# Initial clinical results of BT-001, an oncolytic virus expressing an anti-CTLA4 mAb, administered as single agent and in combination with pembrolizumab in patients with advanced solid tumors

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## BACKGROUND

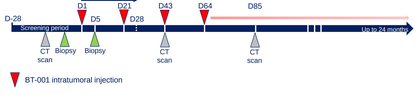
- Intra-tumoral (IT) administration of an oncolytic virus has been shown to induce local and systemic antitumor effects through direct tumor cell killing and adaptive cytotoxic T cell response.
- BT-001 is an oncolytic vaccinia virus with enhanced replication selectivity in tumor cells and genetically engineered to express GM-CSF and a novel full-length anti-CTLA-4 hIgG1 mAb.
- BT-001 showed strong antitumoral activity in various murine tumor models, including immunologically “cold” tumors, with enhanced activity when combined with an anti-PD-1 agent.
- BT-001.01 is a first-in-human dose-escalation trial, to evaluate safety, tolerability, and antitumor activity of IT injections of BT-001 alone and in combination with intravenous pembrolizumab in patients with advanced/metastatic solid tumors.

## METHODS

- A total of 24 patients received IT injections of BT-001 every 3 weeks as monotherapy (Part A) at doses of $10^6$ pfu/mL (cohort 1, n=6), $10^7$ pfu/mL (cohort 2, n=6) or $10^8$ pfu/mL (cohort 3, n=6), or combined to 200 mg of IV pembrolizumab (Part B) at the dose of $10^7$ pfu/mL (n=6).
- Treatment was administered until disappearance of all injectable lesions (for BT-001), confirmed disease progression per iRECIST, or unacceptable toxicity (for BT-001 and pembrolizumab), for a maximum of 24 months.
- Translational analyses were performed in part A, and consisted of
  - Virus detection by qPCR in (1) tumor biopsies at baseline and on Day 5 or 50, (2) blood at baseline and from Day 1 to 64, (3) skin swabs (4) saliva (5) urine and (6) feces on Days 2, 8, 15, 43 and 64.
  - Measures of (1) GM-CSF by Luminex assay in serum at baseline, on Days 5, 8, 15, 29 and 36, (2) anti-CTLA-4 mAb by ELISA concomitantly to virus detection in blood and, in tumor biopsies at baseline and on Days 5 or 50, and (3) anti-vaccinia virus antibodies in serum (parts A and B).
- Tumor response was assessed by the investigator using iRECIST and RECIST v1.1 on Days 43 (week 6), 85 (week 12), then every 8 weeks the first year and every 12 weeks thereafter.

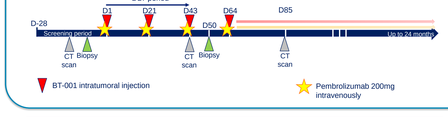
## TRIAL SCHEDULE

**BT-001 monotherapy (Part A)**




IT injection

**BT-001 combination with pembrolizumab (Part B)**




| Longest diameter (mm) | Volume mL |
|---|---|
| 25 to 50 | Up to 2.0 |
| 15 to 24 | Up to 1.0 |
| 5 to 14 | Up to 0.5 |
| <5 | No injection |

**Volume of BT-001 per injection according tumor size**

## KEY ELIGIBILITY CRITERIA

- Age ≥18 years
- Advanced/metastatic solid tumors having failed and/or intolerant to standard therapeutic options
- At least one injectable and measurable cutaneous, subcutaneous or nodal lesion
- Longest diameter of the injected lesions ≤ 50 mm (except in part A, cohort 1)
- ECOG performance status 0 or 1

## PATIENT AND DISEASE CHARACTERISTICS

24 patients from 5 sites in France and Belgium were enrolled in this trial, mostly with melanoma (n=13) and soft tissue sarcoma (n=5).

| Characteristics | BT-001 monotherapy (n=18) | BT-001 in combination with pembrolizumab (n=6) | Overall (n=24) |
|---|---|---|---|
| **Age**, years, med. (range) | 59 (31-77) | 51 (28-62) | 57 (28-77) |
| **Male** (No)/**Female** (No) | 9/9 | 3/3 | 12/12 |
| **Type of cancer**, No (%) | | | |
| Melanoma | 8 (44.4) | 5 (83.3) | 13 (54.2) |
| Soft tissue sarcoma | 4 (22.2) | 1 (16.7) | 5 (20.8) |
| Other: 2 breast cancers, 1 Merkel cell carcinoma, 1 anal SSC, 1 ovarian cancer, 1 larynx SSC | 6 (33.3) | 0 (0.0) | 6 (25.0) |
| **Overall stage at baseline**, No (%) | | | |
| IIIB-C/IV | 2 (11.1)/16 (88.9) | 0 (0.0)/6 (100.0) | 2 (8.3)/22 (91.7) |
| **Number of prior lines of antineoplastic therapy** | 3 (1-7) | 2.5 (2-6) | 3 (1-7) |
| **Number of patients with prior exposure to ICIs,** No (%) | 11 (61.1) | 5 (83.3) | 16 (66.7) |
| **Time from diagnosis to enrollment**, months, med. (range) | 60.7 (10.8-145.6) | 34.2 (13.8-75.4) | 56.7 (10.8-145.6) |
| **Smallpox vaccinated**, No (%) | 10 (55.6) | 3 (50.0) | 13 (54.2) |

## ACKNOWLEDGMENT

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## SAFETY DATA

**Exposure**

- Patients received a median number of 4 BT-001 injections (range, 2-19).
- Median size of injected lesions was 27 mm (range, 15-65)
- Median volume of BT-001 per administration was 2.0 mL (range, 1.0-4.0).
- In the combination part, median number of pembrolizumab infusions was 7.5 (range, 2-11).

**Safety**

- No dose-limiting toxicity was observed.
- No AEs led to BT-001 and/or pembrolizumab discontinuation
- Most common BT-001-related AEs are reported in the table below.

**BT-001-related AEs occurring in more than 2 patients**

| | BT-001 monotherapy ($10^6$ to $10^8$ pfu/mL) N=18 | | BT-001 in combination with pembrolizumab ($10^7$ pfu/mL) N=18 | | Overall N=24 | |
|---|---|---|---|---|---|---|
| Event | N | % | N | % | N | % |
| Pyrexia | 7 | 38.9 | 3 | 50.0 | 10 | 41.7 |
| Chills | 3 | 16.7 | 1 | 16.7 | 4 | 16.7 |
| Skin ulcer | 4 | 22.2 | 0 | 00.0 | 4 | 16.7 |
| Injection site inflammation | 1 | 5.6 | 2 | 33.3 | 3 | 12.5 |
| Injection site ulcer | 3 | 16.7 | 0 | 00.0 | 3 | 12.5 |
| Eosinophilia | 1 | 5.6 | 2 | 33.3 | 3 | 12.5 |

- A total of 18 episodes of BT-001 related pyrexia (15 of grade 1, 3 of grade 2) were observed in 10 patients.
- Injection or biopsy site AEs were reported in 15 patients, including all 6 patients of cohort 1 in the monotherapy part, and mainly consisted of grade 1-2 skin ulcer, and injection site inflammation, pain and ulcer. The trial Safety Review Committee considered that some of them were rather due to disease progression.
- A total of 2 grade ≥3 BT-001-related AEs were observed: one grade 3 skin ulcer located at the injection/biopsy site, and one grade 3 transient lymphocyte count decrease.
- Five of the 6 patients treated with BT-001 in combination with pembrolizumab presented a total 13 AEs related to pembrolizumab, none of them being grade ≥ 3. Most common AEs were pyrexia (n=3), pruritus (n=2), and eosinophilia (n=2).

## EFFICACY DATA

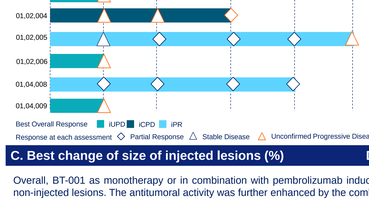
### A. Best Overall Response according to iRECIST

| Part | N | iPR n (%) | iSD n (%) | iUPD n (%) | iCPD n (%) |
|---|---|---|---|---|---|
| BT-001 monotherapy | 18 | 0 (0.0) | 4 (22.2) | 8 (44.4) | 6 (33.3) |
| BT-001 in combination with pembrolizumab ($10^7$ pfu/mL) | 6 | **2 (33.3)** | 0 (0.0) | 3 (50.0) | 1 (16.7) |

**2 of the 6 patients treated with BT-001 in combination with pembrolizumab exhibited PR**

iUPD: Unconfirmed Progressive Disease
iCPD: Confirmed Progressive Disease
iPR: Partial Response
iSD: Stable Disease

### B. BT-001 in combination with pembrolizumab: swimming plot (iRECIST)



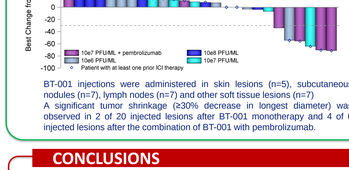

PR was observed in:
- one patient (01.02.005) with PD(L)-1 resistant melanoma
- one patient (01.04.008) with leiomyosarcoma treated with 5 prior lines of therapies.

Both showed a sustained PR.

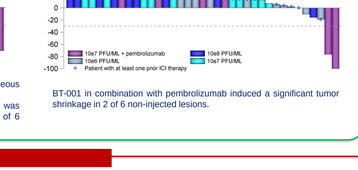
### C. Best change of size of injected lesions (%)

### D. Best change of size of non-injected lesions (%)

Overall, BT-001 as monotherapy or in combination with pembrolizumab induced tumor shrinkage in 9 of 26 injected lesions and in 5 of 37 non-injected lesions. The antitumoral activity was further enhanced by the combination with pembrolizumab.




BT-001 injections were administered in skin lesions (n=5), subcutaneous nodules (n=7), lymph nodes (n=7) and other soft tissue lesions (n=7)
A significant tumor shrinkage (≥30% decrease in longest diameter) was observed in 2 of 20 injected lesions after BT-001 monotherapy and 4 of 6 injected lesions after the combination of BT-001 with pembrolizumab.




BT-001 in combination with pembrolizumab induced a significant tumor shrinkage in 2 of 6 non-injected lesions.

## EFFICACY DATA

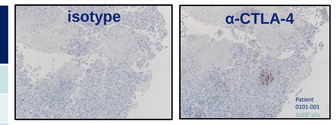
## TRANSLATIONAL ANALYSES

### A. Tumor biopsy analyses (part A)

| Patient | Dose (pfu/mL) | Tumor | Injected lesion/Size mm | Biopsy collection | qPCR (copies/µg DNA) | RT-qPCR (ΔCt α-CTLA-4) |
|---|---|---|---|---|---|---|
| 01.03.001 | $10^6$ | Anal squamous cell carcinoma | Perineal skin/20 | D5 | Not detected | 10.2515 |
| 01.03.002 | $10^6$ | Breast cancer | Breast nodule/30 | D5 | 8.06E+02 | Not evaluable |
| 01.01.001 | $10^6$ | Melanoma | Axillary lymph node/39 | D5 | 1.31E+06 | 1.833 |
| 01.03.003 | $10^6$ | Larynx carcinoma | Subcutaneous sternal node/25 | D5 | 1.68E+07 | 3.212 |
| 01.01.002 | $10^6$ | Melanoma | Inguinal lymph node/59 | D5 | 1.15E+03 | Not done |
| 01.02.001 | $10^6$ | Melanoma | Skin lesion/65 | D5 | 7.64E+03 | -0.1625 |
| 01.03.011 | $10^8$ | Leiomyosarcoma | Muscular lesion (trapezius)/25 | D50 | 4.65E+02 | Not detected |

(A) (B) Virus backbone and/or α-CTLA-4 payload were found in all tumor biopsies.

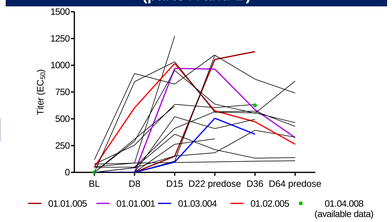
### B. IHC Intratumoral detection of α-CTLA-4




### C. Biological fluids analyses (part A)

| Analyses | | Number of tested samples | Number of positive samples |
|---|---|---|---|
| Detection in blood | Virus detection | 198 | 4 |
| | GM-CSF | 96 | 0 |
| | α-CTLA-4 monoclonal antibody | 350 | 0 |
| Shedding | Skin swab | 53 | 4 |
| | Saliva | 54 | 2 |
| | Urine | 53 | 0 |
| | Feces | 40 | 0 |

### D. Anti-vaccinia virus neutralizing antibodies (parts A and B)




(C) BT-001 oncolytic virus replicated within the tumor with rare and sporadic shedding in biological fluids or excreta.
(D) Anti-vector neutralizing antibodies were induced in all patients including those who had PR (01.02.005, and 01.04.008) and those with significant decrease (≥30%) in size of injected lesions (01.01.001, 01.01.005, and 01.03.004).

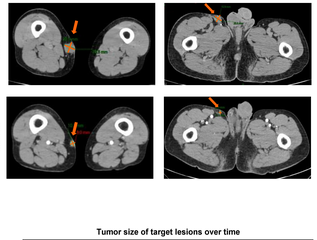
## PATIENTS WITH PARTIAL RESPONSE

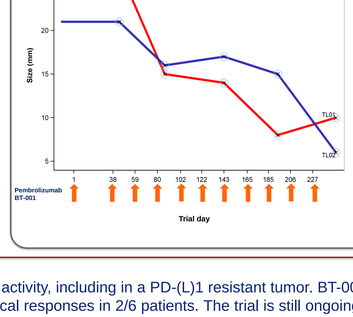
### PD(L)-1 resistant melanoma

48-year-old male
Stage IV melanoma diagnosed in 2020
Three prior lines of antineoplastic therapy (nivolumab-ipilimumab x2 and durvalumab-ceralasertib).

BT-001 was injected at the dose of $10^7$ pfu/mL in 2 right thigh subcutaneous lesions in combination with IV pembrolizumab. Longest diameter of target lesions (TL) progressively decreased over time. PR was observed from week 6 to 28.

At week 36, a new metastatic pancreatic lesion was observed.

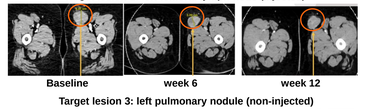


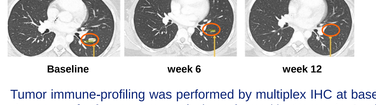

### Multiresistant leiomyosarcoma

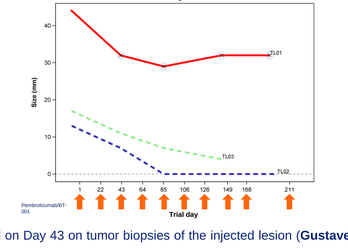
28-year-old female
Stage IV intraosseous dorsolumbar junction leiomyosarcoma diagnosed in 2021
Radiation therapy, then 5 lines of antineoplastic therapy (Cisplatin-ifosfamide-adriamycin/pazopanib/trabectedin/ dacarbazine/olaparib) and radiotherapy of a pulmonary cardiophrenic angle lesion one month before inclusion
BT-001 was injected at the dose of $10^7$ pfu/mL in a left femoral adenopathy in combination IV pembrolizumab.
Longest diameter of TL progressively decreased over time. PR was observed from week 6 to 28+ with a clear reduction in the size of multiple and bilateral non-injected pulmonary lesions.

**Target lesion 1: left femoral lymph node (injected)**




**Target lesion 3: left pulmonary nodule (non-injected)**

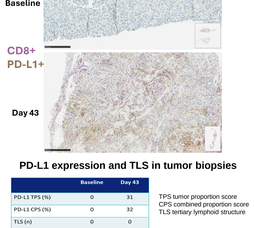



Tumor immune-profiling was performed by multiplex IHC at baseline and on Day 43 on tumor biopsies of the injected lesion (**Gustave Roussy Unlock program**, analysis performed by **Veracyte, Inc**.).

At baseline, tumor cells were PD-L1 negative and immune profile was cold with a low T cell infiltrate, a dominant M2 macrophage infiltrate, and lack of tertiary lymphoid structure (TLS).

**CD8+/PD-L1 IHC in tumor biopsies (Immunoscore® CD8 PD-L1)**

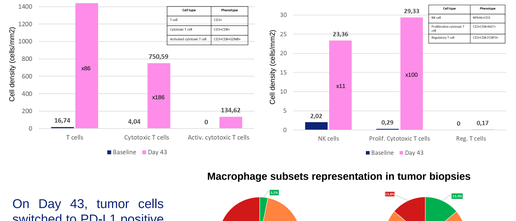



**PD-L1 expression and TLS in tumor biopsies**

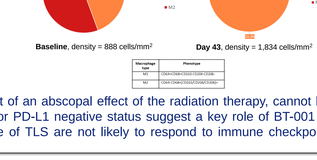
| | Baseline | Day 43 |
|---|---|---|
| PD-L1 TPS (%) | 0 | 31 |
| PD-L1 CPS (%) | 0 | 32 |
| TLS (n) | 0 | 0 |

TPS tumor proportion score
CPS combined proportion score
TLS tertiary lymphoid structure

**Immune contexture analysis in tumor biopsies**




**Macrophage subsets representation in tumor biopsies**




On Day 43, tumor cells switched to PD-L1 positive and immune profile significantly warmed up with a high T cell infiltrate and a switch to dominant M1 macrophage infiltrate.

Even if an antitumoral effect of pembrolizumab alone, or to a lesser extent of an abscopal effect of the radiation therapy, cannot be ruled out, the absence at baseline of T cell infiltrate or TLS, and the tumor PD-L1 negative status suggest a key role of BT-001 in observed activity. Moreover, leiomyosarcomas, particularly in the absence of TLS are not likely to respond to immune checkpoint inhibitor monotherapies.

## CONCLUSIONS

IT BT-001 alone or in combination with IV pembrolizumab was well tolerated and showed antitumoral activity, including in a PD-(L)1 resistant tumor. BT-001 replicated in the tumor and expressed its GM-CSF and anti-CTLA-4 transgenes. The combination of BT-001 at the dose of $10^7$ PFU/mL with pembrolizumab showed first signs of efficacy with documented radiological responses in 2/6 patients. The trial is still ongoing to further evaluate BT-001 at the dose of $10^8$ pfu/mL combined with pembrolizumab (cohort 2). In a patient with a heavily pre-treated sarcoma, BT-001 treatment in combination with pembrolizumab turned cold to hot the tumor microenvironment with a high T cell infiltrate, a higher M1/M2 ratio, and a shift to PD-L1 positivity.

Trial sponsored by TRANSGENE, 400 Boulevard Gonthier d’Andernach - Parc d’Innovation - CS80166 67405 Illkirch Graffenstaden Cedex – France. This trial is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

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