# Biomarker analysis from phase 2 study of agenT-797 (invariant natural killer T-cells), botensilimab (a Fc-enhanced CTLA-4 Inhibitor) with balstilimab (anti-PD-1) in PD-1 refractory gastroesophageal cancer (GEC)

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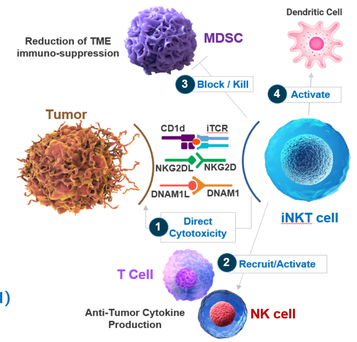
## Background

### Gastroesophageal (GE) cancer

- Gastroesophageal (GE) cancer is the 2nd leading cause of cancer-related mortality, with 1.3M deaths/year
- PD-1 ICB + chemotherapy is approved, but most patients progress
- Second-line treatment with ramucirumab and paclitaxel has a median PFS is 4.4 mo, and the ORR is 28%[1]
- We are performing a phase II trial of agenT-797, botensilimab (BOT), and balstilimab (BAL), with ramucirumab and paclitaxel in patients with advanced GE adenocarcinoma

### agenT-797 – an iNKT cell therapy

- A cell therapy product of allogeneic human unmodified invariant natural killer T (iNKT) cells, isolated from healthy donors and expanded *ex vivo*
- Induces direct cellular cytotoxicity via CD1d recognition and indirectly via IL-12 and IFN-γ upregulation
- Enables T and NK cell tumor infiltration and peripheral immune activation
- Mediates repolarization of immune-suppressive cells
- Activates dendritic cells, promoting T cell priming
- In a ph1 trial of patients with PD-1 refractory tumors, agent-797 + aPD-1 showed activity in gastric cancer[2] and other solid tumors (NCT05108623)




### Botensilimab (Fc-enhanced aCTLA-4) & Balstilimab (aPD-1)

- Fc-enhancement improves anti-tumor T cell responses
- Promotes depletion of suppressive T regulatory cells
- In a phase I trial of heavily pre-treated, immunotherapy-refractory patients, botensilimab + balstilimab demonstrated significant efficacy[3] (NCT03860272)

## Methods

**TRIAL SCHEMA:** An investigator-initiated, single-arm phase II trial of agenT-797, botensilimab, and balstilimab, with ramucirumab and paclitaxel in patients with advanced GE adenocarcinoma who have received one prior line of therapy, performed at Memorial Sloan Kettering Cancer Center (NCT06251793).

**Key Inclusion Criteria**
- Advanced GE adenocarcinoma
- Progressed on 1L treatment

N=37 patients

Longitudinal tissue and peripheral blood collection at baseline and throughout treatment

| | Cycle 1 | Cycle 2+ |
|---|---|---|
| **agenT-797 Induction** | agenT-797 | Botensilimab (3 total doses) + Balstilimab + Ramucirumab + Paclitaxel |
| **agenT/BOT/BAL Induction** | agenT-797 + Botensilimab + Balstilimab | Botensilimab (3 total doses) + Balstilimab + Ramucirumab + Paclitaxel |
| **No Induction** | agenT-797 + Botensilimab + Balstilimab + Ramucirumab + Paclitaxel | Botensilimab (3 total doses) + Balstilimab + Ramucirumab + Paclitaxel |

Treatment continued until disease progression or intolerable toxicity

**Primary Endpoint:** ORR

**Secondary Endpoints:**

PFS/OS, DCR. duration of response, safety / tolerability

**Exploratory Endpoints:**
- Changes to immune cell subsets within tumor microenvironment
- Changes in peripheral T cell populations and cytokines

| Drug | Dose | Dose Frequency |
|---|---|---|
| agenT-797 | 1.4x10$^7$ cells/kg | Day 1 of treatment, cycle one only |
| Botensilimab[i] | 75 mg fixed dose | Day 1 of each 28-day cycle for three cycles |
| Balstilimab | 240 mg fixed dose | Days 1 and 15 of each 28-day cycle |
| Ramucirumab | 8 mg/kg | Days 1 and 15 of each 28-day cycle |
| Paclitaxel[ii] | 80 mg/m$^2$ | Days 1, 8, and 15 of each 28-day cycle |

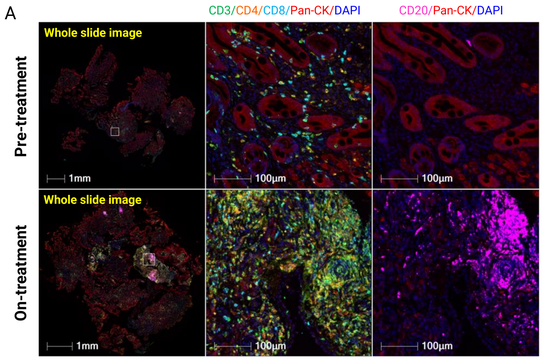
[i]Starting dose of BOT is 75 mg on day 1 of a 28-day cycle (three doses total). Following treatment of the first 10 patients, a planned safety review determined that all subsequently enrolled patients will be treated with botensilimab at 50 mg (dose level -1) and will begin treatment without an induction cycle. [ii]Patients may be treated with 70 mg/m$^2$ if deemed necessary.

## Patient Characteristics

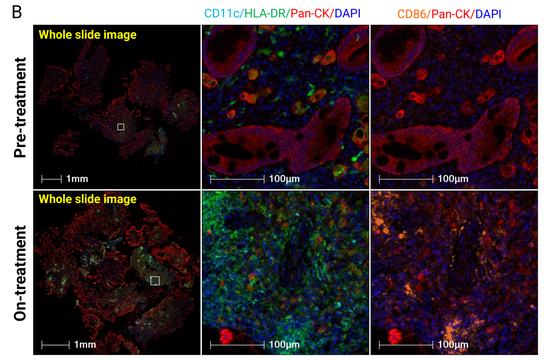
| Characteristics | Number of Patients (N = 15) |
|---|---|
| Median age (IQR), years | 63 (39-73) |
| Male<br>Female | 11 (73%)<br>4 (27%) |
| Esophageal / GEJ<br>Gastric | 11 (73%)<br>4 (27%) |
| Number metastatic sites<br>1<br>≥ 2 | <br>5 (33%)<br>10 (67%) |
| Liver metastases<br>Peritoneal metastases | 9 (60%)<br>5 (33%) |
| **Frontline Treatment:**<br>PD-1 + Chemotherapy<br>PD-1 + Trastuzumab + Chemotherapy<br>PD-1 + TKI + Chemotherapy<br>PD-1 + TIGIT + Chemotherapy<br>PD-1 + CLDN Ab + Chemotherapy<br>PD-1 + FGFR2 Ab/Placebo + Chemotherapy | <br>8 (53%)<br>2 (13%)<br>2 (13%)<br>1 (7%)<br>1 (7%)<br>1 (7%) |
| **Induction Treatment:**<br>AgenT-797 cells alone<br>AgenT-797 / Botensilimab / Balstilimab<br>No induction | <br>2 (13%)<br>5 (33%)<br>8 (53%) |

## Results

### Tumor immune infiltration following combination treatment (agenT-797/BOT/BAL)




### Activation of APCs within tumors following combination treatment (agenT-797/BOT/BAL)




### Summarized infiltration data from all treated patients

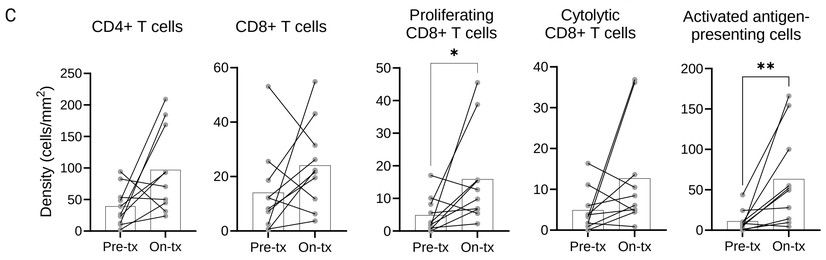
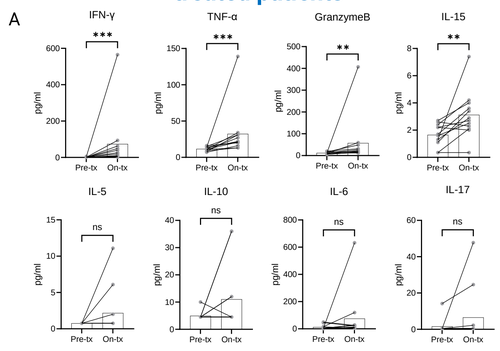



**Figure 1: Increased immune cell infiltration following treatment** (A-B) Multiplex immuno-flourescence on biopsies collected pre- and on-treatment (end of cycle 1) for a patient treated with an induction cycle of agenT-797 + BOT/BAL. Position of zoomed panels on whole slide image is indicted. (C) Data from all treated patients with pairwise biopsies (n=10). Statistical analysis using a Non-parametric Wilcoxson matched-pairs signed rank test on GraphPad Prism 10.

### Biomarkers of enhanced cytolytic function in all treated patients




### Sustained increase of IFN-γ in response to treatment with agenT-797 + BOT/BAL

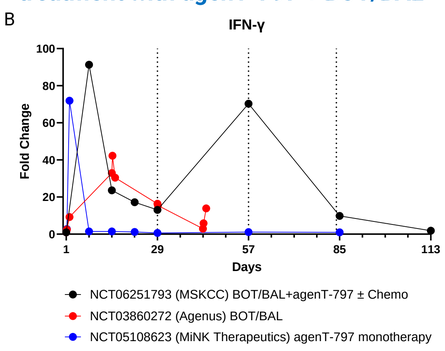



**Figure 2: Increased serum cytokines indicates activation of pro-inflammatory and cytolytic immune responses.** (A) Biomarker levels as measured by ELISA. For each marker, the On-tx value is defined as the peak concentration measured during cycle 1. Data from all patients with paired Pre-tx and On-tx serum samples (n=12). Statistical analysis using a Non-parametric Wilcoxson matched-pairs signed rank test on GraphPad Prism 10.
(B) Comparison of IFN-γ fold change following treatment with agenT-797 in combination with BOT/BAL with that of BOT/BAL (Agenus phase 1 data; advanced cancers; NCT03860272) or agenT-797 monotherapy (MiNK phase 1 data; solid tumors; NCT05108623).

### Increased Inducible Co-Stimulator (ICOS) and HLA-DR expression on peripheral T cells in all treated patients

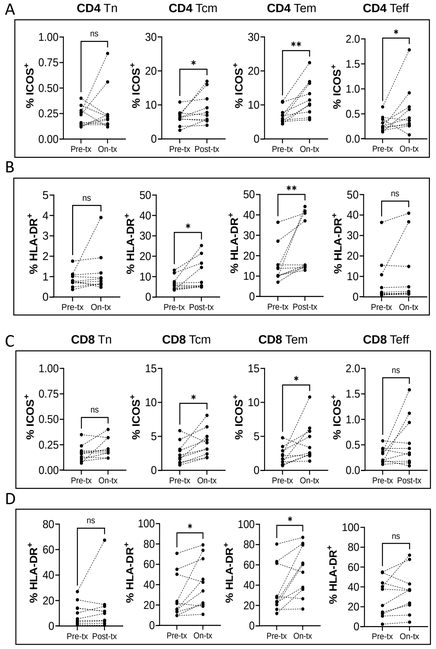



Figure 3: Treatment induced activation of peripheral T cells. Assessment by flow cytometry to assess the activation status of peripheral (A-B) CD4 and (C-D) CD8 T cells. Tn, naïve T cells; Tcm, central memory T cells; Tem, effector memory T cells; Teff, effector T cells. Data from all patients with paired Pre-tx and On-tx PBMC samples (n=10). Statistical analysis using a Non-parametric Wilcoxson matched-pairs signed rank test on GraphPad Prism 10.

## Conclusions and Future Directions

The combination of agenT-797/BOT/BAL with Ram/Pac drives significant immune modulation in patients with PD-1-refractory GEC, characterized by:
- Robust tumor T cell infiltration and intra-tumoral APC activation
- Increased activation of circulating effector-memory and effector T cells
- Enhanced IFN-γ response, indicative of iNKT cell, T cell, and NK cell activation
- A cytokine release profile suggestive of heightened cytolytic function

These findings highlight the therapeutic potential of agenT-797 in combination with BOT/BAL to overcome resistance in PD-1-refractory tumors and drive meaningful clinical benefit.

**1)** Wilke H, Muro K, Van Cutsem E et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. Lancet Oncol. 2014 Oct;15(11):1224-35. **2)** Hadfield MJ, Safran H, Purphoo MA, Grossman JE, Buell JS, Carneiro BA. Overcoming resistance to programmed cell death protein 1 (PD-1) blockade with allogeneic invariant natural killer T-cells (iNKT). Oncogene. 2024 Mar;43(10):758-762. **3)** Bullock AJ, Schlechter BL, Fakih MG et al. Botensilimab plus balstilimab in relapsed/refractory microsatellite stable metastatic colorectal cancer: a phase 1 trial. Nat Med. 2024 Sep;30(9):2558-2567.