Immuno-Oncology Agent CB-1158 is a Potent and Selective Arginase Inhibitor and Causes an Immune-Mediated Anti-Tumor Response

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Abstract

L-arginine is a critical metabolite for T-cell receptor signaling and subsequent T-cell proliferation, and depletion of arginine arrests T-cell growth. In the tumor microenvironment, infiltrating myeloid-derived suppressor cells (MDSCs), macrophages, and neutrophils produce arginase, which depletes local arginine concentrations and dampens T cell-mediated immune surveillance. Pharmacological inhibition of arginase is expected to restore arginine levels and allow T-cells to proliferate, thereby leading to an immune-mediated anti-tumor response. CB-1158 is a potent inhibitor of human arginase (IC_{50} = 98 nM). In culture, human granulocytes release arginase and deplete media arginine to levels that inhibit T-cell proliferation. In a co-culture system of human granulocytes and T-cells, CB-1158 potently blocks granulocyte-derived arginase activity, maintains extracellular arginine levels, and restores proliferation of T-cells.

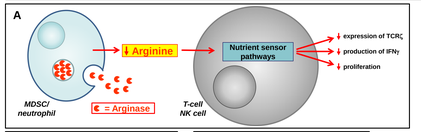
CB-1158 has high oral bioavailability in rodents and is very well tolerated. BID oral dosing of CB-1158 leads to dose-dependent pharmacodynamic increases in plasma and tumor arginine levels resulting in single agent anti-tumor efficacy in mouse syngeneic tumor models including Lewis Lung carcinoma (LLC) and Madison 109. The anti-tumor effects of CB-1158 are consistent with promoting a proinflammatory tumor microenvironment. Following CB-1158 treatment, multiple Th1 T-cell, NK-cell, and M1 macrophage-associated chemokines, cytokines, and activation markers are elevated in the LLC tumor microenvironment. The anti-tumor efficacy of CB-1158 requires an intact tumor microenvironment since CB-1158 has no effect on LLC cell growth in vitro. Furthermore, CB-1158 treatment of immunocompromised C57/SCID mice bearing LLC tumors has no anti-tumor effect, supporting an immune-mediated anti-tumor mechanism.

Immunosuppression in the tumor microenvironment can occur via multiple mechanisms, including arginine depletion, and our data support the combination of checkpoint inhibitors and arginase inhibition by CB-1158. In mice bearing LLC tumors, CB-1158 in combination with checkpoint inhibitors reduced tumor growth, increased the number of tumor infiltrating CD8+ T-cells, and increased the level of Th1/NK/M1-associated chemokines, cytokines, and activation markers in the tumor microenvironment. In mice bearing 4T1 tumors, a tumor type that is highly refractory to checkpoint inhibition, the combination of CB-1158 with anti-PD-1 and anti-CTLA-4 reduces tumor growth and lung metastases. These results support the 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.

Arginine Depletion Blocks T-cell and NK cell Activation

Arginase plays a major immunosuppressive role in human cancer:

- **Arginase-expressing MDSCs/neutrophils are found in many tumor types and are associated with poor prognosis**
- **Arginase depletes arginine, required for the activation/proliferation of T- and NK cells**
- **Arginase inhibition is a novel strategy to target myeloid-mediated immunosuppression**



- **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**

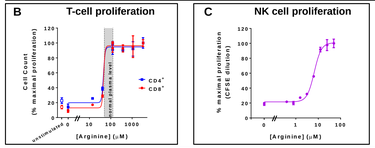
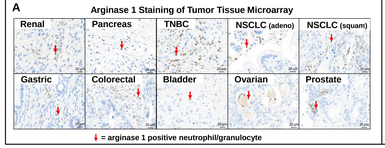
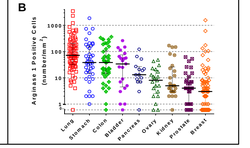


Figure 1: (A) Mechanism of immunosuppression by tumor infiltrating myeloid cells. **(B)** Proliferation of αCD3/αCD28-stimulated human T-cells measured by flow cytometry with αCD4 and αCD8 staining after a 4 day incubation in media containing varying concentrations of arginine. **(C)** Proliferation of human rIL-2-stimulated human NK cells measured by CFSE dilution after 3 day incubation in media containing varying concentrations of arginine.

Arginase 1 Expression in Cancer Patients

Arginase 1 is expressed in tumor-associated myeloid cells in cancer patients





Altered arginase 1 and arginine levels in cancer patient plasma

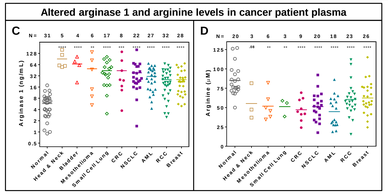
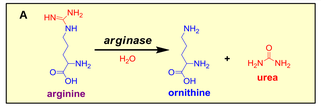


Figure 2: (A) Immunohistochemistry (IHC) staining for arginase 1 in sections of human tumor tissues (N = 12 tumor histologies analyzed). Representative images are shown (red arrows point to arginase-expressing myeloid cells). **(B)** Digital histopathology quantitation of IHC staining of tumor tissues. **(C)** ELISA determination of arginase 1 levels in plasma samples from normal donors and cancer patients. **(D)** Analysis of plasma arginine concentrations by LC/MS in normal and cancer patient samples. (**** P < 0.0001; *** P < 0.001; ** P < 0.01; ns, vs. normal)

CB-1158 Potently Inhibits Arginase

- **CB-1158 is a potent inhibitor of arginase 1 and 2**
- **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)**
- **CB-1158 is a selective arginase inhibitor (minimal off-target activity at 50 μM in a Eurofins panel)**



B

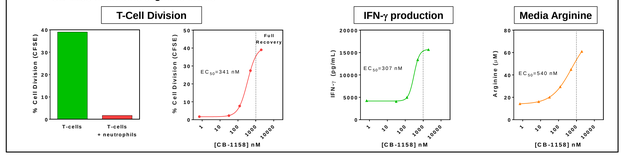
Human Arginase Source	IC_{50}
Arginase 1 (recombinant)	98 nM
Arginase 2 (recombinant)	274 nM
Neutrophil lysate	162 nM
Red blood cell lysate	116 nM
Hepatocyte lysate	139 nM
RCC patient plasma #1	127 nM
RCC patient plasma #2	174 nM

Figure 3: (A) Schema of arginase reaction. **(B)** IC_{50} values for inhibition of arginase reaction by CB-1158 using various sources of arginase as indicated. Activity was measured by urea and/or ornithine production using a dose titration of CB-1158.

CB-1158 Reverses Myeloid Cell Mediated T-cell Suppression

CB-1158 reverses human T-cell immunosuppression by neutrophils or cancer patient-derived MDSCs *ex vivo*

- **Purified neutrophils were mixed with T-cells a from healthy volunteer; neutrophils suppress T-cell proliferation**
- **CB-1158 relieves neutrophil-induced suppression of T-cell proliferation and IFN-γ production**
- **CB-1158 maintains arginine levels**



- **Cancer patient MDSCs purified from PBMCs with α-CD66b antibodies (3 separate patients)**
- **MDSC-conditioned media suppresses T-cell proliferation**

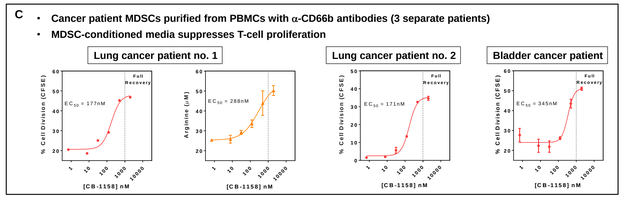


Figure 4: (A) Schema of myeloid mediated suppression of T-cell proliferation via arginase. **(B)** Proliferation (CFSE) and IFN-γ (ELISA) production of αCD3/αCD28-stimulated human T-cells after a 4 day incubation in the presence or absence of neutrophils. **(C)** T-cell proliferation in the presence of CD66b MDSCs. **(B/C)** Arginine concentrations from neutrophils or MDSCs pre-incubated with media and CB-1158 for 2 days prior to T-cell addition.

CB-1158 Elevates Arginine in Tumors

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

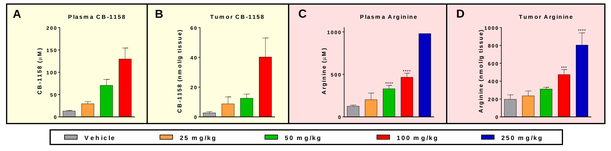


Figure 5: (A-B) Concentration of CB-1158 in (**A**) plasma or (**B**) Lewis Lung Carcinoma (LLC) tumor lysates from C57.Bl/6 mice dosed orally with 5 doses of CB-1158 on a BID schedule. Samples were collected 2 h after the last dose of CB-1158 (N = 5 per group). **(C-D)** Arginine concentration in (**C**) plasma or (**D**) tumor of samples in **A-B**. (**** P < 0.0001; *** P < 0.001 vs. vehicle)

CB-1158 Anti-tumor Efficacy is Immune Based

CB-1158 anti-tumor activity in Lewis Lung Carcinoma Model (LLC)

- **Single agent CB-1158 reduces the growth of LLC in immuno-competent mice**
- **CB-1158 has no anti-tumor activity in immuno-compromised mice**
- **NK cell or CD8 depletion partially reverses the anti-tumor effects of CB-1158**

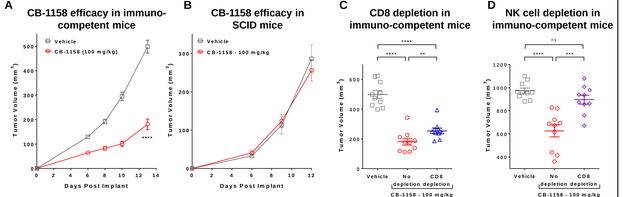
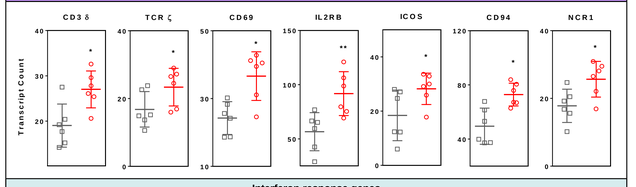


Figure 6: (A) LLC cells were implanted in C57.Bl/6 mice or **(B)** B6.CB17-Prkdc (SCID)/SzJ mice. Mice were dosed orally twice daily with vehicle or 100 mg/kg CB-1158. **(C)** An additional group of mice in (A) were treated with anti-CD8. (**D**) In a separate experiment, tumor-bearing mice were treated with vehicle or CB-1158 in the presence or absence of anti-NK1.1 (N = 10 per group; **** P < 0.0001; *** P < 0.001; ** P < 0.01; ns, not significant)

CB-1158 increases Th1, NK, and IFN-related gene expression in LLC tumors

T-cell and NK cell markers



Interferon response genes

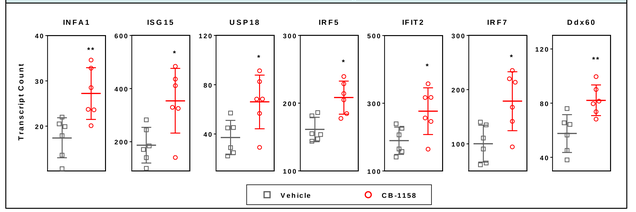


Figure 7: Levels of mRNA transcripts (determined by Nanostring) in LLC tumors from mice treated with vehicle or CB-1158 twice daily (100 mg/kg) for 14 days. (N = 6 per group; ** P < 0.01; * P < 0.05)

CB-1158 increases Th1 and M1 cytokines and decreases MDSC cytokines in LLC tumors

↑ Th1 Cytokines | **↑ M1 Macrophage Cytokines** | **↓ MDSC Cytokines**

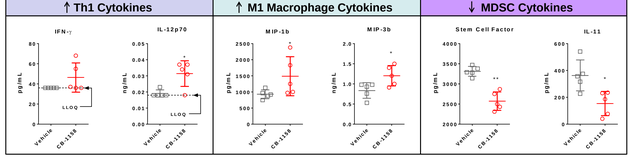


Figure 8: Levels of cytokines and chemokines (determined by Luminex) in LLC tumors from mice treated with vehicle or CB-1158 twice daily (200 mg/kg) for 14 days. (N = 5 per group; ** P < 0.01; * P < 0.05)

CB-1158 increases CD8⁺ T-cells and decreases M2 macrophages in LLC tumors

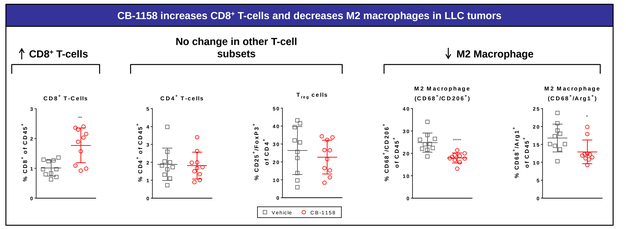


Figure 9: Levels of immune cell subsets (determined by flow cytometry) in LLC tumors from mice treated with vehicle or oral CB-1158 (100 mg/kg) twice daily for 14 days. (N = 10 per group; **** P < 0.0001; ** P < 0.01; * P < 0.05)

CB-1158 single agent efficacy in B16.F10 melanoma and Madison 109 lung carcinoma models

B16.F10 melanoma model | **Madison 109 lung cancer model**

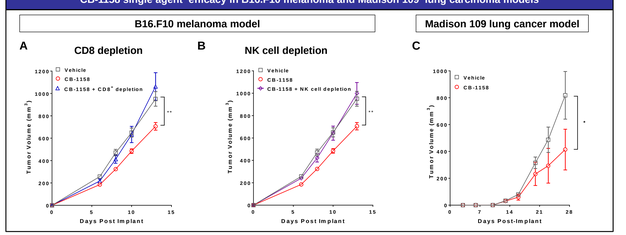


Figure 10: (**A**) B16.F10 cells were implanted in C57.Bl/6 mice and mice were dosed orally twice daily with vehicle or 100 mg/kgCB-1158. One group of mice were treated with anti-CD8. (**B**) A separate group of mice in (A) were treated with anti-NK1.1. **(C)** Madison109 cells were implanted in balb/c mice and were dosed orally twice daily with vehicle or 100 mg/kg CB-1158. (N = 10 per group; ** P < 0.01; * P < 0.05 vs. vehicle control)

Increased Activity of CB-1158 with Checkpoint Inhibitors

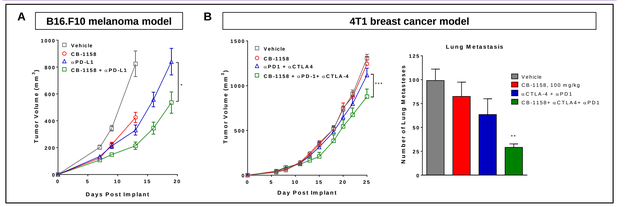


Figure 11: (A) B16.F10 cells were implanted in C57.Bl/6 mice. CB-1158 (100 mg/kg) was dosed BID. anti-PD-L1 (10F.9G2): 5 mg/kg IP Days 3, 5, 7, 9, 11, 13 **(B)** 4T1 mammary carcinoma cells implanted orthotopically into female balb/c mice and treated with either vehicle, CB-1158 (100 mg/kg PO BID), anti-CTLA-4 (5 mg/kg IP on Days 2, 5, 8) plus anti-PD-1 (5 mg/kg IP on days 3, 6, and 9), or the combination of CB-1158 with anti-CTLA-4 and anti-PD-1. (N = 10 per group; *** P < 0.001; ** P < 0.01; * P < 0.05 vs. vehicle control)

Conclusions

- **Cancer patient tumors have arginase-containing myeloid infiltrates and show increased plasma arginase and decreased plasma arginine compared to healthy normal individuals**
- **CB-1158 potently inhibits arginase and reverses MDSC/neutrophil induced suppression of T-cell proliferation**
- **CB-1158 increases tumor and plasma arginine levels and has single agent efficacy in syngeneic models**
- **CB-1158 increases inflammation and lymphocyte activation in the tumor microenvironment**
- **The addition of CB-1158 to anti-PD-1 and anti-CTLA-4 results in further tumor growth inhibition**
- **CB-1158 is currently in IND-enabling studies**

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