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Poster #470

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BOTENSILIMAB, A NOVEL FC-ENHANCED ANTI-CTLA-4 ANTIBODY ENHANCES T CELL: APC FUNCTIONALITY AND PROMOTES SUPERIOR ANTI-TUMOR IMMUNITY

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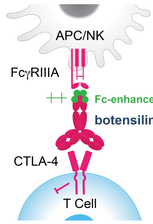
Botensilimab demonstrates unprecendented clinical activity by enhancing innate and adaptive immune activation



Design:

- Enhanced blockade of CTLA-4
- Improved binding to activating FcγRs on APCs and NK cells
- Reduced complement binding

Function (relative to first-generation anti-CTLA-4):

- ↑ T cell priming/activation[1] and memory formation
- ↑ Intratumoral Treg depletion[1]
- ↑ Frequency of activated APCs
- ↓ Complement mediated toxicity

Clinical responses across 9 different solid tumors, including in cold and I-O refractory cancers, and in prior anti-PD-1 +/- CTLA-4 failures[2,3]

Here we show that botensilimab leverages novel mechanisms of action to extend curative benefits of I-O to 'cold' and poorly immunogenic tumor types and promotes more effective immune activation in a large cohort of patients with advanced cancer (NCT03860272)

Botensilimab^{ms} promotes superior efficacy to first-generation anti-CTLA-4 Extends curative benefits of I-O to 'cold' and poorly immunogenic mouse tumors

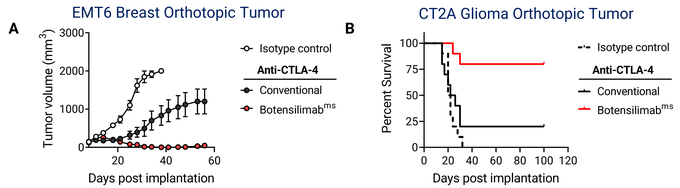


Figure 1. **(A)** Tumor growth (mean ± SEM) in BALB/c mice bearing EMT6 breast carcinoma in the mammary fat pad (~60 mm^3; n=10 mice/group) were treated with 100 µg of a mouse surrogate of botensilimab (botensilimab^{ms}; Fc-enhanced anti-CTLA-4, clone 9D9, mIgG2b.DLE), first-generation anti-CTLA-4 (clone 9D9, mIgG2b) or isotype control (clone MPC-11, mIgG2b) antibodies once a week for three weeks. **(B)** Survival of C57BL/6 mice bearing CT2A glioma tumors (n=10 mice/group) implanted intracranially and treated intravenously with 100 µg of the indicated antibodies twice a week for 2 weeks.

Botensilimab^{ms} promotes superior combination potential with anti-PD-1 or chemotherapy in treatment-resistant tumor models

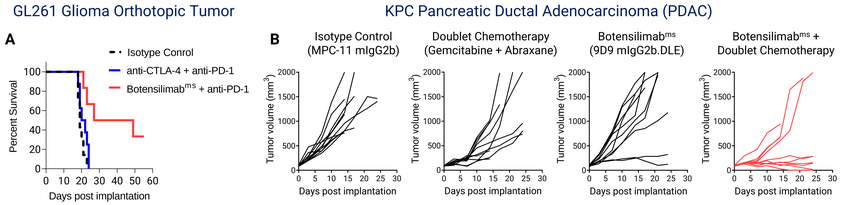


Figure 2: **(A)** Survival of C57BL/6 mice bearing GL261 glioma tumors implanted intracranially and treated with ultrasound and botensilimab^{ms} (clone 9D9, mIgG2b.DLE) or first-generation anti-CTLA-4 (clone 9D9, mIgG2b) in combination with anti-PD-1 (RMP1-14) or isotype control antibodies twice a week for 2 weeks. **(B)** C57BL/6 mice implanted subcutaneously with KPC (KrasG12D, P53-/- Pdx1-Cre) tumor fragments (100 mm^3) isolated from KPC tumor-bearing mice, were treated with botensilimab^{ms} or isotype control antibodies alone or in combination with doublet chemotherapy (Gemcitabine and Abraxane). Antibodies (100 µg/dose) were administered on day 1 intraperitoneally (i.p), twice a week for three weeks. Chemotherapy-treated mice received gemcitabine (70 mg/kg) i.p and Abraxane (25 mg/kg) intravenously on days 1 and 4.

References:

1. Waight et al. *Cancer Cell*. 2018;33(6): 1033-1047
2. El-Khoueiry AB. SITC 2021 Annual Meeting. Poster #479.
3. El-Khoueiry AB. ESMO GI 2022

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Botensilimab enhances T cell priming and activation superior to first-generation anti-CTLA-4

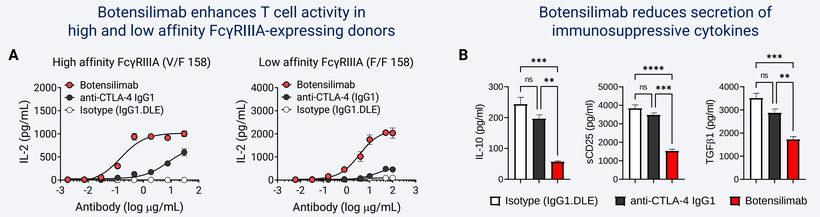

Figure 3. Staphylococcal Enterotoxin A (SEA)-stimulated healthy donor PBMCs were treated with botensilimab, parental anti-CTLA-4 IgG1 (first-generation), or isotype control antibodies. **(A)** IL-2 secretion from donors heterozygous for high affinity FcγRIIIA V/F or low affinity FcγRIIIA F/F haplotype were measured by AlphaLISA. **(B)** IL-10, sCD25, and TGFβ1 were measured in supernatants by Luminex. Error bars indicate standard error of the mean (SEM). Statistical significance was calculated using a one-way ANOVA. ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001.

Botensilimab increases the frequency of activated APCs and depletes Tregs, distinct from first-generation anti-CTLA-4

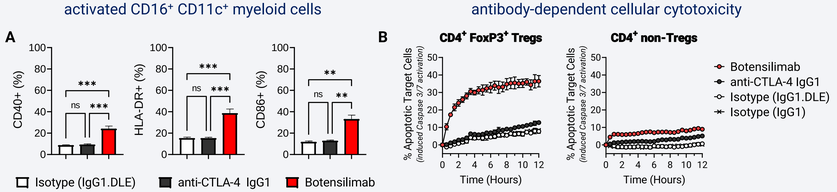

Figure 4 **(A)** SEA-stimulated healthy donor PBMCs were treated with botensilimab, parental anti-CTLA-4 IgG1 (first-generation), or Fc-engineered isotype control antibodies. Activation of CD16$^+$ CD11c$^+$ myeloid cells was assessed by flow cytometry. **(B)** Primary CD4$^+$ FoxP3$^+$ Tregs or CD4$^+$ non-Tregs were co-cultured with NK92 cells engineered to express FcγRIIIA in the presence of botensilimab, first-generation anti-CTLA-4 IgG1, or isotype control antibodies. Live cell imaging was performed by high-content confocal microscopy. Error bars indicate SEM. Statistical significance was calculated using a one-way ANOVA. ** p ≤ 0.01; *** p ≤ 0.001.

Botensilimab reduces the frequency of intratumoral Tregs and increases CD8$^+$ T cell infiltration in patients with advanced solid cancers

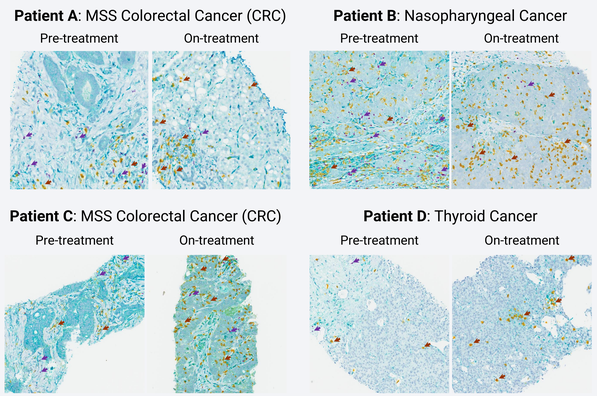

Figure 5: Triplex chromogenic immunohistochemistry on pre-treatment and on-treatment FFPE tumor biopsies from patients treated with botensilimab monotherapy or in combination with balstilimab (anti-PD-1). **Patient A, MSS-CRC** (1.0 mg/kg botensilimab Q6W + 3 mg/kg balstilimab Q2W); **Patient B, nasopharyngeal cancer** (2.0 mg/kg botensilimab Q3W); **Patient C, MSS-CRC** (2.0 mg/kg botensilimab Q6W + 3 mg/kg balstilimab Q2W); **Patient D, thyroid cancer** (2.0 mg/kg botensilimab Q3W). On-treatment biopsies were taken on cycle 2 Day 1 for Q6W cohort or cycle 3 Day 1 for Q3W cohort. CD8 (yellow), FoxP3 (purple) CD68 (turquoise) are shown. Tregs were defined as FoxP3$^+$/CD8$^-$ cells.

Botensilimab expands new peripheral TCR clones in patients with advanced solid cancers

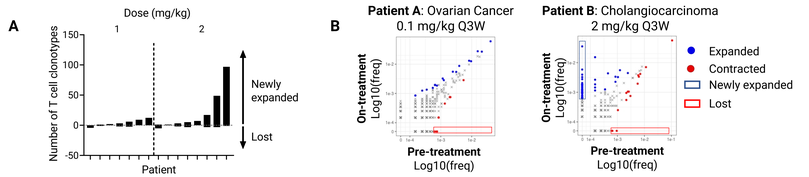

Figure 6. **(A)** Peripheral TCR-sequence and number of newly expanded (↑) and lost (↓) clonotypes based on differential abundance analysis between baseline (pre-treatment; cycle 1 day 1) and 3-4 weeks post-dose from patients treated with 1 or 2 mg/kg of botensilimab Q3W or Q6W. CDR3 regions of human T-cell receptor (TCR) β chains sequencing was performed using the immunoSEQ assay (Adaptive Biotechnologies). **(B)** Representative clonotype abundance from patients treated with 0.1 mg/kg or 2mg/kg Q3W of botensilimab. Blue dots (•) indicate expanded T cell clones; red dots (•) indicate contracted T cell clones.

Botensilimab enhances the frequency and activation of effector and memory T cells in patients with advanced solid cancers

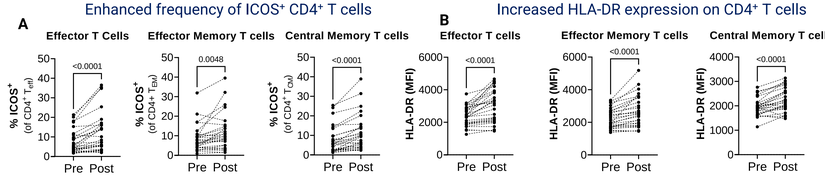

Figure 7. Pre- and post-treatment blood samples were obtained from patients with advanced solid cancers (n = 28) treated with 1 mg/kg or 2 mg/kg botensilimab. Post-treatment blood was drawn 7 days after the first dose. Samples were analyzed by flow cytometry from frozen samples to assess **(A)** the frequency of ICOS$^+$, and **(B)** HLA-DR expression on CD4$^+$ effector (CXCR3$^+$), effector memory (CD45RO$^+$ CCR7$^-$) and central memory (CD45RO$^+$ CCR7$^+$) T cells. Statistical significance was calculated using a Wilcoxon matched-pairs signed rank test.

Clinical benefit from botensilimab is independent of tumor neoantigen burden and FcγRIIIA genotype

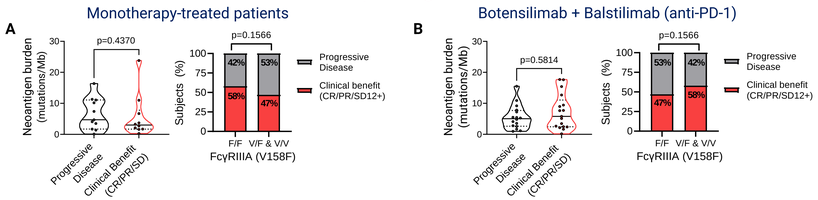

Figure 8. Response rate by tumor neoantigen burden (TNB) and FcγRIIIA genotype at baseline (pre-treatment) in patients with advanced solid cancers treated with **(A)** botensilimab monotherapy (TNB: n=21; FcγRIIIA genotype: n=31) or **(B)** botensilimab in combination with balstilimab (TNB: n=33; FcγRIIIA genotype: n=48). Clinical benefit was defined as patients who had complete response (CR), partial response (PR) or stable disease (SD) for ≥12 weeks as per RECIST 1.1. Statistical significance was calculated using a Mann-Whitney test. For FcγRIIIA genotype analysis statistical significance was calculated using a two-tailed Fisher's exact test.

Summary

- Botensilimab demonstrates superior preclinical and clinical (**Wilky et al., SITC 2022, Abstract: #778**) monotherapy and combination activity in 'cold' and poorly immunogenic tumor types.
- Botensilimab leverages novel Fc-dependent mechanisms of action to enhance innate and adaptive immune functions superior to first-generation or conventional anti-CTLA-4 mAbs.
- Botensilimab reduces intratumoral Tregs, enhances the frequency of peripheral activated effector and memory T cells and promotes emergence of new TCR clones.
- Botensilimab is advancing in Phase 1/2 clinical studies alone and in combination with balstilimab (anti-PD-1; NCT03860272), AGEN2373 (anti-CD137; NCT04121676) and AGEN1571 (anti-ILT2; NCT05377528).