

**BOTENSILIMAB MODULATES INNATE AND ADAPTIVE GENE EXPRESSION PROGRAMS RESULTING IN SUPERIOR IMMUNE STIMULATION RELATIVE TO A FIRST-GENERATION ANTI-CTLA-4 ANTIBODY**<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0941>

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**Background** Botensilimab is a next-generation fragment crystallizable (Fc)-enhanced anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibody that promotes optimized T cell priming, activation, memory formation, and intratumoral regulatory T cell depletion. In patients with advanced solid tumors, botensilimab alone and in combination with balstilimab (anti-programmed cell death protein 1 [PD-1] antibody) demonstrated durable clinical responses in nine different immunotherapy-resistant or poorly immunogenic tumor types. We hypothesize that botensilimab engages multiple immune cell types, including macrophages, dendritic cells (DCs) and natural killer (NK) cells through its enhanced Fc region, to drive deep and broad clinical responses. Here, we applied high-resolution single-cell profiling to demonstrate the differentiated molecular mechanisms of botensilimab relative to a first-generation IgG1 (non-Fc-enhanced) anti-CTLA-4 antibody (bot-IgG1).

**Methods** Human *ex vivo* peripheral blood mononuclear cells were stimulated with staphylococcus enterotoxin antigen followed by treatment with botensilimab, bot-IgG1 or an IgG1 Fc-enhanced isotype control. Secreted cytokines and single-cell measurements of RNA and protein were evaluated over multiple time points and across various immune cell populations. Treatment-specific changes were evaluated within and across donors.

**Results** Enhanced interleukin-2 (IL2) secretion in botensilimab-treated cultures relative to bot-IgG1 or isotype control was confirmed across donors. To identify the upstream mechanisms of botensilimab-induced immune activation, RNA and protein expression patterns were interrogated at different time points leading up to maximum IL2 secretion. Sub-clustering of myeloid cells revealed monocyte-, macrophage-, and DC-like populations. Within the myeloid fraction, botensilimab increased the frequency of DCs relative to monocytes and macrophages, and upregulated innate immune signaling pathways distinct from that of first-generation IgG1 anti-CTLA-4. Notably, botensilimab increased the frequency of leukocyte immunoglobulin like receptor B1 (LILRB1 or ILT2)-expressing myeloid cells. Sub-clustering of lymphocytes revealed CD4, CD8, Treg, NK, NKT, and B cell-like populations. Botensilimab increased activation and effector gene expression, including granzyme B and tumor necrosis factor receptor superfamily 9 (TNFRSF9; CD137) in lymphocyte subsets superior to that observed with an IgG1 anti-CTLA-4.

**Conclusions** The differentiated ability of botensilimab to reshape myeloid transcriptional programs is consistent with its clinical activity in poorly immunogenic tumors where improved antigen presentation or cross-priming could potentiate responses. Increased expression of LILRB1 and TNFRSF9 in botensilimab-treated immune subsets supports the combination strategies with AGEN1571 (anti-ILT2) and AGEN2373 (anti-CD137) currently under clinical investigation.

**Ethics Approval** This study was approved by WCG IRB Ethics Board; approval number 120160614.