Arginase Inhibitor CB-1158 Alleviates Immunosuppression And Enhances Anti-Tumor Responses As A Single Agent And In Combination With Other Immunotherapies

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Abstract

Background. T cells and natural killer (NK) cells require L-arginine for proliferation. Arginine depletion by arginases in the tumor microenvironment induces immunosuppression and is associated with tumor immune evasion. Arginase is expressed by myeloid-derived suppressor cells (MDSCs) and granulocytes, and its pharmacological inhibition is expected to resolve arginase-mediated immunosuppression, leading to anti-tumor responses.

Materials and Methods. We developed CB-1158, a potent and selective small molecule inhibitor of arginase (IC50 = 0.9 nM). The activity of CB-1158 was examined ex vivo using immune cells isolated from healthy volunteers or cancer patients, and in vivo using murine syngeneic tumor models. Arginase abundance in cancer patient plasma and in tumor tissue microarrays was also examined.

Results. In a coculture system of T cells with granulocytes or MDSCs, CB-1158 reverses granulocyte- or MDSC-mediated immunosuppression by blocking arginase depletion, thereby allowing T cells to proliferate. T cells activated in the presence of granulocyte-conditioned media show suppressed production of cytokines involved in Th1-type adaptive immunity, and this effect is reversed by the addition of CB-1158. CB-1158 has high oral bioavailability and is very well tolerated. In tumor-bearing mice, twice daily dosing of CB-1158 causes dose-dependent pharmacodynamic increases in arginase and tumor arginase levels associated with single agent anti-tumor efficacy in multiple syngeneic models. The antitumor efficacy of CB-1158 is abrogated in immunocompromised mice or via depletion of either CB-2 T cells or NK cells, confirming an immune-mediated mechanism of action. Moreover, CB-1158 enhances CD8 T cell infiltration into tumors and increases expression of Th1 cytokines, T cell and NK cell activation markers, and interferon-inducible genes in the tumor microenvironment.

The immunomodulatory activity of CB-1158 supports the potential of its combination with other immunotherapies and/or standard-of-care therapies. CB-1158 enhances the anti-tumor efficacy of adoptive cell therapies such as T cell therapy in the CB-1158 model. CB-1158 also enhances checkpoint inhibitors, including anti-PD-L1 in the CB-1158 model. Moreover, CB-1158 enhances the antitumor efficacy of chemotherapeutics with immunomodulatory activity such as Gemcitabine in several models. To assess the clinical potential of CB-1158, the abundance of arginase in tumors and plasma from cancer patients across multiple cancer histotypes was surveyed. Arginase-expressing granulocytes/immune-stains are abundant in multiple tumor types. Plasma arginase levels are elevated in cancer patients compared to healthy controls, and are associated with decreased plasma arginase. In an ongoing Phase I clinical trial (NCT03203394) for patients with solid tumors, CB-1158 shows significant PK/PD effects as a monotherapy at the first dose of 50 mg BID.

Conclusions. These results support the clinical development of CB-1158, a first-in-class arginase inhibitor, as a novel immunomodulatory agent antagonizing myeloid-mediated immunosuppression alone or in combination with other immunotherapies.

CB-1158 Synergizes with Adoptive T Cell Therapy

Figure 6: (A) B16F10 cells were implanted in C57Bl/6 mice. Non-arginase inhibitor treatment (Cyclophosphamide 250 mg/kg and Fludarabine 50 mg/kg) was dosed (i.p. on day 7 (orange arrow)) to all groups. Pretreatment of CD8 T cells (1 x 106) were adoptively transferred i.v. on days 10 and 11 in mice receiving T cells (i.e. effect on tumor growth; data not shown). CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. (N = 9-10 per group. *** P < 0.001). (B) Survival curves. P = 0.0137 by Mantel-Cox test (experiment is ongoing).

CB-1158 Synergizes with Adoptively-Transferred Antigen-Specific T cells to Inhibit Tumor Growth

Figure 4: (A) B16F10 cells were implanted in C57Bl/6 mice. Non-arginase inhibitor treatment (Cyclophosphamide 250 mg/kg and Fludarabine 50 mg/kg) was dosed (i.p. on day 7 (orange arrow)) to all groups. Pretreatment of CD8 T cells (1 x 106) were adoptively transferred i.v. on days 10 and 11 in mice receiving T cells (i.e. effect on tumor growth; data not shown). CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. (N = 9-10 per group. *** P < 0.001). (B) Survival curves. P = 0.0137 by Mantel-Cox test (experiment is ongoing).

CB-1158 Combines with Checkpoint Blockade

Figure 9: (A) Immunofluorescent staining (white arrow) shows CD8+ and CD25+ T cells in a tumor section from a patient with head & neck cancer. (B) Percentage of arginase 1 positive cells that co-express the immune checkpoint marker CD25 or macrophage marker CD163 in tumor tissue microarrays as determined by quantitation of Multispan immunofluorescence. (C) Percent survival. P = 0.01 by Mantel-Cox test (experiment is ongoing).

CB-1158 Combines with Anti-PD-1, anti-CTLA-4 and IDO Inhibitors to Inhibit Tumor Growth

Figure 5: (A) Proliferation (MTS assay) of CD3+CD8+CD25−/− CD4+CD25−/− stimulated human T cells after 48-hour incubation in the presence or absence of neutrophils or CB-1158 (10 µM). (B) Human T cells from a healthy donor were cultured in tissue media pre-conditioned for 2 days with granulocytes isolated from a patient with head & neck cancer. The media was collected and analyzed for the indicated analytes by Cytometric Bead Array.

Higher Arginase and Lower Arginine Levels are Observed in Cancer Patient Plasma

Figure 10: (A) Arginase 1 protein levels in plasma from healthy donors and cancer patients as determined by ELISA. (B) Plasma arginine concentrations in healthy donors and cancer patient samples as determined by LC/MS (*** P < 0.001; ** P < 0.01; vs. healthy).

CB-1158 Elevates Plasma Arginine in Patients at the Lowest Dose Level in Trial CX-1158-101

Figure 11: (A) Plasma levels of CB-1158 in the first dose cohort (50 mg BID) of clinical trial CX-1158-101 (ClinicalTrials.gov NCT03203394) evaluated after the first dose (Day 1) and at steady state (Day 15). (B) Fasted plasma arginine levels (absolute concentration or normalized to total plasma amino acid levels) in samples collected pre-dose (t rush) on the indicated days. Analytes were quantitated by LC/MS/MS.

Conclusions

CB-1158 potently inhibits arginase and reverses MDSC/granulocyte-induced suppression of T cell proliferation

CB-1158 increases tumor and plasma arginine levels and has single agent efficacy in multiple syngeneic and mouse models

CB-1158 increases inflammation and lymphocyte activation in the tumor microenvironment

Addition of CB-1158 to adoptive cell therapy, checkpoint blockade, or chemotherapy results in further tumor growth inhibition

Cancer patients have arginase-containing tumor immune cell infiltrates, increased plasma arginase, and decreased plasma arginine compared to healthy individuals

CB-1158 is currently in a Phase I clinical trial in solid tumor patients (CX-1158-101)

CB-1158 shows significant pharmacodynamic effects in patients at the first dose level (For more details on the design of clinical study CX-1158-101, see Poster # 155)