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

Transgene and BioInvent are collaborating in the development of a next generation of armed oncolytic viruses and bring respectively the Invir.IO® oncolytic Vaccinia virus (oVV) based platform and the n-CoDeR®/F.I.R.S.T™ platforms for antibody discovery.

The first product, named **BT-001, is based on the Copenhagen oVV strain, deleted in Thymidine Kinase (TK) and Ribonucleotide Reductase (RR) viral genes involved in nucleotide synthesis to restrict its replication to replicating cells. BT-001 encodes the human IgG1 4-E03 recognizing the human CTLA4**. 4-E03 has an improved Treg-depleting activity compared with ipilimumab. BT-001 also encodes GM-CSF, the cytokine expressed in the clinically approved product T-Vec (Imlygic).

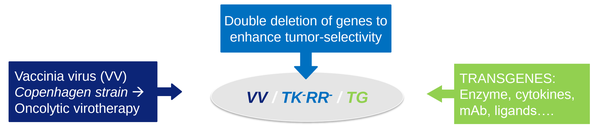
Our studies demonstrated that **the expression of both transgenes was sustained, at least up to 20 days, in tumors after intratumoral (IT) administration and reached 10-fold the IT concentration obtained after intraperitoneal (IP) injection of a therapeutic dose (i.e. 3 mg/kg)** of recombinant 4-E03. Yet, 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. These results confirm that the oncolytic vectorization allows an increase of the specificity of the delivery of therapeutic proteins into the tumor while sparing the rest of the organism.

The murine surrogate of BT-001, expressing anti-murine CTLA4 mAb and murine GM-CSF also displayed a very potent anti-tumoral activity, leading to outstanding cure rate in CT26, EMT6, A20, and C38 syngeneic tumor murine models. Furthermore, sub-optimal dose of BT-001 reinforced the therapeutic activity of anti-PD-1 mAb.

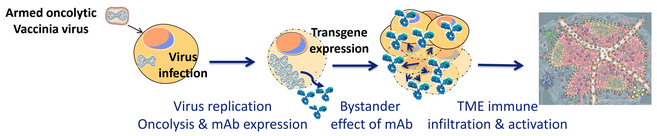
Clinical batch of BT-001 has been produced and toxicological evaluation is ongoing in non-human primates. Transgene and BioInvent have applied for a multicenter clinical trial targeting injectable superficial tumors.

INVIR.IO PLATFORM

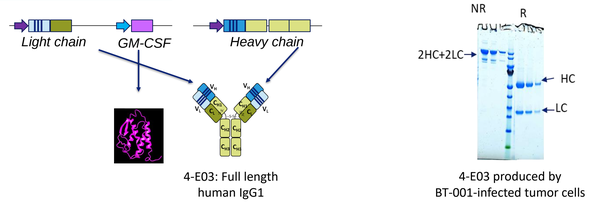
Differentiating factors of Transgene's proprietary oncolytic platform :



- 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 escalation 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

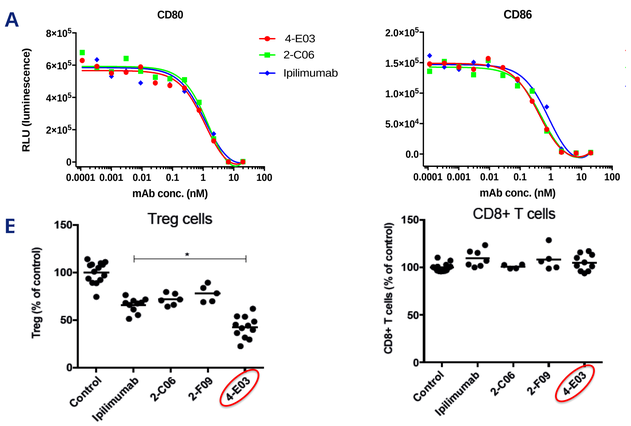


GENERATION OF BT-001



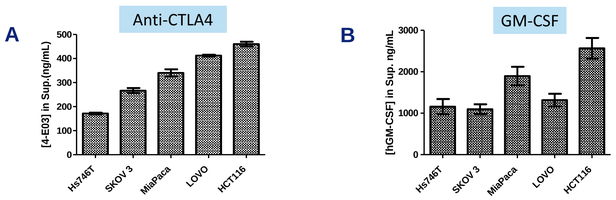
RESULTS

1: 4-E03 binds hCTLA4 and depletes human Treg cells, but not CD8+ T 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 human PBMC transplanted NOG/SCID mice (B) Of note the Treg depletion potency of 4-E03 is significantly higher than the one of Ipilimumab in this xenograft model.

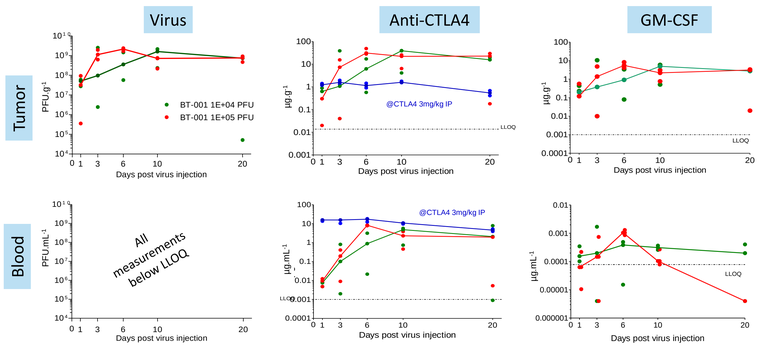
2: BT-001-infected tumor cells express 4-E03 & GM-CSF



Concentrations of 4-E03 (A) and GM-CSF (B) in supernatants of human tumor cells infected by BT-001 at MOI 0.05 for 48 hours.

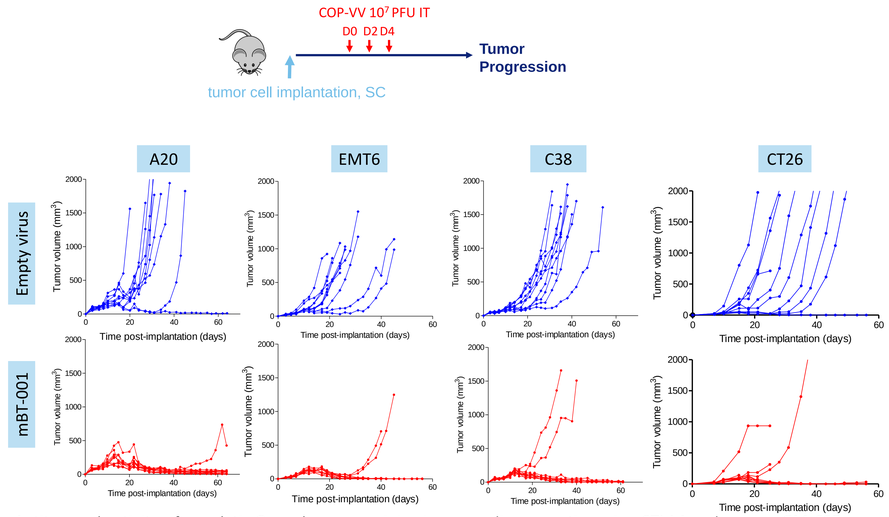
3: 4-E03 and GM-CSF accumulate in tumor with low systemic exposure.

4-E03 tumor concentrations after IT administration of BT-001 is more than 10-fold higher than after IP administration of 3 mg/kg of 4-E03 in xenograft tumor model while systemic concentrations remain lower.



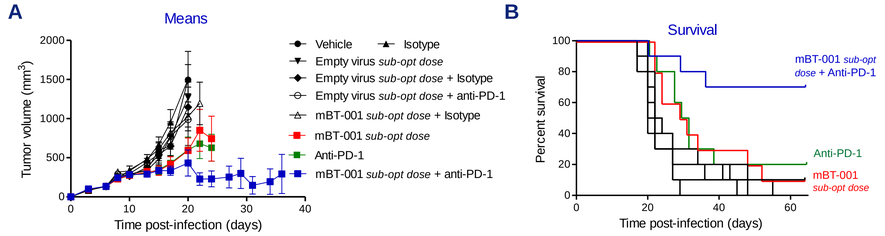
Virus, 4-E03 and GM-CSF concentrations time course in tumor (graphs above) and blood (graphs below) after a single intratumoral (IT) administration of 10^4 (green line) or 10^5 (red line) PFU of BT-001.
LoVo human colorectal tumor cells were implanted subcutaneously to Nude mice and virus was injected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

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



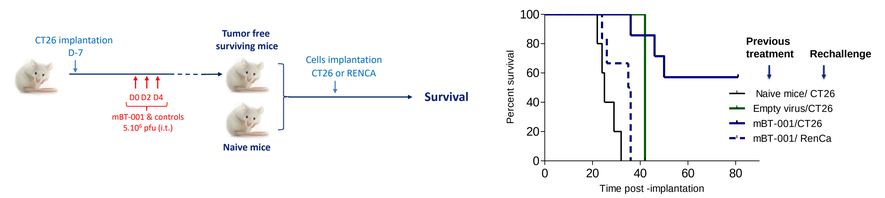
Antitumoral activity of oncolytic Copenhagen vaccinia virus encoding an anti-murine CTLA4 and murine GM-CSF was assessed in four different syngeneic tumor models after 3 IT administrations of 10^7 PFU. Each line represents the individual tumor volume over the duration of the experiment. The upper and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

5: mBT-001 and anti-PD-1 display additive anti-tumoral activities



Sub-optimal dose (i.e. 10^5 PFU) of viruses was given IT in A20 model in the same conditions as described above and in combination with a twice a week IP administration of 250 µg of anti-mPD1 or corresponding isotype. Mean tumor volume in 10 animals is represented in (A) and the corresponding survival is shown in (B).

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



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

REFERENCES

- 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.
- Kleinpeter *et al.* Oncoimmunology. 2016 Sep 9;5(10):e1220467
- PCT/EP2019/073488 Novel Antibodies and nucleotides sequences, and uses thereof
- WO2009/065546 Poxviral Oncolytic Vectors
- WO2016/008976 Oncolytic virus for expression of immune checkpoint modulators

CONCLUSIONS

- **BT-001 is a multifunctional oncolytic vaccinia virus that encodes a Treg-depleting anti-CTLA4 antibody as well as the cytokine GM-CSF.**
- **BT-001 combines potent oncolytic activities with the production of high intratumoral concentrations of anti-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. Furthermore, BT-001 at sub-optimal dose reinforces therapeutic activity of an anti-PD-1 treatment and leads to a specific and long-lasting tumor specific immune response.**
- **The first clinical trial application for BT-001 was filed and the first-in-human trial is expected to start before the end of 2020, in combination with an anti-PD-1.**