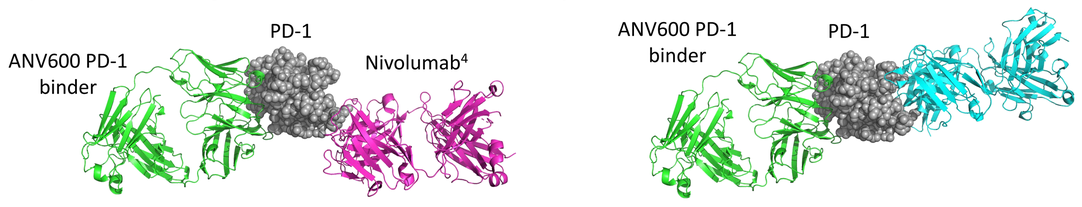




Cytokine agonism and PD-1 blockade are difficult to combine pharmacologically Dose/pharmacokinetic incompatibilities of PD-1 blockers and targeted cytokine agonists led ANAVEON to develop a non-blocking anti-PD-1 targeting antibody.

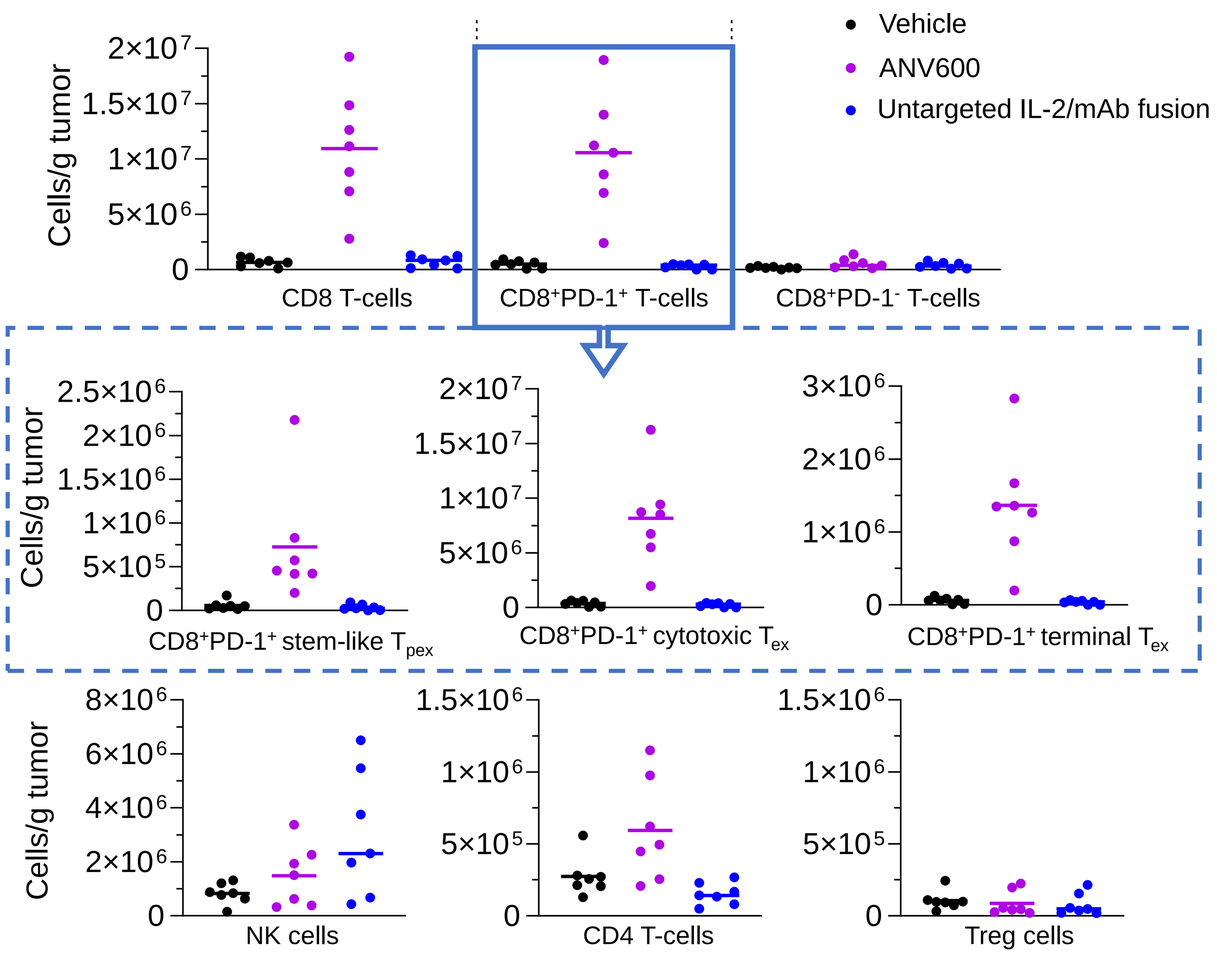


ANAVEON's targeting antibody binds a distinct epitope on PD-1 Modeling of the binding site of ANV600 to PD-1 and *in vitro* binding assays demonstrate that its PD-1 binding epitope does not compete with a range of established PD-1 checkpoint inhibitors.

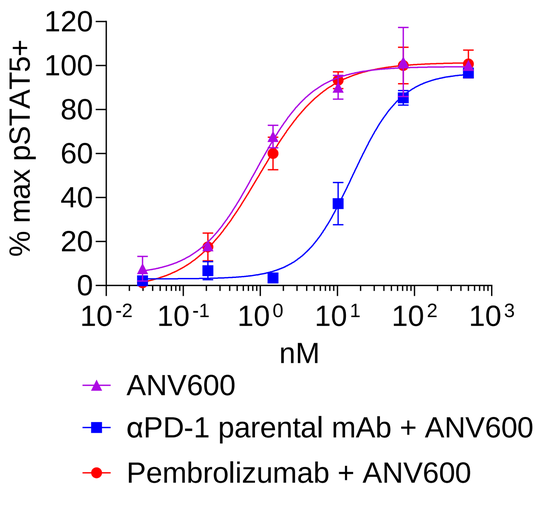


Results

ANV600 preferentially increases the number of PD-1+ CD8 T cells in B16F10 tumors and has no effect on infiltrating Treg cells [1] Compared to an untargeted bispecific IL-2/mAb fusion, ANV600 treatment of subcutaneous (s.c.) B16F10 tumor bearing human PD-1 (hPD-1) transgenic mice induces expansion of intratumoral CD8+ T cells, which is driven by the expansion of PD-1+ stem-like (T_{pex}) and cytotoxic exhausted T (T_{ex}) cells. The number of tumor infiltrating NK cells and CD4+ T cells is slightly increased, no changes in Treg cells are observed.

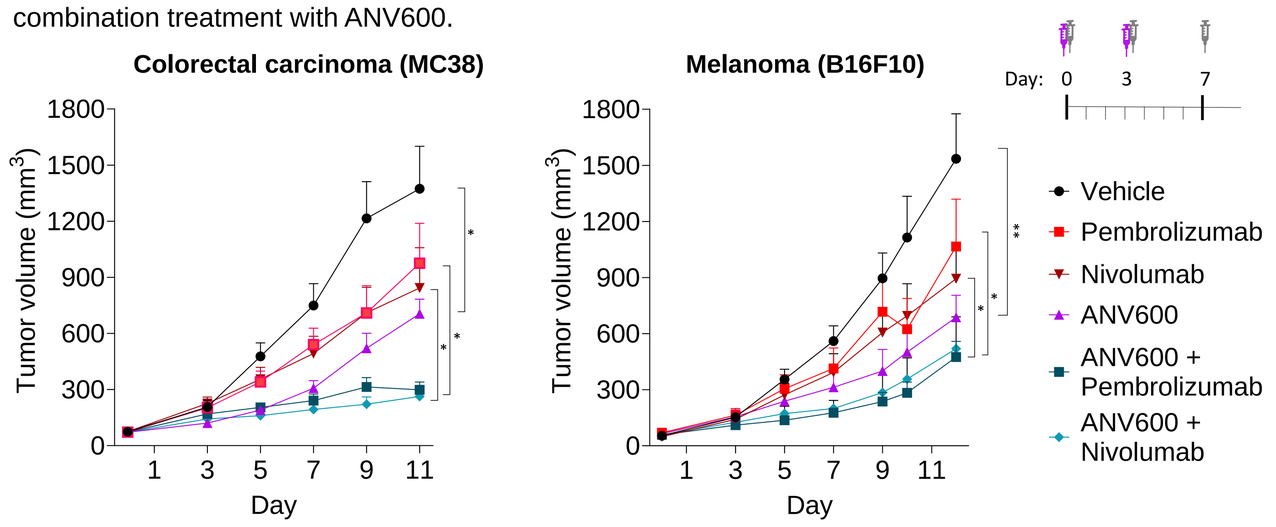


The potency of ANV600 on PD-1+ Jurkat cells in presence of PD-1 blocking antibodies remains unchanged[2] Pre-incubation of PD-1+ Jurkat cells with saturating amounts of pembrolizumab, nivolumab or other existing PD-1 checkpoint inhibitors does not reduce the ANV600 potency in inducing STAT5 phosphorylation. By contrast, pre-incubation of the target cells with the parental αPD-1 antibody of ANV600 markedly reduces IL-2R signaling.

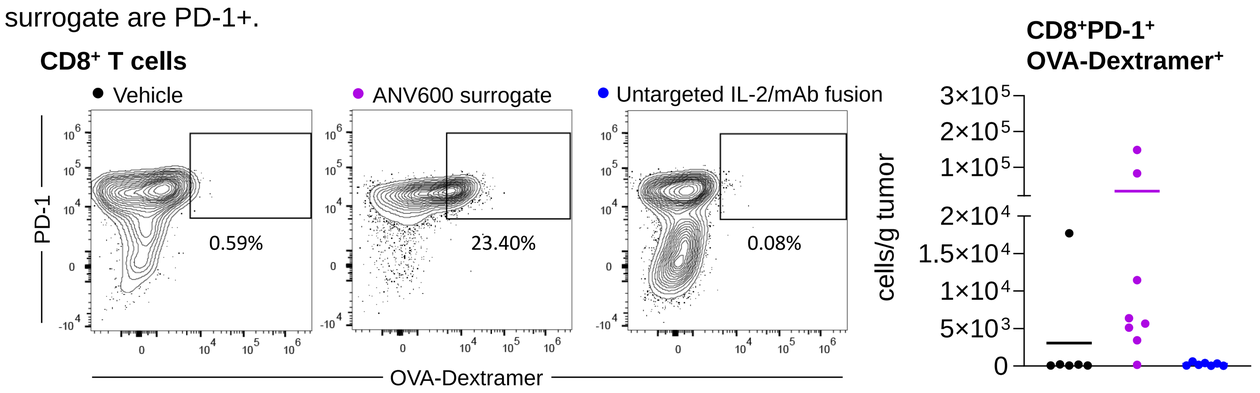


Compound	fold reduction ANV600 potency	Combinable with ANV600
αPD-1 parental mAb	18.19	N/A
Pembrolizumab	1.20	Yes
Nivolumab	1.28	Yes
Cemiplimab	1.95	Yes
Dostarlimab	1.12	Yes
Zeluvalimab	1.03	Yes
Tislelizumab	1.72	Yes
Ezabenlimab	2.00	Yes
Toripalimab	0.77	Yes
Cetrelimab	0.89	Yes

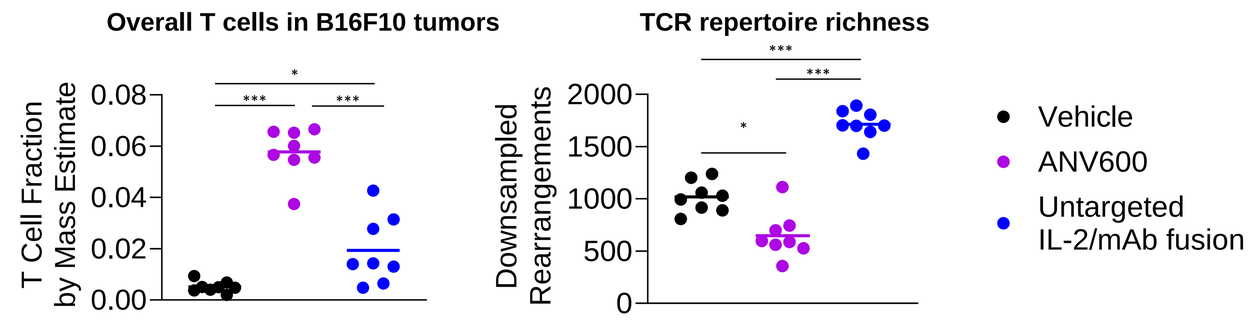
ANV600 enhances anti-tumor efficacy of pembrolizumab and nivolumab in mouse models of cancer[3] In transgenic hPD-1 mice ANV600 monotherapy (↓0.2 mg/kg) induces strong tumor growth retardation in the MC38 and B16F10 s.c. tumor models. The moderate inhibitory effect on tumor growth of the PD-1 inhibitors pembrolizumab and nivolumab (↓10mg/kg) is significantly increased by combination treatment with ANV600.



ANV600 mouse surrogate expands neoantigen specific CD8+PD-1+ tumor infiltrating lymphocytes[1] compared to vehicle or non-PD-1 targeted αIL-2/IL-2 fusion in C57BL/6 mice bearing s.c. B16F10-OVA tumors. All OVA-specific CD8+ T cells in tumors of mice treated with ANV600 mouse surrogate are PD-1+.



ANV600 expands a focused T cell repertoire dominated by few specific clones[1]
T cell receptor beta (TCRβ) chain sequencing of tumor infiltrating lymphocytes from s.c. B16F10 tumor bearing hPD-1 mice confirms the overall increase of intratumoral T cells upon ANV600 treatment. While the TCR repertoire is enriched in tumors with untargeted IL-2, ANV600 expands a restricted subset of TCR specificities of likely tumor-antigen specific clones.



Unpaired Wilcox Test used for statistical analyses

Conclusions

- ANV600 is a PD-1 targeted IL-2Rβ/γ agonist that can be combined with PD-1 blocking antibodies.
- The potency of ANV600 in inducing STAT5 phosphorylation in PD-1$^+$ cells is strongly increased compared to an untargeted IL-2Rβ/γ agonist and is not influenced by the presence of therapeutic PD-1 antibodies.
- ANV600 monotherapy in mice bearing poorly immunogenic tumors leads to significant increase in CD8+PD-1+ effector T cells and to strong inhibition of tumor growth.
- Combination treatment with ANV600 and PD-1 blockers at independent/compound-appropriate doses leads to further significant gains in efficacy in two mouse models of cancer.
- Taken together our data suggests that ANV600 selectively promotes the expansion of a focused anti-tumor specific repertoire of T cells.
- Based on these preclinical results, clinical exploration of ANV600 as a monotherapy or in combination with anti-PD-1 therapy is planned.

Methods and References

1. For tumor infiltrating lymphocyte analyses by flow cytometry and for TCR sequencing, compounds were administered i.v. on study day 0 (tumor volumes 70-100mm^3) and day 3. Tumors were analyzed on day 5. Transgenic human PD-1 mice (C57BL/6N-*Pdcd1*tm$^{1.1(PDCD1)Geno}$) were provided by genOway.
2. Jurkat PD-1 cells were pre-incubated for 30 min with 200nM of PD-1 blocking or parental anti-PD-1 antibody. Without washing ANV600 was added for 15 min at 37°C. After fixation, the cells were analyzed by flow cytometry.
3. For tumor efficacy studies C57BL/6N-*Pdcd1*tm$^{1.1(PDCD1)Geno}$ mice were administered ANV600 i.v. on study day 0 (tumor volumes 70-100mm^3) and day 3 and αPD-1 antibodies were injected i.p. at 10mg/kg on days 0, 3 and 7. Tumor volumes are plotted + SEM. n=9 (MC38) and n=10 (B16F10) at study start. *: p ≤ 0.05; **: p ≤ 0.01; ***: p ≤ 0.001; ****: p ≤ 0.0001. One-way ANOVA multiple comparison against Vehicle, pembrolizumab or nivolumab treatment.
4. Lee J. et al. Nat Commun 7, 13354 (2016).