ANV600 is a potent, *cis*-signaling, IL-2Rβ/γ directed IL-2 which efficiently expands intratumoral stem-like CD8 T cells

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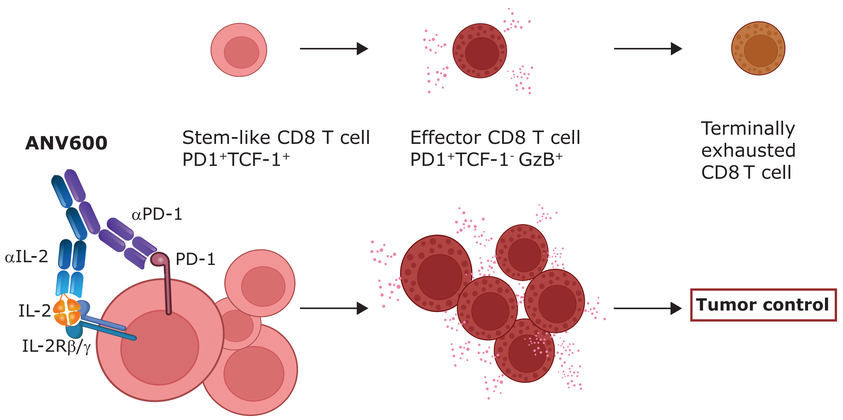
Background

ANV600 is a novel PD-1 targeted, IL-2Rβ/γ directed IL-2

ANV600 consists of a proprietary PD-1 binding moiety and an IL-2Rβ/γ directed interleukin-2/anti-IL-2 fusion protein, thus targeting the cytokine to PD-1 expressing T cells. The IL-2 agonist arm of ANV600 includes an anti-IL-2 antibody with high affinity to the IL-2Rα binding domain of IL-2. The cytokine is directly fused to the light chain of the antibody through a peptide linker, allowing ANV600 to present IL-2 to the dimeric β/γ IL-2 receptor on CD8 PD-1⁺ T cells, while sterically excluding binding to the high affinity trimeric α/β/γ IL-2 receptor.



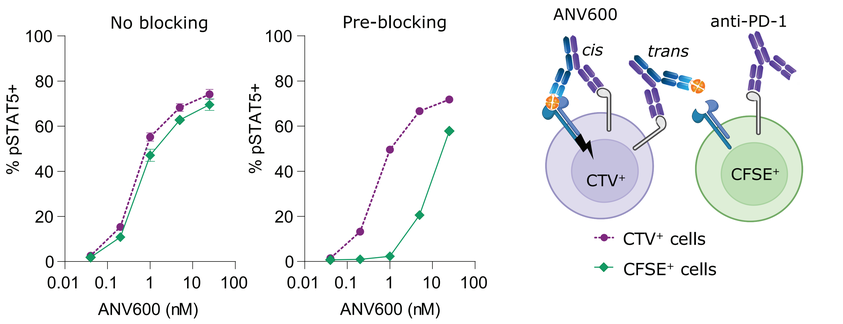
Adapted from Siddiqi I., et al. Immunity, 2019 with Biorender.com

In the tumor microenvironment, the pool of PD-1⁺ T cells is primarily composed of tumor antigen experienced cells. ANV600 potently and selectively proliferates tumor specific PD-1⁺ stem-like CD8 T cells and effector cells, and markedly reduces tumor growth in poorly immunogenic syngeneic mouse tumor models.

Results

ANV600 signals preferentially in *cis* on PD-1⁺ Jurkat cells

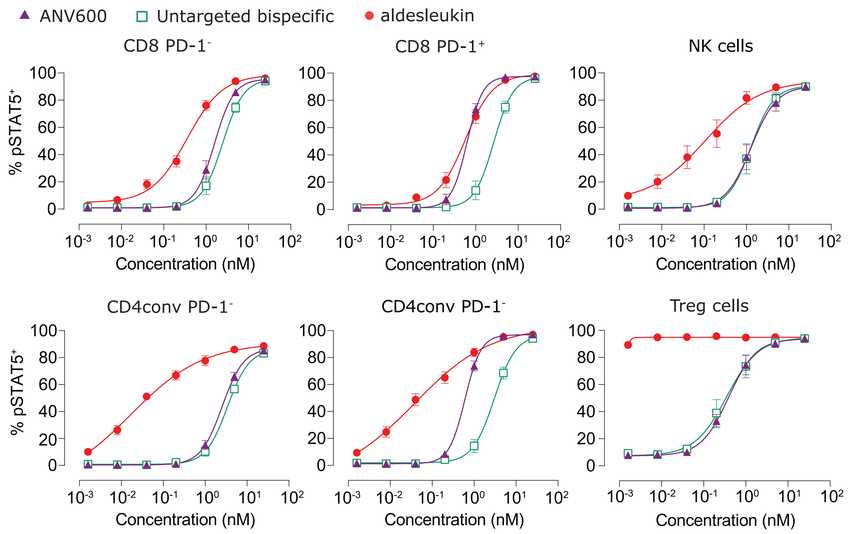
ANV600 enhances STAT5 phosphorylation in *cis*, when PD-1 and the IL-2Rβ/γ are expressed on the same cell, but not in *trans* when PD-1 binding is blocked. This suggests that PD-1 targeting of the ANV600 IL-2 cytokine enhances its ability to bind to the IL-2Rβ/γ on the same cell.



Jurkat PD-1 expressing cells were labeled with either CFSE or CTV. CFSE labeled cells were then pre-incubated with the parental anti-PD-1 mAb of ANV600. Both CTV and CFSE labeled cells were mixed at a 1:1 ratio, stimulated with ANV600 and pSTAT5 was measured by flow cytometry.

ANV600 anchoring to PD-1 increases its potency for STAT5 phosphoryation in PD-1 expressing primary human T cells

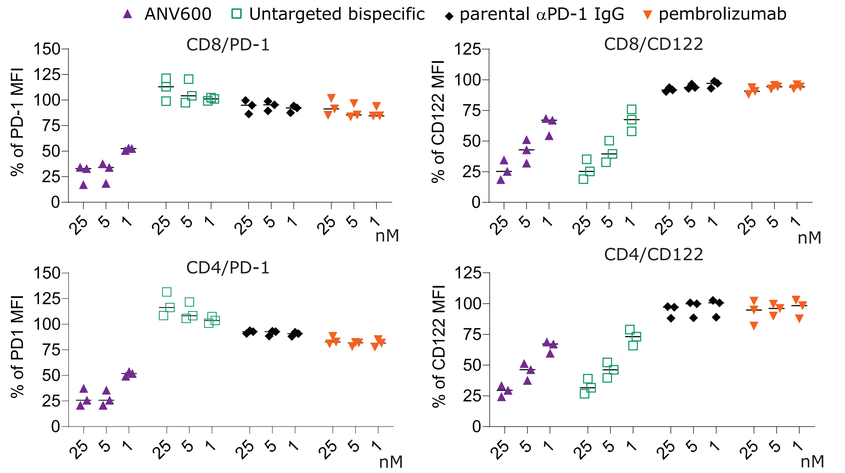
Consistent with a *cis*-signaling mode of action, ANV600 is more potent towards PD1⁺ vs. PD1⁻ CD8⁺ and CD4⁺ T cells in human PBMCs. ANV600 is equipotent to aldesleukin in inducing STAT5 phosphorylation in PD-1⁺ CD8 T cells, but has markedly reduced potency towards NK cells, Treg cells and PD-1⁻ T cells.



Human PBMCs were incubated with ANV600, untargeted bispecific, or IL-2 (aldesleukin). Phosphorylation of STAT5 was measured in PD-1⁺ and PD-1⁻ T cells, NK cells, and Tregs by flow cytometry (n=6 donors).

ANV600 decreases surface PD-1 levels on human T cells

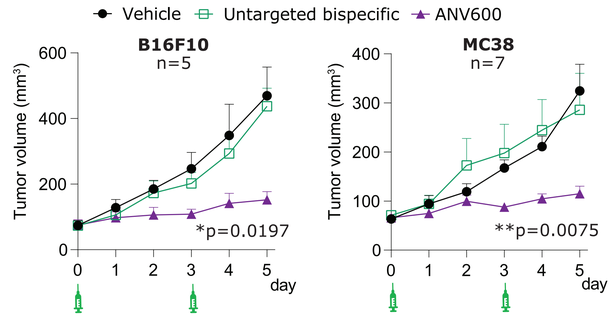
Incubation of ANV600 with activated human PBMCs leads to a concentration-dependent simultaneous decrease of cell surface PD-1 and IL-2Rβ (CD122), possibly induced by receptor co-internalisation. The untargeted IL-2Rβ/γ directed IL-2 control reduces only surface CD122, while the parental anti-PD-1 IgG control or pembrolizumab result in a minimal reduction of surface PD-1.



PD-1 expression was induced in human PBMCs by anti-CD3/anti-CD28 activation for 48h. Activated cells were then incubated with ANV600 or control compounds and surface PD-1 and CD122 expression were determined by flow cytometry (n=3 donors).

ANV600 treatment leads to tumor growth inhibition in poorly immunogenic mouse models of cancer

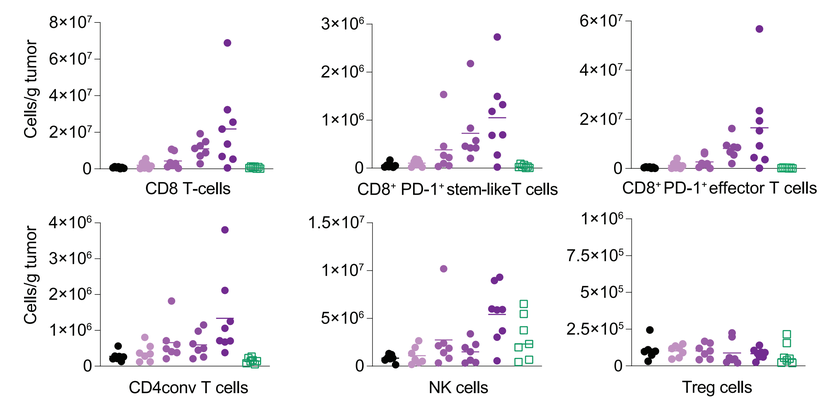
In transgenic human PD-1 (hPD-1) C57BL/6 mice ANV600 induces strong tumor growth retardation in the B16F10 and MC38 subcutaneous (s.c.) tumor models compared to vehicle or to treatment with untargeted bispecific carrying the IL-2Rβ/γ directed IL-2.



Mice were injected s.c. with tumor cells. Treatment was started when tumors reached 70-100mm³ volume. Compound was administered i.v. on study days 0 and 3 at 0.2 mg/kg.

ANV600 increases the number of PD-1⁺ stem-like and effector CD8 T cells in B16F10 tumors and has no effect on infiltrating Treg cells

Compared to vehicle or untargeted bispecific, ANV600 treatment of s.c. B16F10 tumor bearing mice revealed a dose-dependent increase of intratumoral CD8 T cells, which was driven by the expansion of PD-1⁺ stem-like and effector T cells. The number of tumor infiltrating NK cells was increased at the highest dose of ANV600 and no changes in Treg cells were observed at any dose level.



hPD-1 mice bearing s.c. B16F10 tumors (70-100mm³) were injected i.v. with ANV600 or untargeted non-alpha fusion protein on study days 0 and 3. On day 5 mice were sacrificed and intratumoral lymphocytes were characterized by flow cytometry (n=7-8).

Conclusions

- ANV600 is a fusion protein targeting an IL-2Rβ/γ directed IL-2 to PD-1 expressing tumor antigen experienced T cells.
- Through *cis*-mediated signaling it has enhanced potency for STAT5 phosphorylation in PD-1⁺ CD8 and CD4 T cells and leads to decreased surface PD-1 levels.
- Treatment of hPD-1 mice bearing poorly immunogenic tumors with ANV600 leads to a significant increase in intratumoral antigen-experienced effector T cells and to strong tumor growth inhibition compared to vehicle or untargeted bispecific treatment.
- ANV600 may be a promising treatment against tumors that are resistant to current immunotherapies and is expected to enter clinical development in the near future.