Arginase Inhibitor CB-1158 Elicits Immune-Mediated Anti-Tumor Responses as a Single Agent and Enhances the Efficacy of other Immunotherapies

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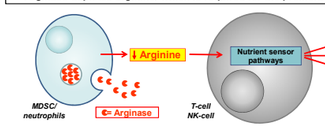
Abstract

Myeloid derived suppressor cells (MDSCs) and polymorphonuclear cells (PMNs) limit effective anti-tumor immune responses; however there are no approved clinical agents that directly antagonize the activity of these cells. One of the immunosuppressive mechanisms of MDSCs and PMNs is the expression and secretion of the enzyme arginase into the tumor microenvironment resulting in local depletion of the amino acid arginine, a key nutrient required by T-cells and natural killer (NK)-cells to proliferate and mount an effective anti-tumor response. To assess the potential of arginase inhibition as a therapeutic strategy, we surveyed the abundance of arginase in tumor and plasma from cancer patients across multiple histotypes. Consistent with previous reports, we observed that multiple tumor types have substantial arginase-expressing PMN infiltrates and that cancer patients have higher levels of plasma arginase and lower levels of plasma arginine compared to healthy controls. CB-1158 is a potent, selective, and orally-bioavailable small molecule inhibitor of arginase (IC_{50}=98 nM). In a co-culture system, neutrophils or patient-derived MDSCs strongly suppressed T-cell proliferation. The addition of CB-1158 blocked arginase activity, maintained arginine levels, and allowed T-cells to proliferate in the presence of MDSCs/PMNs, highlighting arginase as a prominent immunosuppressive mechanism of these myeloid cells. CB-1158 has high oral bioavailability in mice and rats. Twice-daily oral dosing of CB-1158 produced dose-dependent pharmacodynamic increases in plasma and tumor arginine levels and resulted in single-agent anti-tumor efficacy in several murine syngeneic tumor models including Lewis Lung Carcinoma (LLC), Madison-109 lung carcinoma, and B16F10 melanoma. Immunodepletion of either CD8⁺ T-cells or NK-cells partially antagonized the anti-tumor effect of CB-1158 in the LCC and B16F10 models indicating that CB-1158 acts by an immune cell-mediated mechanism. Consistent with immune-mediated anti-tumor efficacy, CB-1158 dosing of LLC-tumor bearing mice resulted in increases in tumor infiltrating CD8⁺ T-cells, increased levels of tumor Th1 T-cell cytokines, and increased expression of T-cell and NK-cell activation markers. Based on its novel mechanism of action, there is potential for CB-1158 to enhance the activity of other immunotherapies or standard-of-care therapeutics. We observed improved anti-tumor activity when CB-1158 was combined with either epacadostat or anti-PD-L1 in the B16F10 model, with low dose ionizing radiation in the Madison-109 model, and with gemcitabine in the LLC model. These results support the clinical development of CB-1158, a first-in-class arginase inhibitor, as a novel immuno-oncology agent targeting the immunosuppressive effects of tumor-infiltrating myeloid cells.

Arginase is an Immunosuppressive Enzyme

- Arginase-expressing MDSCs/neutrophils are present in multiple tumor types and associated with poor prognosis
- Arginase depletes arginine, which is required for the proliferation of T-cells and NK-cells



- Normal plasma arginine levels are 50-130 μM in humans
- Arginine levels <40 μM suppress T-cell proliferation
- A small decrease in arginine is immunosuppressive

CB-1158 Potently Inhibits Arginase

- CB-1158 is a potent and selective inhibitor of arginase 1 and 2 (minimal off-target activity at 50 μM in a Eurofins panel)
- CB-1158 inhibits extracellular arginase in plasma and cell lysates
- CB-1158 is not cytotoxic to cancer cell lines or primary T-cells (up to 1 mM)

Figure 1: (A) Schema of arginase reaction. **(B)** CB-1158 IC_{50} values for arginase inhibition using various sources of arginase. Activity was measured by urea and/or ornithine production

Human Arginase Source	IC_{50}
Arginase 1 (recombinant)	98 nM
Arginase 2 (recombinant)	274 nM
Granulocyte lysate	162 nM
Red blood cell lysate	116 nM
Hepatocyte lysate	139 nM
RCC patient plasma #1	127 nM
RCC patient plasma #2	174 nM

CB-1158 Reverses Myeloid Cell-Mediated T-cell Suppression

- In co-cultures, CB-1158 reverses the suppression of T-cell proliferation mediated by neutrophils
- CB-1158 reverses the suppression of T-cell proliferation by media conditioned with cancer patient neutrophils/G-MDSCs

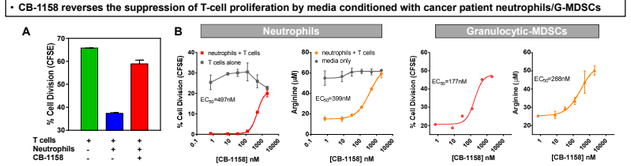


Figure 2: (A) Proliferation of αCD3/αCD28-stimulated human T-cells after a 4 day incubation in the presence or absence of neutrophils and CB-1158 (1.6 μM). **(B)** Proliferation of human T-cells from a healthy donor in media pre-conditioned for 2 days with either neutrophils isolated from a patient with head & neck cancer or CD66b⁺ G-MDSCs isolated from a patient with lung cancer. Arginine levels were measured in the media by LC/MS.

CB-1158 Elevates Arginine in Tumors

- Increasing oral doses of CB-1158 increase drug exposure in plasma and tumor
- Elevated exposure of CB-1158 increases plasma and tumor arginine levels

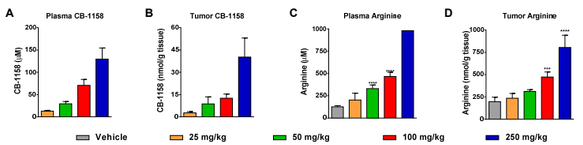


Figure 3: (A-B) Concentration of CB-1158 in (**A**) plasma or (**B**) Lewis Lung Carcinoma (LLC) tumor lysates from mice dosed orally with 4 doses of CB-1158 on a BID schedule. Samples were collected 2 h after the last dose of CB-1158 (N = 5 per group). **(C-D)** Arginine concentration in (**C**) plasma or (**D**) tumor of samples in **A-B**. (**** P < 0.0001; *** P < 0.001 vs. vehicle)

CB-1158 has Single-Agent Anti-Tumor Efficacy

CB-1158 Efficacy is Dependent on an Intact Immune System

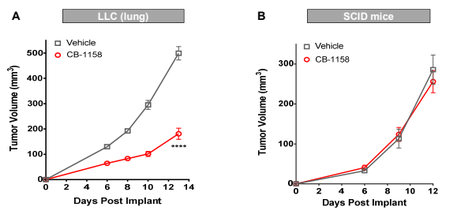
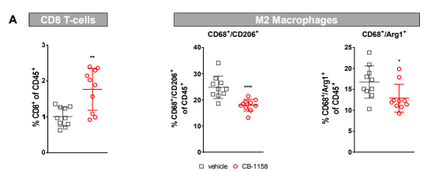


Figure 4: (**A**) C57.Bl/6 mice or (**B**) B6.CB17-Prkdc (SCID)/SzJ mice were implanted with Lewis lung carcinoma (LLC) cells. CB-1158 was dosed 100 mg/kg PO BID. (**** P < 0.0001 vs.vehicle)

CB-1158 Increases Inflammation in the Tumor

CB-1158 Increases CD8 T-cells and Decreases M2 macrophages



CB-1158 Increases T-cell gene expression

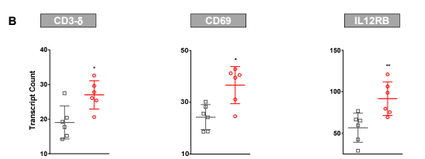


Figure 5: (A) Levels of immune cell subsets (determined by flow cytometry, N = 10 per group). **(B)** Levels of mRNA transcripts (determined by Nanostring, N = 6 per group) in LLC tumors from mice treated with vehicle or 100 mg/kg CB-1158 twice daily for 14 days. (** P < 0.01; * P < 0.05)

CB-1158 Enhances PD-L1 Blockade

CB-1158 in Combination with α-PD-L1 Increases Survival of CT-26 Tumor-Bearing Mice

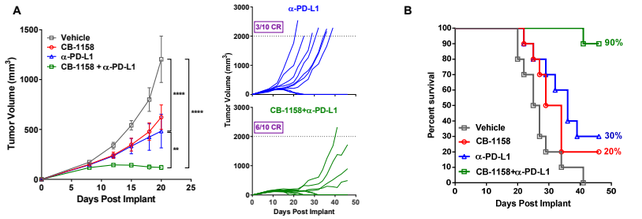


Figure 6: (A) CT26 cells were implanted in Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. α-PD-L1 (10F.9G2) was dosed at 5 mg/kg IP on days 5, 7, 9, 11, 13 and 15. Mice were sacrificed when tumor volumes reached 2000 mm³. *CR*: complete regression. (N = 10 per group; **** P < 0.0001; ** P < 0.01). **(B)** Survival curves. P < 0.0001 by Mantel-Cox test.

CB-1158 Enhances Gemcitabine Efficacy

CB-1158 in Combination with Gemcitabine Decreases Myeloid Cells and Increases NK cells and Efficacy in the CT-26 Model

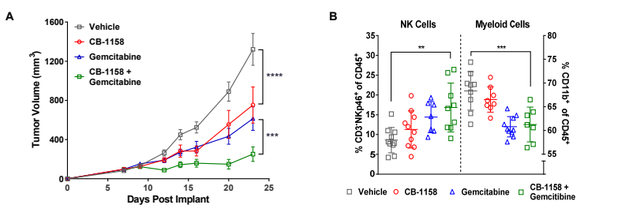


Figure 7: (A) CT26 cells were implanted in Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. Gemcitabine was dosed at 50 mg/kg IP on days 10 and 16. **(B)** Levels of immune cell subsets determined by flow cytometry in CT26 tumors from mice treated for 13 days. (N = 10 per group; **** P < 0.0001; *** P < 0.001; ** P < 0.01).

CB-1158 Synergizes with Adoptive T-Cell Therapy

CB-1158 in Combination with Adoptive T-Cell Transfer Increases Survival of B16-F10Tumor-Bearing Mice

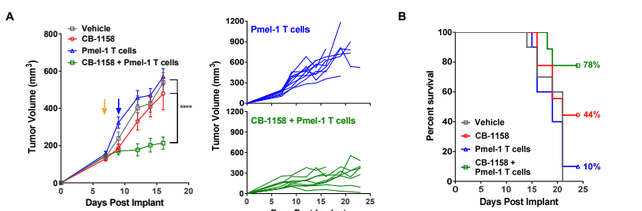
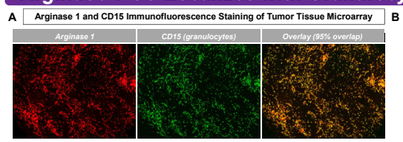


Figure 8: (A) B16-F10 cells were implanted in C57.Bl/6 mice. Non-myeloablative chemotherapy (cyclophosphamide 250 mg/kg and fludarabine 50 mg/kg) was dosed IP on day 7 (orange arrow) to all groups. Pmel-1 CD8 T-cells (1 x 10^6) were adoptively transferred I.V. on day 9 (blue arrow). Recombinant human IL-2 (200,000 U/i) was dosed IP BID on days 8, 9, 10 in mice receiving T-cells. CB-1158 was dosed 100 mg/kg PO BID. (N = 9-10 per group; **** P < 0.0001). **(B)** Survival curves. P = 0.0137 by Mantel-Cox test.

Arginase 1 Co-Localizes with Granulocytes in Human Tumors



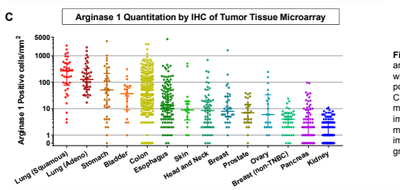


Figure 9: (A) Immunofluorescent staining (MultiOmyx) of arginase 1 and CD15 in a tumor section from a patient with head & neck cancer. **(B)** Percentage of arginase 1 positive cells that co-express the granulocyte marker CD15 or macrophage marker CD68 in tumor tissue microarrays as determined by quantitation of MultiOmyx immunofluorescence. **(C)** Human tumor tissue microarrays were stained with anti-arginase 1 antibody by immunohistochemistry. Arginase 1-positive infiltrating granulocytes were quantified by digital histopathology.

Higher Arginase 1 and Lower Arginine in Cancer Patient Plasma

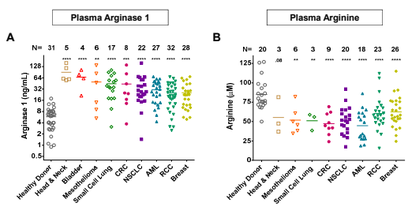


Figure 10: (A) Arginase 1 protein levels in plasma from healthy donors and cancer patients as determined by ELISA. **(B)** Plasma arginine concentrations in healthy donor and cancer patient samples determined by LC/MS. (**** P < 0.0001; *** P < 0.001; ** P < 0.01; vs. healthy)

CB-1158 Elevates Plasma Arginine in Patients

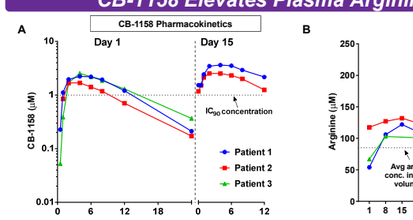


Figure 11: (A) Plasma levels of CB-1158 in the first dose cohort (50 mg BID) of clinical trial CX-1158-101 (ClinicalTrials.gov #NCT02903914) evaluated after the first dose (Day 1) and at steady state (Day 15). **(B)** Fasted plasma arginine levels (absolute concentration or normalized to total plasma amino acid levels) in samples collected pre-dose (trough) on the indicated days. Analytes were quantitated by LC/MS/MS.

Conclusions

- CB-1158 inhibits arginase and reverses MDSC/neutrophil-induced suppression of T-cell proliferation
- CB-1158 increases tumor arginine levels and has single agent efficacy in multiple syngeneic 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

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