Comprehensive preclinical studies of BT-001: an oncolytic vaccinia virus armed with Treg-depleting @CTLA4 and GM-CSF.

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ABSTRACT

Background
Immune checkpoint blockade (ICB) is a clinically proven concept to treat cancer. Still, a majority of cancer patients including those with poorly immune infiltrated "cold" tumors are resistant to currently available ICB therapies. **Oncolytic viruses** are natural immunomodulators able **to turn "cold" tumors "hot"** and, for some of them, to **deliver therapeutic payloads intratumorally**. Payloads of choice are those with tolerability and/or pharmacokinetic/biodistribution issues, such as **@CTLA4 antibodies and GM-CSF** respectively. Here, Transgene and BioInvent present a preclinical characterization of a highly efficacious and potentially safe strategy to target CTLA4 and GM-CSF using vectorization in an oncolytic vaccinia virus.

Methods
A novel human IgG1 CTLA4 antibody (4-E03) was identified using function-first screening for mAbs and targets associated with checkpoint blocking and superior Treg depleting activity. A tumor-selective **oncolytic Vaccinia vector** was then engineered to encode this **novel @CTLA4 as full-length antibody** (i.e. distinct transgenes for light and heavy chains) **and GM-CSF** (VV_{GM}-@hCTLA4, BT-001). Viruses encoding matching mouse surrogate payloads were additionally generated, enabling proof-of-concept studies in syngeneic immune competent mouse tumor models.

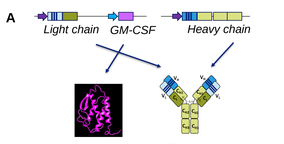
Results
Our studies demonstrate that two chains cloning approach allowed **to express @CTLA4 at a high level** in numerous infected tumor cell lines without affecting viral cycle. **Intratumoral (i.t.) administration** of VV_{GM}-@CTLA4 (mBT-001) **achieved tumor-restricted virus replication and CTLA4 receptor saturation** at least for 10 days, stronger systemic expansion of tumor-specific $CD8^+$ T cells and **antitumor immunity compared with systemic @CTLA4 antibody** or unarmed virus therapies. Remarkably, in a several syngeneic mouse models, mBT-001 given i.t. induced a strong anti-tumoral response, synergized with @PD-1 to reject tumors (data not shown) and induced an abscopal effect in a two-tumor model (data not shown).

Conclusion
Our findings demonstrate in vivo proof-of-concept for tumor delivery by Vaccinia virus of GM-CSF and strongly Treg-depleting, immune checkpoint blocking, vectorized @CTLA4. This vector-based delivery is a highly effective and safe strategy to target CTLA4 which overcomes current limitations of approved @CTLA4 regimens. **A clinical trial evaluating i.t. VV_{GM}-@hCTLA4 (BT-001) alone and in combination with @PD-1 in metastatic or advanced solid tumors has been initiated.**

BT-001 FEATURES

- → Copenhagen Vaccinia virus (VACV) attenuated by thymidine kinase and ribonucleotide reductase deletions
- → Encodes full length IgG1 blocking @CTLA4 (4-E03) with improved Treg depletion compared to Ipilimumab & GM-CSF
- → Murine surrogate (@mCTLA4 and mGM-CSF) virus generated for preclinical experiments in mice
- → Clinical trial ongoing with i.t. administrations in solid tumors

BT-001 encodes @CTLA4 heavy and light chains and GM-CSF

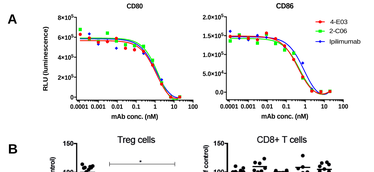


4-E03: Full length human IgG1

4-E03 produced by BT-001-infected tumor cells

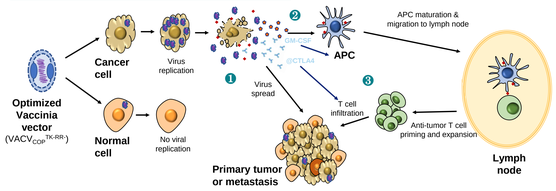
A) The heavy (HC) and light chains (LC) of the @CTLA4 are cloned separately in VACV genome to ensure the optimal expression, antibody assembly and genetic stability of virus.
B) The antibody produced by the tumor cells infected by BT-001 is indistinguishable of the benchmark 4-E03 mAb

4-E03 binds hCTLA4 and depletes human Treg cells



4-E03 is a new @hCTLA4 human IgG1 that blocks the CD80-CD86/CTLA4 interactions with the same IC50 as Ipilimumab (A) and depletes human Treg cells, but not $CD8^+$ T cells, in human PBMC transplanted mice (B) Of note, the Treg depletion potency of 4-E03 is significantly higher than the one of Ipilimumab in this xenograft model.

BT-001 MODE OF ACTION



❶ DIRECT VIRUS-MEDIATED TUMOR CELL LYSIS
Immunogenic cell death, release of tumor antigens, DAMPs, & PAMPs
+
❷ RELEASE OF TRANSGENE PRODUCTS IN TME
↗ T Cell eff - ↘ T Reg
Stimulation of immune cells (incl APC)
+
❸ STIMULATION OF ANTI-TUMOR IMMUNITY

= STRONG ANTI-TUMOR ACTIVITY

REFERENCES

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- Arce Vargas et al. Cancer Cell. 2018 Apr 9;33(4):649-663
- Foloppe *et al.* Mol Ther Oncolytics. 2019 Mar 27;(14):1-14.
- WO2020/049001 Novel Antibodies and nucleotides sequences, and uses thereof
- WO2009/065546 Poxviral Oncolytic Vectors
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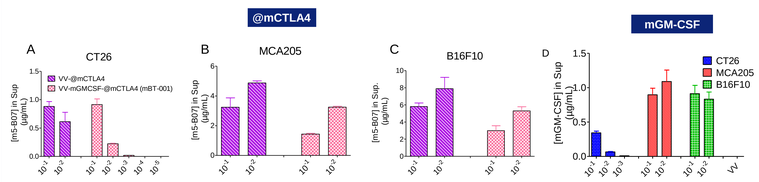
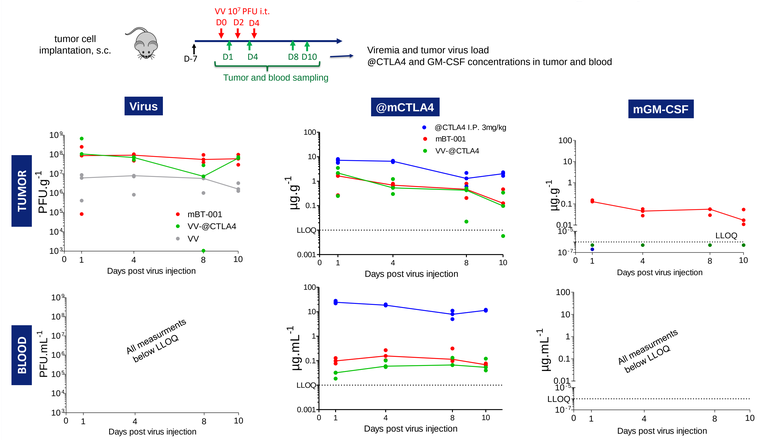
RESULTS

1: @CTLA4 mAb and GM-CSF are expressed at high levels by mBT-001-infected tumor cells



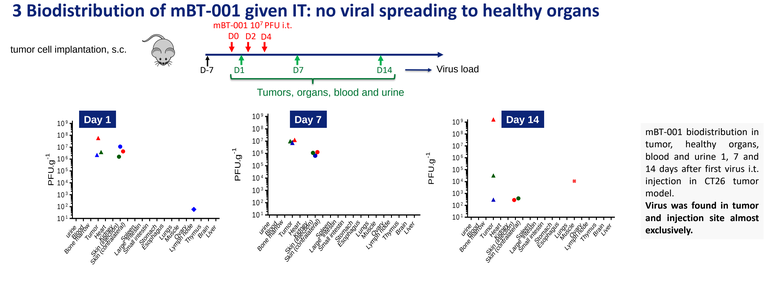
Concentrations of murine @mCTLA4 (m5-B07) in culture supernatants of 3 murine tumor cells (A-C) infected at different multiplicity of infection (MOI, ratio: virus/cellules) by mono- or bi-armed (mBT-001) vaccinia virus. Concentration of murine GM-CSF (D) in culture supernatants of CT26, MCA205, and B16F10 infected at different MOI by mBT-001. All measurements were performed after 5 days of infection.
High level of expression of both transgenes in all murine cell lines with a high variability of expression depending on cell and MOI

2: Intratumoral mBT-001 allows a sustained and tumor restricted transgene expression



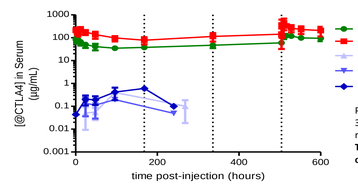
Virus, @mCTLA4 mAb and mGM-CSF concentrations time course in tumor (upper graphs) and serum (lower graphs) after three i.t. administrations 2 days apart of 10^7 PFU of mBT-001 (red line) or VACV encoding only @mCTLA4 mAb (green line). CT26 colorectal tumor cells were implanted subcutaneously to Balb/c mice and virus was injected (IT) when the tumor reached ~20-50 mm^3. One injection intraperitoneally of @mCTLA4 at 3 mg/kg was used as benchmark (blue line).
- **High concentration of transgene products in tumor -while very low concentration in blood**
- **No impact of transgene on virus replication in vivo**

3 Biodistribution of mBT-001 given IT: no viral spreading to healthy organs



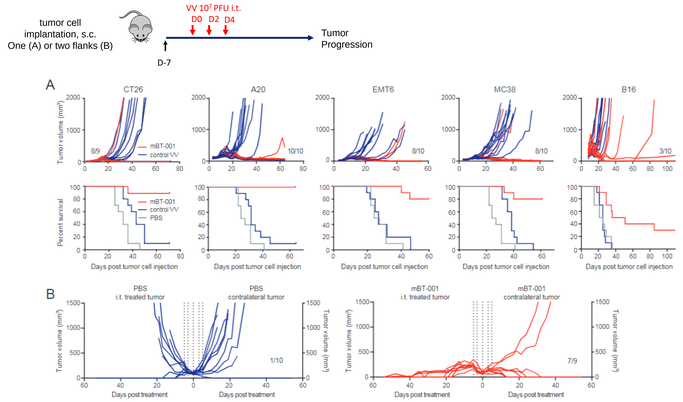
mBT-001 biodistribution in tumor, healthy organs, blood and urine 1, 7 and 14 days after first virus i.t. injection in CT26 tumor model.
Virus was found in tumor and injection site almost exclusively.

4: IV injection in NHP of BT-001: circulating [@CTLA4] ~100-fold lower than therapeutic dose



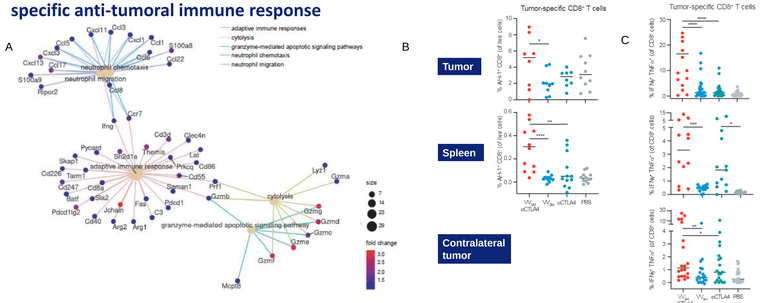
Pharmacokinetic profiles of @CTLA4 antibody in serum of macaques after i.v. injections of either 3 and 10 mg/kg-(each week for 4 weeks, dashed vertical lines indicate antibody injection) of recombinant 4-E03 mAb or a single injection of BT-001 at the three indicated doses.
The level of circulating 4-E03 mAb after IV injection of BT-001 is ~100-fold lower than the one obtained after injection of therapeutic dose of 4-E03.

5: mBT-001 induces strong anti-tumoral activity in several syngeneic tumor models



Antitumoral activity of mBT-001 was assessed in five different syngeneic tumor models after 3 i.t. administrations of 10^7 PFU. The graphs in (A) show the antitumoral activity of mBT-001 versus control VV (*non treated tumor not shown in upper graphs but only for survival*) and the corresponding survival. (B) abscopal effect was observed in a CT26 twin-tumor model where only one tumor was treated as in A.

6: mBT-001 induces a robust, systemic and specific anti-tumoral immune response



Change in immune signature into treated, untreated (contralateral) CT26 tumors and spleen of mice treated by mBT-001 (VV_{GM} @CTLA4), VACV control, @CTLA4 or PBS. CT26 tumors were treated as described above. For 3' RNA seq, tumors were harvested 4 days after the first virus injection and RNA extracted for 3' RNA seq analysis. (A) Network view of the differentially expressed genes associated with the five most enriched GO terms in the set of 352 differentially expressed genes, either upregulated or downregulated, in CT26 tumors treated with mBT-001 versus VV empty. Genes upregulated were found associated with these five enriched GO terms. Tumor cell suspensions and splenocytes were restimulated ex vivo CT26 (AH- 1)- specific peptide and the percentage of IFN-γ+ and TNFα+ $CD8^+$ T cells, or MHC class I- labeled multimer positive $CD8^+$ T cells was quantified by FACS. Quantification of (B) antigen- specific and (C) IFN-γ+/TNFα+ $CD8^+$ T cells in indicated organs. Each dot represents one mouse (n=3–6 experiments)

CONCLUSIONS

- → **BT-001 is a multifunctional oncolytic vaccinia virus co-developed by Transgene and BioInvent that encodes a Treg-depleting @CTLA4 antibody as well as the cytokine GM-CSF.**
- → **BT-001 combines potent oncolytic activities with the production of high intratumoral concentrations of @CTLA4 antibody and GM-CSF, and very low systemic exposure.**
- → **BT-001 murine surrogate has demonstrated an outstanding antitumoral activity in several syngeneic tumor models. An abscopal effect has been observed on several models. This antitumoral activity is further enhanced by a combination with @PD-1 treatment.**
- → **BT-001 treatment leads to a remodeling of tumor microenvironment with an inflamed phenotype along with specific and long-lasting antitumoral immune response.**
- → **A clinical trial evaluating i.t. BT-001 alone and in combination with @PD-1 in metastatic or advanced solid tumors is ongoing.**

See also Semmrich et al. J. ImmunoTherapy of Cancer. 2022 10:e003488. doi:10.1136/jitc-2021-003488 for more inforamtions