FC-ENHANCED ANTI-CTLA-4 ANTIBODY, BOTENSILIMAB^{MS}, ENHANCES THE EFFICACY OF MULTIPLE THERAPEUTIC MODALITIES IN IMMUNOTHERAPY-REFRACTORY TUMOR MODELS

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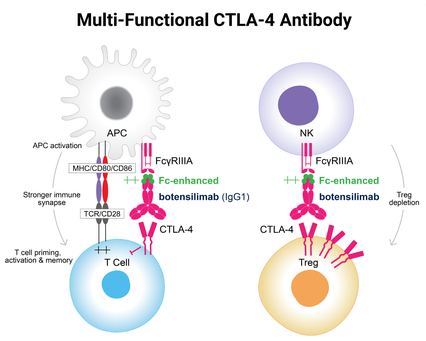
Botensilimab is a Novel Innate & Adaptive Immune Activator



1. **Enhances T Cell Priming, Activation and Memory**
   Primes and expands a diverse set of tumor-reactive T cells that can infiltrate the tumor; establishes memory[1,2]
2. **Activates APCs/Myeloid cells**
   Upregulates co-stimulatory and antigen presentation machinery on dendritic cells and other myeloid cells[2]
3. **Reduces Regulatory T Cells**
   Removes intratumoral regulatory T cells that suppress the activity of cytotoxic T cells[2]
4. **Avoids Difficult-To-Treat Adverse Events**
   Mitigates complement-mediated toxicities associated with conventional anti-CTLA-4 therapy[2]

Key Findings: Preclinical evaluation of botensilimab-based combinations with standard-of-care and novel therapies demonstrates significantly improved response rates and survival across multiple immunotherapy-refractory and difficult-to-treat mouse tumor models, including glioblastoma, melanoma, colorectal, pancreatic and breast cancers, providing a strong rationale for further clinical investigation.

Botensilimab^{ms} promotes superior anti-tumor efficacy in combination with focal radiation in checkpoint-blockade resistant GBM and breast cancer

GL261 (anti-PD-1 resistant) Glioblastoma

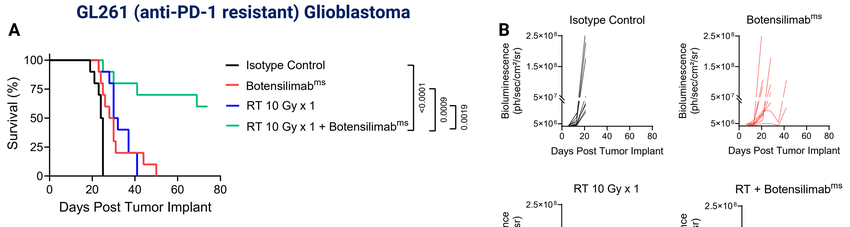


Figure 1: C57BL/6 mice with intracranial PD-1-resistant GL261 glioma cells received focal radiotherapy (RT, 10 Gy) on day 6 post-implantation. Botensilimab mouse surrogate (botensilimab^{ms}; 100 µg) was administered from day 7, twice weekly for three weeks. Graphs show, **(A)** Overall survival , analyzed by Log-rank (Manel-Cox) test and **(B)** Tumor bioluminescence in individual mice.

4T1 Breast Carcinoma

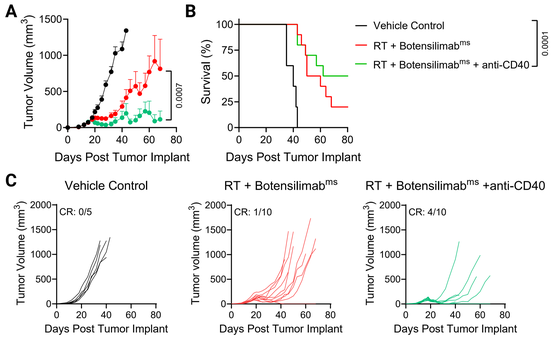


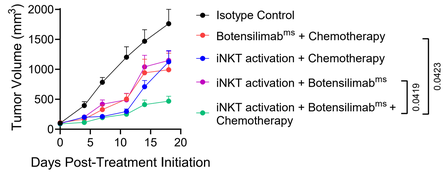
Figure 2: BALB/c mice with subcutaneous 4T1 tumors (50-70 mm³) were randomized (n=5-10/group) and treated with: radiotherapy (RT, 8 Gy x 3 days), botensilimab^{ms} (100 µg, with 2nd RT dose + 2 weekly doses), and/or CD40-agonist mAb (100 µg, 2 days post-RT + every other day x 3). Graphs show, **(A)** Mean tumor volume, analyzed by paired t-test; $p < 0.05$, **(B)** Overall survival, analyzed by Log-rank (Manel-Cox) test; and **(C)** Individual tumor growth kinetics. CR: complete response.

Combination Rationale:

1. Radiation therapy (RT) activates cGAS/STING pathway, enhancing antigen presentation and CD8 T cell priming.
2. Botensilimab upregulates CD40 on CD11c+ cells[2], addressing suboptimal CD40-CD40L signaling that limits RT effectiveness[3]
3. Botensilimab-enhanced T cell priming and Treg depletion may boost RT-induced in situ immunization[2,4].

Botensilimab^{ms} enhances the efficacy of iNKT activation and chemotherapy in treatment-resistant pancreatic ductal adenocarcinoma

KPC Pancreatic Ductal Adenocarcinoma (PDAC)



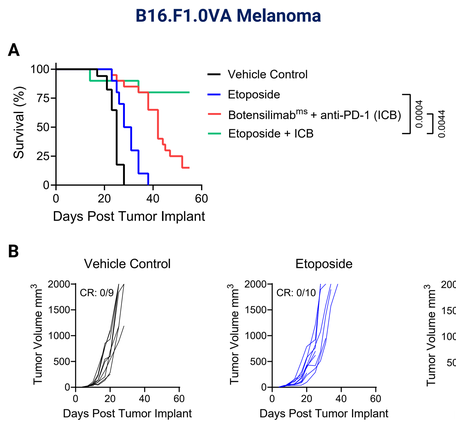
Combination Rationale:

1. α-GalCer-activated iNKT cells promote potent anti-tumor activity, producing IFN-γ, IL-4, and IL-12, and cytolytic effects.
2. Gemcitabine/Nab-paclitaxel chemotherapy induces immunogenic cell death (ICD), enhancing T cell priming.
3. Together with botensilimab, this triple combination maximizes anti-tumor immunity by complementarily activating innate and adaptive immune responses.

Figure 3: C57BL/6 mice were implanted subcutaneously with minced KPC (Kras^{G12D}, Trp53$^{-/-}$ Pdx1-Cre) tumor fragments derived from KPC-tumor bearing mice. Once tumors reached ~100 mm³, mice were randomized and treated with biweekly intraperitoneal (i.p.) injections of either isotype control or botensilimab^{ms}, (100 µg) in combination with chemotherapy and a single dose of α-Galactosylceramide (α-GalCer) to activate iNKT cells. Chemotherapy consisted of i.p. gemcitabine (70 mg/kg) and intravenous Nab-paclitaxel (25 mg/kg) administered on days 1 and 4.

Botensilimab^{ms} and anti-PD-1 enhance the efficacy of topoisomerase II inhibitor etoposide in poorly immunogenic, refractory melanoma

B16.F1.0VA Melanoma



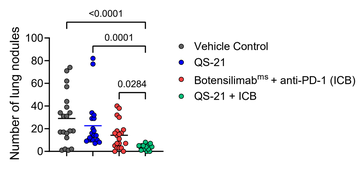
Combination Rationale:

1. Etoposide, a DNA-damaging agent, induces an 'injury response', enhancing antigen cross-presentation and T cell activation.
2. Co-administration of botensilimab is expected to enhance T cell priming and effector function against tumor cells

Figure 4: C57BL/6 mice with subcutaneous B16.F1.OVA tumors (~60 mm³) were treated with biweekly i.p. injections of botensilimab^{ms} (100 µg) and anti-PD-1 mAb (200 µg; RMP1-14), combined with three daily i.p. doses of etoposide (400 µg). Graphs show, **(A)** Overall survival, analyzed by Log-rank (Manel-Cox) test, and **(B)** individual tumor growth kinetics.

Local immune priming with QS-21, a saponin-based adjuvant, combines with Botensilimab^{ms} and anti-PD-1 to enhance metastatic tumor control

4T1 Breast Carcinoma Lung Metastases



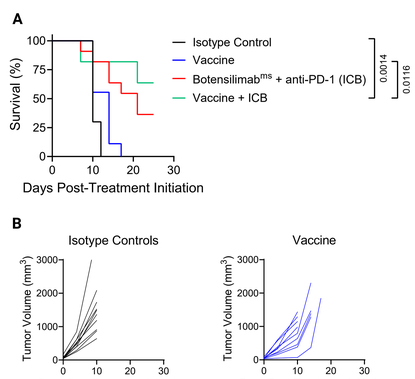
Combination Rationale:

1. QS-21 activates the inflammasome, producing IL-1β, IL-18, and other pro-inflammatory cytokines to enhance innate immunity[5].
2. QS-21's membrane-disrupting properties facilitate tumor antigen release and cross-presentation to T cells[5], complimenting immune checkpoint blockade therapy

Figure 5: BALB/c mice with orthotopic 4T1 tumors (~80mm³, day 8) received intratumoral (IT) QS-21 (5 µg) and/or systemic immune checkpoint blockade (ICB) therapy (botensilimab^{ms}, 100 µg + anti-PD-1, 200 µg). Lung metastases were quantified on day 32. Data analyzed by one-way ANOVA, followed by a Kruskal-Wallis multi-comparisons test.

Neoantigen vaccination in combination with Botensilimab^{ms} and anti-PD-1 promotes superior tumor control

B16.F10 Melanoma



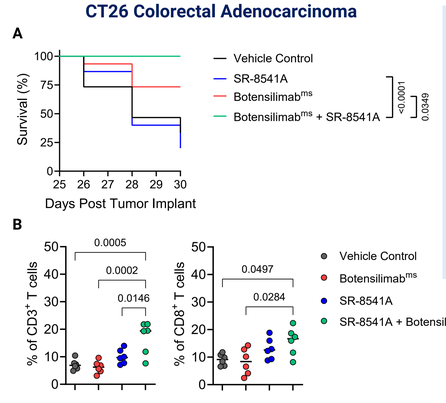
Combination Rationale:

1. B16.F10 tumors are immunologically "cold," with limited immune infiltration but substantial suppressive regulatory T cell (Treg) presence.
2. Botensilimab depletes intratumoral Tregs, and in combination with a polyvalent neoantigen vaccine (GAd), enhances priming and activation of tumor-specific T cell responses.

Figure 6: C57BL/6 mice with subcutaneous B16.F10 tumors (60-80mm³) received intramuscular GAd neoantigen vaccine (5x10^{8} viral particles), weekly botensilimab^{ms} (100 µg, i.p.), and biweekly anti-PD-1 (200 µg, i.p.). Graphs show, **(A)** Overall survival analyzed by Log-rank (Manel-Cox) test and **(B)** Individual tumor growth kinetics.

Botensilimab enhances the anti-tumor efficacy of ENPP1 inhibition-mediated STING pathway activation

CT26 Colorectal Adenocarcinoma



Combination Rationale:

1. ENPP1 negatively regulates STING pathway activation by degrading cGAMP.
2. High ENPP1 levels correlates with reduced immune cell infiltration and resistance to chemo- and immunotherapy.
3. ENPP1 inhibition with SR-8541A may sensitize tumors to botensilimab by enhancing STING-mediated immune activation.

Figure 7: BALB/c mice with subcutaneous CT26 tumors (75-100 mm³) received 0.2 mg/kg oral SR-8541A twice daily (days 8-30) and a single dose of botensilimab (100 µg, i.p., day 12). Graphs show, **(A)** Overall survival, analyzed by Log-rank (Manel-Cox) test and **(B)** T cell infiltration by IHC/QPath analysis. Data analyzed by one-way ANOVA, followed by a Tukey's multi-comparisons test.

Summary

- Botensilimab^{ms} combines with both standard-of-care and novel therapies to enhance anti-tumor immunity across multiple 'cold' and treatment-refractory preclinical cancer models, including glioblastoma, melanoma, colorectal, pancreatic, and breast cancers.
- Combination strategies presented here address key barriers in the tumor microenvironment (TME) by blocking tumor survival pathways, re-polarizing pro-tumor immune cells, enhancing anti-tumor effector cell functionality, and selectively targeting the tumor stroma.
- Consistent with these preclinical findings, clinical trials are evaluating botensilimab in combination with SR-8541A (NCT06589440), Gemcitabine/Abraxane (NCT05630183), KRAS-targeted vaccine (NCT06411691), Doxorubicin/Ultrasound (NCT05864534) and iNKT cell therapy (NCT06251973).

References: **1.** Waight et al. *Cancer Cell*, 2018; **2.** Chand et al. *Cancer Discovery*, 2024; **3.** Rudqvist et al., *Nature Communications*, 2023; **4.** Rudqvist et al., Oncoimmunology, 2023; **5.** Lv et al., *iScience*, 2024

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