Vectorized Treg-depleting αCTLA-4 elicits antigen cross-presentation and CD8⁺ T cell immunity to reject „cold“ tumors

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Abstract #746

1. Background

Treatment with checkpoint inhibitor antibodies results in long-lasting antitumor responses in a variety of cancers [1]. However, only a small fraction of patients responds to the treatment, probably due to inadequate tumor infiltration with immune cells. While combination therapy with anti-CTLA-4 and anti-PD-1 antibodies significantly improves efficacy, concerns with tolerability has limited wide-spread clinical use [2].

Here we present a potentially safe and more efficacious strategy to combine anti-CTLA-4 and anti- PD-1/PDL1 checkpoint inhibition in the context of oncolytic virotherapy. A Treg-depleting anti-CTLA-4 antibody has been vectorized alongside GM-CSF into the Invir.IO™ oncolytic Vaccinia virus (oVV) based platform. This product named BT-001 (VV_{GM}-αhCTLA4) consists of the Copenhagen oVV strain - deleted in J2R and I4L viral genes allowing restricted replication in proliferating cells - and the human CTLA-4-specific antibody 4-E03 IgG1, which shows improved Treg-depletion compared with ipilimumab.

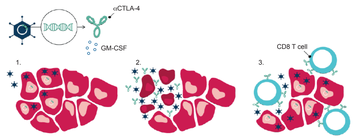


Figure 1. Local administration of BT-001 combines oncolytic activities with high intratumoral concentrations of anti-CTLA-4 antibody and GM-CSF eliciting a stronger antitumor response with improved safety profile.

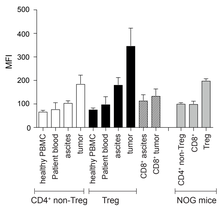


Figure 2. In primary patient material, CTLA-4 is highest expressed on intratumoral Treg cells which makes them a good target for Treg depleting antibodies.

2. Generation of BT-001 and mouse surrogate VV_{GM}-αCTLA4

Figure 3. Treg depleting and blocking anti-CTLA-4 mAb 4-E03

BioInvent's F.I.R.S.T. discovery platform [3] was used to isolate scFv antibody fragments recognizing human or mouse CTLA-4. Target-specific antibody clones were classified as actives, transferred to full-length IgG format, and further characterized biochemically and functionally. The antibody clone 4-E03 was chosen as candidate for vectorization in Copenhagen oVV. It blocks CD80/CTLA-4 and CD86/CTLA-4 interactions with the same potency as ipilimumab (a) but the Treg depleting activity is improved compared to ipilimumab as demonstrated in a PBMC-NOG/SCID transfer model in vivo (b). An anti-mouse CTLA-4 antibody (clone 5-B07, mouse IgG2a) with similar functional effects in ligand blocking and Treg depletion (data not shown) was selected for the generation of the murine surrogate virus VV_{GM}-αCTLA4 .

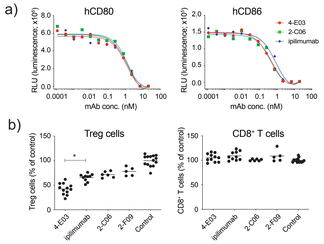
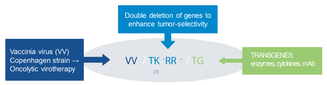
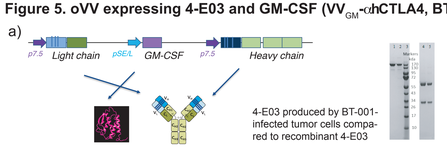


Figure 4. Oncolytic Vaccinia virus Copenhagen strain

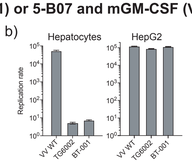


Copenhagen oVV strain (Invir.IO™ platform, Transgene)
- is deleted in J2R (TK locus) and I4L (RR locus) viral genes involved in nucleotide synthesis to restrict replication in proliferating cells
- allows large DNA insertions with successful vectorization of various expression cassettes
- has the best oncolytic activity among VACV strains
- induces Immune Cell Death

Figure 5. oVV expressing 4-E03 and GM-CSF (VV_{GM}-αhCTLA4, BT-001) or 5-B07 and mGM-CSF (VV_{GM}-αCTLA4)



4-E03 produced by BT-001-infected tumor cells compared to recombinant 4-E03

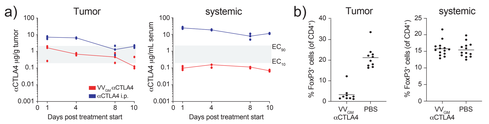


a) Anti-CTLA-4 mAb and GM-CSF were vectorized in Copenhagen oVV. Thereby deleted J2R and I4L genes were replaced by heavy and light chain of anti-CTLA-4, respectively. The expression cassette encoding GM-CSF was also placed at the I4L locus. b) BT-001 selectively replicated in tumor cells and not in normal cells, similar to the clinically validated oVV TG6002 (Transgene).

3. Anti-tumor activity in vivo

Figure 6. Vectorization in oVV allows intratumoral accumulation of transgenes with low systemic exposure

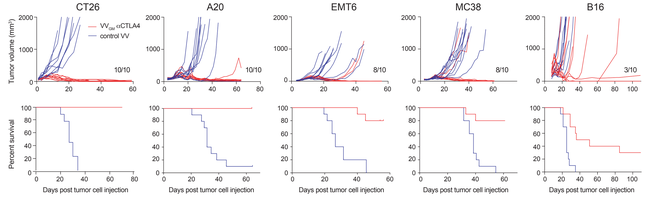
Amount of transgenes in tumor vs blood was measured for both anti-CTLA-4 mAb and GM-CSF (not shown) in syngeneic CT26 model.



a) Concentration of anti-mouse CTLA-4 mAb 5-B07 was monitored in syngeneic CT26 tumor model. CT26 colorectal tumor cells were implanted subcutaneously to Balb/c mice and virus was injected 3 times i.t. One injection intraperitoneally of 5-B07 at 3 mg/kg was used as benchmark.

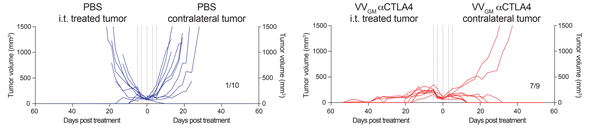
b) Intratumoral administration of VV_{GM}-αCTLA4 was associated with sustained intratumoral antibody exposure and intratumoral Treg depletion, systemic exposure was orders of magnitude lower and not associated with peripheral Treg depletion.

Figure 7. Improved survival after treatment with VV_{GM}-αCTLA4 in several syngeneic tumor models



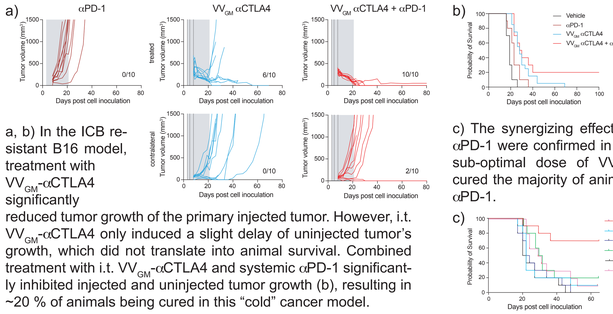
Antitumoral activity of Copenhagen oVV encoding an anti-mouse CTLA-4 and murine GM-CSF (VV_{GM}-αCTLA4) was assessed in different syngeneic tumor models after 3 i.t. administrations of 10^7 PFU. Results demonstrate broad and potent antitumor activity of VV_{GM}-αCTLA4 in immune inflamed and immune excluded models.

Figure 8. Intratumoral injection of VV_{GM}-αCTLA4 induces systemic antitumor immunity (abscopal effect)



Local, intratumoral administration of VV_{GM}-αCTLA4 induced complete tumor regression in contralateral uninjected CT26 tumors in 7/9 mice demonstrating the induction of systemic antitumor responses, or the abscopal effect. There was no evidence of viral particle dissemination to uninjected tumors as assessed by plaque assay (data not shown).

Figure 9. Combination of VV_{GM}-αCTLA4 with anti-PD-1 is beneficial

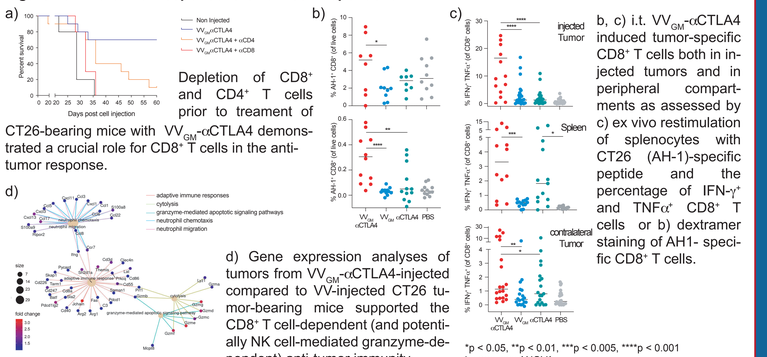


a, b) In the ICB resistant B16 model, treatment with VV_{GM}-αCTLA4 significantly reduced tumor growth of the primary injected tumor. However, i.t. VV_{GM}-αCTLA4 only induced a slight delay of uninjected tumor's growth, which did not translate into animal survival. Combined treatment with i.t. VV_{GM}-αCTLA4 and systemic αPD-1 significantly inhibited injected and uninjected tumor growth (b), resulting in ~20 % of animals being cured in this "cold" cancer model.

c) The synergizing effects of VV_{GM}-αCTLA4 and αPD-1 were confirmed in the A20 model where a sub-optimal dose of VV_{GM}-αCTLA4 (10^5 PFU) cured the majority of animals in combination with αPD-1.

4. Mode-of-action characterization

Figure 10. Anti-tumor response is CD8 T cell-dependent



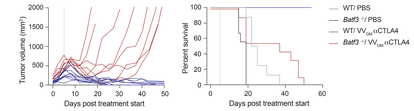
Depletion of $CD8^+$ and $CD4^+$ T cells prior to treament of CT26-bearing mice with VV_{GM}-αCTLA4 demonstrated a crucial role for $CD8^+$ T cells in the anti-tumor response.

d) Gene expression analyses of tumors from VV_{GM}-αCTLA4-injected compared to VV-injected CT26 tumor-bearing mice supported the $CD8^+$ T cell-dependent (and potentially NK cell-mediated granzyme-dependent) anti-tumor immunity.

b, c) i.t. VV_{GM}-αCTLA4 induced tumor-specific $CD8^+$ T cells both in injected tumors and in peripheral compartments as assessed by c) ex vivo restimulation of splenocytes with CT26 (AH-1)-specific peptide and the percentage of IFN-γ^+ and TNFα^+ $CD8^+$ T cells or b) dextramer staining of AH1- specific $CD8^+$ T cells.

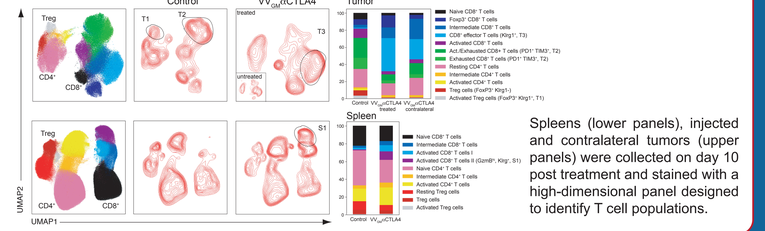
*p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 by one-way ANOVA

Figure 11. Intratumorally induced $CD8^+$ T cell anti-tumor immunity is cDC1-dependent



MC38 tumor-bearing WT and Batf3$^{-/-}$ C57BL/6 mice received i.t. injections of VV_{GM}-αCTLA4 or PBS. Graphs show tumor volume (left and center panels) and mouse survival (right panel). Vertical lines indicate end of the treatment.

Figure 12. I.t. VV_{GM}-αCTLA4 expands effector $CD8^+$ T cells and reduces Treg and exhausted $CD8^+$ T cells



Spleens (lower panels), injected and contralateral tumors (upper panels) were collected on day 10 post treatment and stained with a high-dimensional panel designed to identify T cell populations.

5. Conclusions

- BT-001 is a multifunctional oVV co-developed by Transgene and BioInvent that encodes a Treg-depleting αCTLA-4 antibody as well as the cytokine GM-CSF.
- Intratumoral delivery of a Vaccinia-Virus encoded anti-CTLA4 antibody achieved tumor-restricted exposure and Treg depletion.
- The murine surrogate VV_{GM}-αCTLA4 has demonstrated a robust antitumoral activity in several syngeneic tumor models. This antitumoral activity is $CD8^+$ T cell-dependent and synergized with αPD-1 treatment to reject cold tumors.
- A clinical study investigating i.t. VV_{GM}-αhCTLA4 (BT-001) alone and in combination with αPD-1 in metastatic or advanced solid tumors has been commenced (NCT04725331).

6. References

1. Sharma, P. and J.P. Allison, Science, 2015. 348(6230): p. 56-61.
2. Postow, M.A., et al., N Engl J Med, 2015. 372(21): p. 2006-17.
3. Soderlind, E., et al., Nat Biotechnol, 2000. 18(8): p. 852-6.
4. Arce Vargas, F., et al., Cancer Cell, 2018.
5. Kleinpeter, P., et al., Oncoimmunology, 2016. 5(10): p. e1220467.
6. Fend, L., et al., Cancer Res, 2017. 77(15): p. 4146-4157.
7. www.transgene.fr, www.bioinvent.se