BT-001, an oncolytic Vaccinia virus armed with a Treg-depletion-optimized recombinant human anti- CTLA4 antibody and GM-CSF to target the tumor microenvironment

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ABSTRACT

Oncolytic viruses have been intensively studied for the treatment of various types of solid tumors over the last years and were shown to combine well with current immunotherapies. The mode of action of armed oncolytic Vaccinia viruses (oVV) associates cell death after selective replication in tumor cells, local inflammation and immune signaling, leading to T-cell infiltration and immune engagement, and intra-tumoral delivery of therapeutic modalities ranging from enzymes to antibodies, as well as cytokines or engineered receptor ligands.

BioInvent and Transgene are collaborating in the development of a next generation armed oncolytic viruses, bringing respectively its n-CoDeR F.I.R.S.T™ platform for antibody discovery, and the Invir.IO oVV based platform. The first product, named **BT-001, involves the Copenhagen oVV strain, deleted in J2R and I4L viral genes involved in nucleotide synthesis to restrict its replication to replicating cells, and the human IgG1 4-E03 recognizing the human CTLA4**. 4-E03 shows improved Treg-depleting activity compared with ipilimumab. BT-001 also encodes GM-CSF, the cytokine expressed in clinically advanced products like T-Vec (Imlygic).

Since virus replication and expression of recombinant transgenes are tightly linked, this armed oVV is an interesting design for the intra-tumoral delivery of therapeutic proteins exhibiting toxicological limitation (e.g. anti-CTLA4) and/or pharmacokinetic issues (e.g. GM-CSF).

Our studies demonstrated that both **the anti-CTLA4 antibody and the GM-CSF were expressed at high level (1 µg/mL range)** as functional molecules after infection by BT-001 of several human tumoral cell lines. **Moreover, the expression of both transgenes was sustained, up to 20 days, in tumors after intratumoral (IT) administration and reached 10-fold the IT concentration obtained after an intraperitoneal (IP) injection of a therapeutic dose (i.e. 3 mg/kg)** of anti-CTLA4 monoclonal antibody (mAb). Yet, the bloodstream concentrations of 4-E03 produced by BT-001 after IT injection remained lower compared to IP administration of 3 mg/kg of 4-E03 as mAb. These results confirm that the oncolytic vectorization allows the specific delivery of therapeutic proteins into the tumor while sparing the rest of the organism.

The murine surrogate of BT-001, expressing anti-murineCTLA4 and murine GM-CSF has also displayed a very potent anti-tumoral activity, leading to up to 100 % of tumor free mice in CT26, EMT6, A20, and C38 syngeneic tumor murine models. The excellent anti-tumoral profile was shown to depend on anti-CTLA4 expression and could be boosted by co-administration of anti-PD-1 mAb. Clinical batch of BT-001 has been produced and toxicological evaluation is ongoing in non-human primates, Transgene and BioInvent are preparing CTA and IND filings for a multicenter clinical trial targeting injectable superficial tumors.

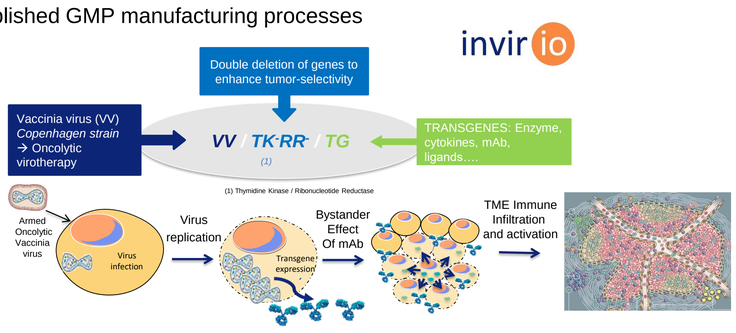
ACHIEVEMENTS

- **Selection of an anti-CTLA4 full human IgG1 with improved Treg-depleting activity compared with ipilimumab**
- **Vectorization in Copenhagen vaccinia virus of anti-CTLA4 mAb and GM-CSF without impairing virus replication or oncolytic activity**
- **Good expression of fully functional transgenes in vitro by infected tumor cells**
- **Sustained expression into the tumor of both transgenes with very low circulating concentrations**
- **Anti-tumoral activity of surrogate virus encoding murine molecules in several syngeneic tumor models with up to 100% tumor free animals.**
- **Additive anti-tumoral activity with anti-PD-1 antibody**
- **Specific and long-lasting immune memory against tumor cells**

INVIR.IO PLATFORM MAIN FEATURES

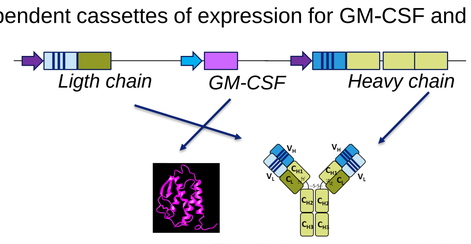
Differentiating factors of Transgene's oncolytic platform :

- Copenhagen strain: best oncolytic activity among VACV strains, and among orthopoxviruses
- Good safety profile and high therapeutic index; thymidine kinase (TK) and ribonucleotide reductase (RR) deletions restrict replication to proliferative cells (e.g. tumor cells)
- Solid track record of clinical use (TG6002 currently in clinical trial, dose esc. up to 10^9 pfu IV)
- Large DNA insertions are possible (up to 25 kb), with successful vectorization of various expression cassettes (enzymes, cytokines, antibodies, etc.)
- Pure cytoplasmic replication (no risk for genome integration or mutagenesis)
- Good immunological balance (anti-tumor vs anti-viral responses, Th1 vs Th2, etc.)
- Well-established GMP manufacturing processes

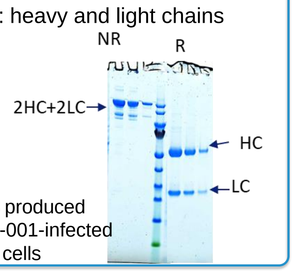


BT-001 MAIN FEATURES

- Vaccinia virus Copenhagen strain TK-RR-
- Independent cassettes of expression for GM-CSF and a full IgG1: heavy and light chains



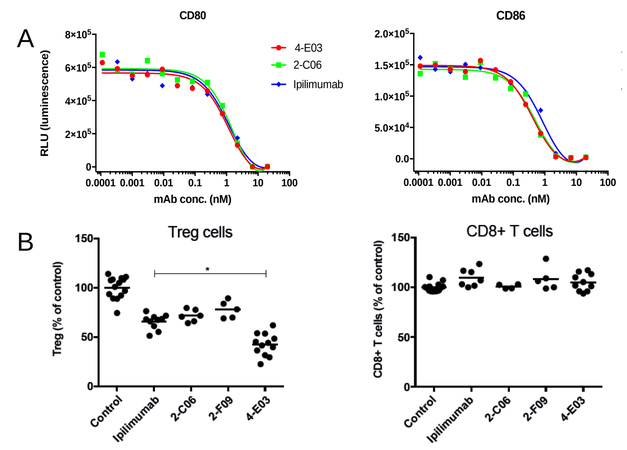
4-E03: Full length human IgG1



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

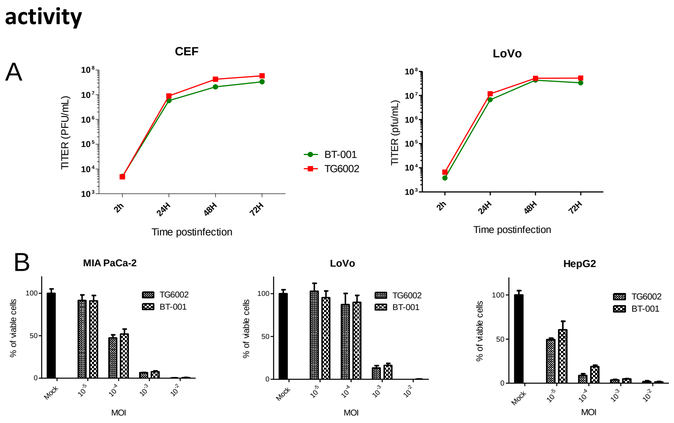
RESULTS

1: 4-E03 binds hCTLA4 and depletes human Treg cells



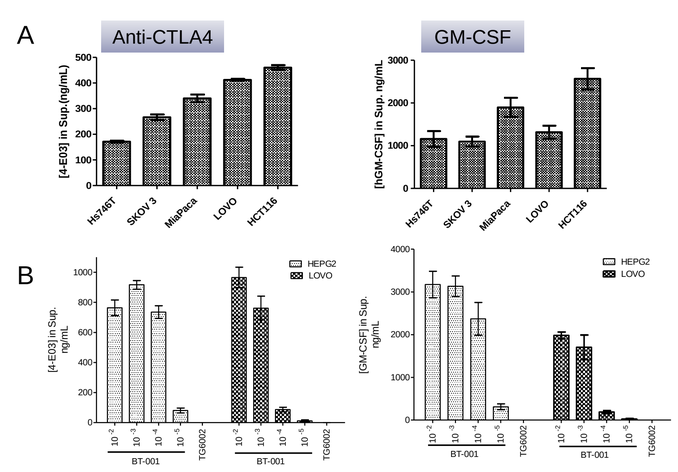
4-E03 is a new anti-hCTLA4 human IgG1 that blocks the CD80/CTLA4 and CD86/CTLA4 interactions with the same IC50 as Ipilimumab (A) and depletes human Treg cells, but not CD8 T cells, in PBMC transplanted mice (B) Of note the Treg depletion potency of 4-E03 is significantly higher than the one of Ipilimumab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

2: Vectorization does not impair virus replication or oncolytic activity



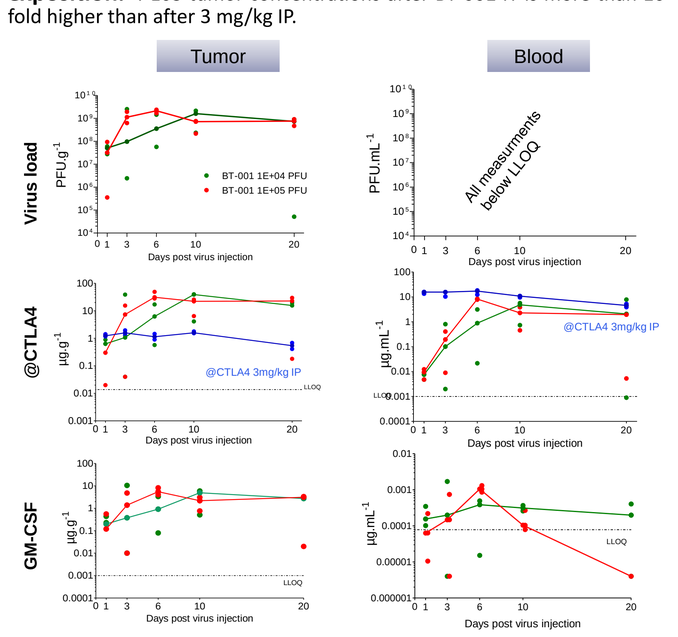
BT-001 replicates as well as the benchmark virus TG6002 in cell of production (CEF) or human tumor cells (LoVo) (A). BT-001 and TG6002 have the same oncolytic activity on three human tumor cells (B)

3: BT-001-infectes tumor cells express 4-E03 and GM-CSF



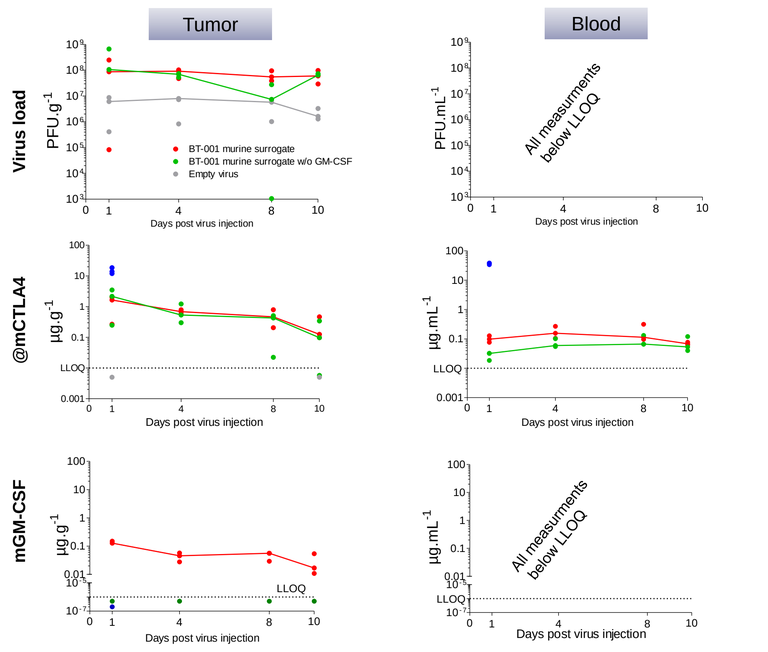
Concentrations of 4-E03 and GM-CSF in supernatants of human tumor cells infected by BT-001 at MOI 0,05 for 48 hours (A) or at different MOI and after 72 hours incubation (B).

4: In xenograft tumor model treated by BT-001: 4-E03 mAb and GM-CSF accumulate in tumor with low systemic exposition. 4-E03 tumor concentrations after BT-001 IT is more than 10-fold higher than after 3 mg/kg IP.



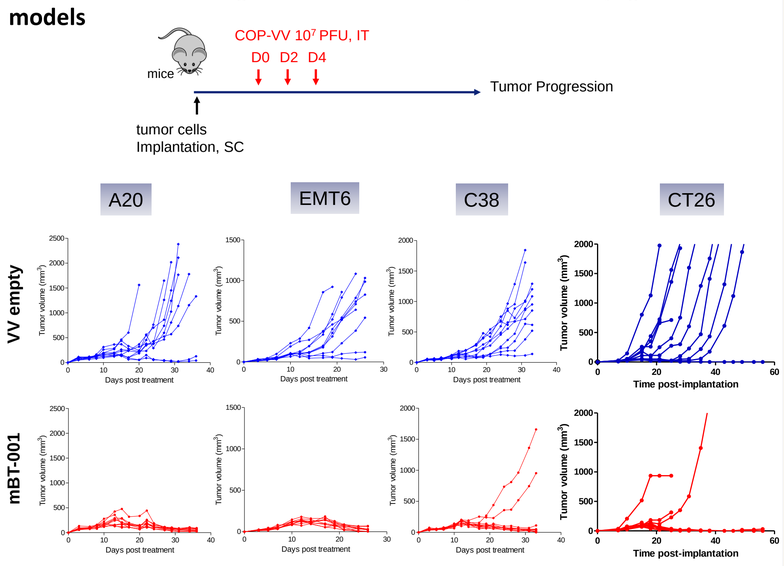
Virus, 4-E03 and GM-CSF concentrations time course in tumor (left graphs) and serum (right graphs) after a single Intratumoral (IT) administration of 10^4 (green line) or 10^5 (red line) PFU of BT-001.
LoVo human colorectal tumor cells were implanted subcutaneously to Nude mice and virus was injected (IT) when the tumor reached ~120 mm³. One injection intraperitoneally of 4-E03 at 3 mg/kg was used as benchmark

5: In CT26 tumor model treated by surrogate viruses (mBT-001): @CTLA4 mAb and mGM-CSF accumulate in tumor with low systemic exposition



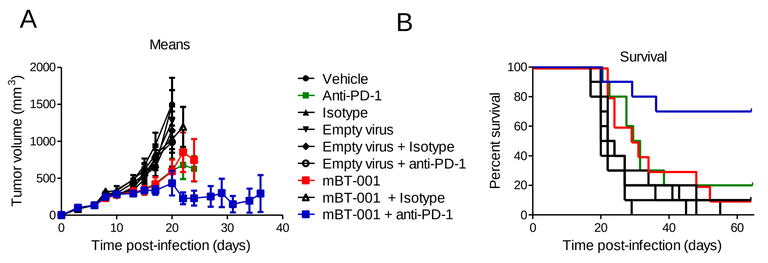
Virus, @mCTLA4 mAb and mGM-CSF concentrations time course in tumor (left graphs) and serum (right graphs) after three IT administrations 2 days apart of 10^7 PFU of viruses encoding either @mCTLA4 mAb (green line) or @mCTLA4 mAb and mGM-CSF (red line). CT26 colorectal tumor cells were implanted subcutaneously to Balb/c mice and virus was injected (IT) when the tumor reached ~20-50 mm³. One injection intraperitoneally of @mCTLA4 at 3 mg/kg was used as benchmark

6: mBT-001 has strong anti-tumoral activity in several syngeneic tumor models



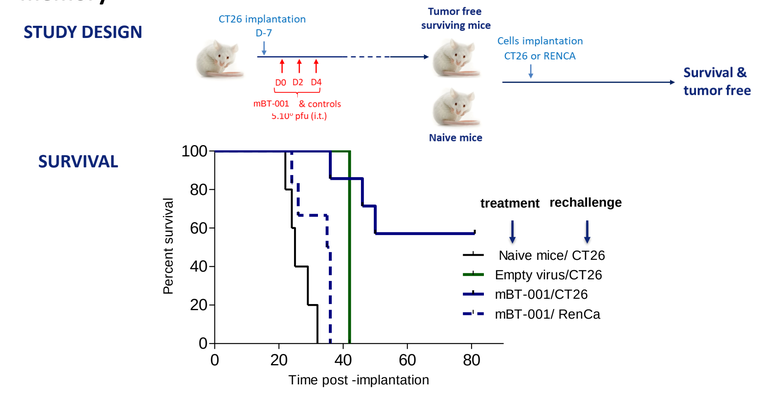
Antitumoral activity of oncolytic Copenhagen vaccinia virus encoding an anti-murine CTLA4 and murine GM-CSF was assessed in four different syngeneic tumor models and after 3 IT administrations of 10^7 PFU. Each line represents the individual tumor volume over the duration of the experiment. The upper and lower graphs represent the antitumoral activity of Copenhagen vaccinia virus without any transgene ("empty") or mBT-001 respectively

7: mBT-001 and anti-PD-1 have additive anti-tumoral activities .



Suboptimal dose (i.e. 10^5 PFU) of Virus was given IT in A20 model in same conditions as described above and in combination with twice a week IP administration of 250 µg/mouse of anti-PD1 or corresponding isotype. Mean tumor volume of 10 animals are represented in (A) and the corresponding survival is shown in (B)

8: Treatment with mBT-001 induces a specific and long-lasting immune memory



Mice that were cured of their CT26 tumor after viral treatment were rechallenged with either CT26 or RENCA tumor cells. CT26 first rejection protects from homologous but not heterologous rechallenge indicating that the treatment with BT-001 has induced a specific and long-lasting antitumoral immune response

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CONCLUSIONS

- **BT-001 is a multifunctional oncolytic vaccinia virus co-developed by Transgene and BioInvent that encodes a Treg-depleting anti-CTLA4 antibody as well as the cytokine GM-CSF**
- **BT-001 combines potent oncolytic activities with production of high intratumoral concentrations of anti-CTLA4 antibody and GM-CSF, and very low systemic exposure.**
- **BT-001 murine surrogate have demonstrated an outstanding antitumoral activities in several syngeneic tumor models. This antitumoral activity is further enhanced by a combination with anti-PD-1 treatment and lead to a specific and long-lasting antitumoral immune response.**
- **The first clinical trial application for BT-001 has been filed and the first-in-human trial of BT-001 is expected to start before the end of 2020.**