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 arginase 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 restore arginine levels and relieve immunosuppression, leading to anti-tumor immune responses.

Materials and Methods. We developed CB-1158, a potent and selective small molecule inhibitor of arginase (IC_{50} = 98 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 co-culture system of T cells with granulocytes or MDSCs, CB-1158 reverses granulocyte- or MDSC-mediated immunosuppression by blocking arginine 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.

In vivo, 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 plasma and tumor arginine levels associated with single agent anti-tumor efficacy in multiple syngeneic models. The anti-tumor efficacy of CB-1158 is abrogated in immunocompromised mice or via depletion of either CD8 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.

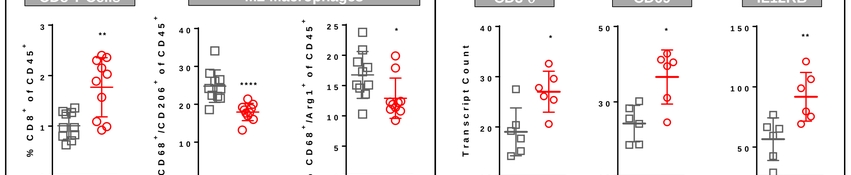
The immunomodulatory activity of CB-1158 supports the potential of its combination with other immunotherapies and/or standard-of-care therapies. CB-1158 enhances the anti-tumor efficacy of adoptive cell therapies such as T cell therapy in the B16F10 model. CB-1158 also enhances checkpoint inhibitors, including anti-PD-L1 in the B16F10 model. Moreover, CB-1158 enhances the anti-tumor efficacy of chemotherapies 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 granulocytic infiltrates are abundant in multiple tumor types. Plasma arginase levels are elevated in cancer patients compared to healthy controls, and are associated with decreased plasma arginine. In an ongoing Phase I clinical trial (NCT02903914) 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.

Arginase is an Immunosuppressive Enzyme

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



CB-1158 Potently Inhibits Arginase

- **CB-1158 is a potent and selective inhibitor of arginase 1 (IC_{50} 98 nM) and arginase 2 (IC_{50} 274 nM)**
- **CB-1158 is not cytotoxic to cancer cell lines or primary T cells at concentrations up to 1 mM**

CB-1158 Reverses Myeloid Cell Mediated T Cell Suppression

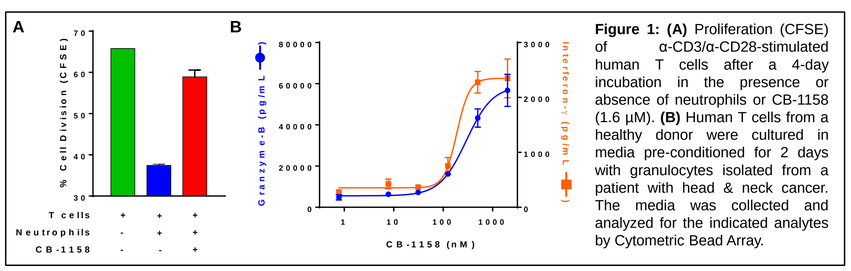


Figure 1: (A) Proliferation (CFSE) of α-CD3/α-CD28-stimulated human T cells after a 4-day incubation in the presence or absence of neutrophils or CB-1158 (1.6 µM). **(B)** Human T cells from a healthy donor were cultured in media pre-conditioned for 2 days with granulocytes isolated from a patient with head & neck cancer. The media was collected and analyzed for the indicated analytes by Cytometric Bead Array.

CB-1158 Increases Arginine Levels and Has Single-Agent Anti-Tumor Efficacy in Animal Models

- **Single agent CB-1158 reduces the growth of LLC, B16F10 and CT26 tumor models**
- **Efficacy of CB-1158 correlates with increases in tumor arginine levels**
- **CB-1158 has no anti-tumor activity in SCID mice or in mice depleted of CD8 T cells or NK cells (LLC model)**

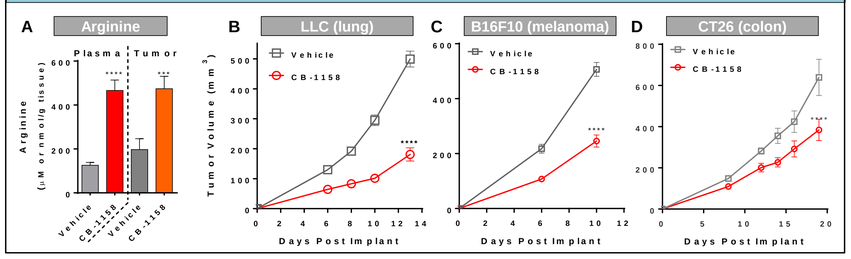


Figure 2: (A) Concentration of arginine in plasma or LLC tumor lysates from C57.Bl/6 mice dosed orally with four doses of CB-1158 at 100 mg/kg BID (samples collected 2 h after the last dose; N = 5 per group). LLC **(B)** and B16F10 **(C)** cells were implanted in C57.Bl/6 mice and CT26 cells **(D)** were implanted in Balb/c mice (N =10 per group). CB-1158 was dosed at 100 mg/kg BID starting on day 2 and continued throughout each study. (N = 10 per group; **** P < 0.0001; *** P < 0.001).

CB-1158 Increases Tumor Inflammation

Figure 3: *(left)* Levels of immune cell subsets (determined by flow cytometry, N = 10 per group), or *(right)* levels of mRNA transcripts (determined by Nanostring, N = 6 per group) in LLC tumors from mice treated with vehicle or CB-1158 at 100 mg/kg BID starting on day 2 for 14 days. (** P < 0.01; * P < 0.05).

CB-1158 Synergizes with Adoptive Cell Therapy

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

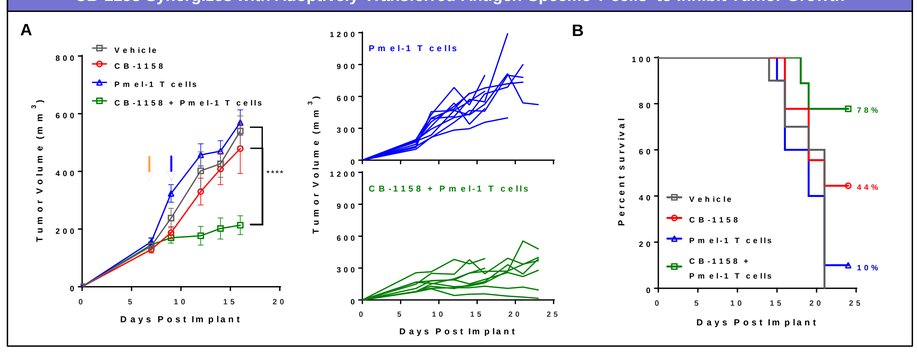


Figure 4: (A) B16F10 cells were implanted in C57.Bl/6 mice. Non-myeloablative chemotherapy (Cyclophosphamide 250 mg/kg and Fludarabine 50 mg/kg) was dosed IP on day 7 (orange arrow) to all groups. Pmel-1 CD8 T cells (1 x 10^6) were adoptively transferred IV on day 9 (blue arrow). Recombinant human IL-2 (200,000 UI) was dosed IP BID on days 9, 10 and 11 in mice receiving T cells (no 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 < 0.0001). **(B)** Survival curves. P = 0.0137 by Mantel-Cox test.

CB-1158 Combines with Checkpoint Blockade

CB-1158 Combines with anti-PD-L1 to Inhibit Tumor Growth

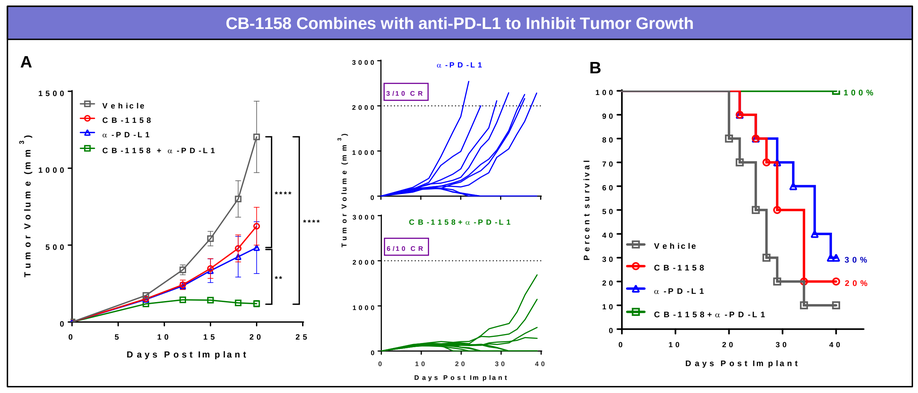


Figure 5: (A) CT26 cells were implanted in Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. α-PD-L1 (10F.9G2) was dosed at 5 mg/kg IP on days 5, 7, 9, 11, 13 and 15. Mice were sacrificed when tumor volumes reached 2000 mm^3. *CR:* complete regression. (N = 10 per group; **** P < 0.0001; ** P < 0.01). **(B)** Survival curves. P < 0.0001 by Mantel-Cox test (*experiment is ongoing*).

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

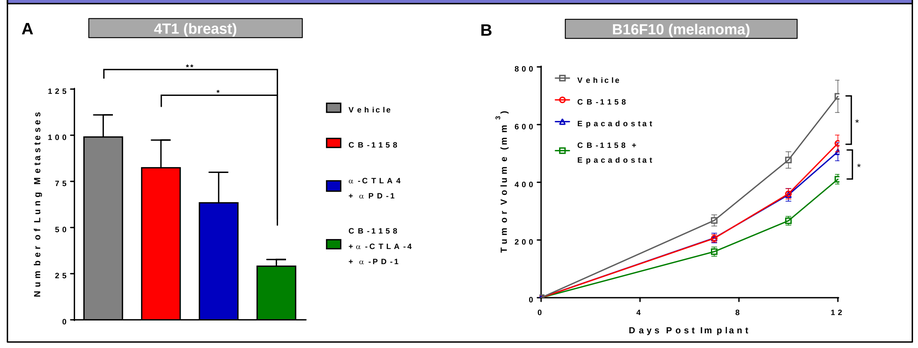


Figure 6: **(A)** 4T1 cells were implanted orthotopically into female Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. α-CTLA-4 (9H10) was dosed at 5 mg/kg IP on days 2, 5 and 8. α-PD-1 (RPMI-14) was dosed at 5 mg/kg IP on days 3, 6 and 9. Lung metastases were counted on day 26. **(B)** B16F10 cells were implanted in C57.Bl/6 mice. CB-1158 and Epacadostat were both dosed at 100 mg/kg PO BID starting on day 2. (N = 10 per group; **** P < 0.0001; ** P < 0.01; * P < 0.05).

CB-1158 Combines with Standard-of-Care Chemotherapy

CB-1158 Combines with MDSC-depleting Gemcitabine to Inhibit Tumor Growth

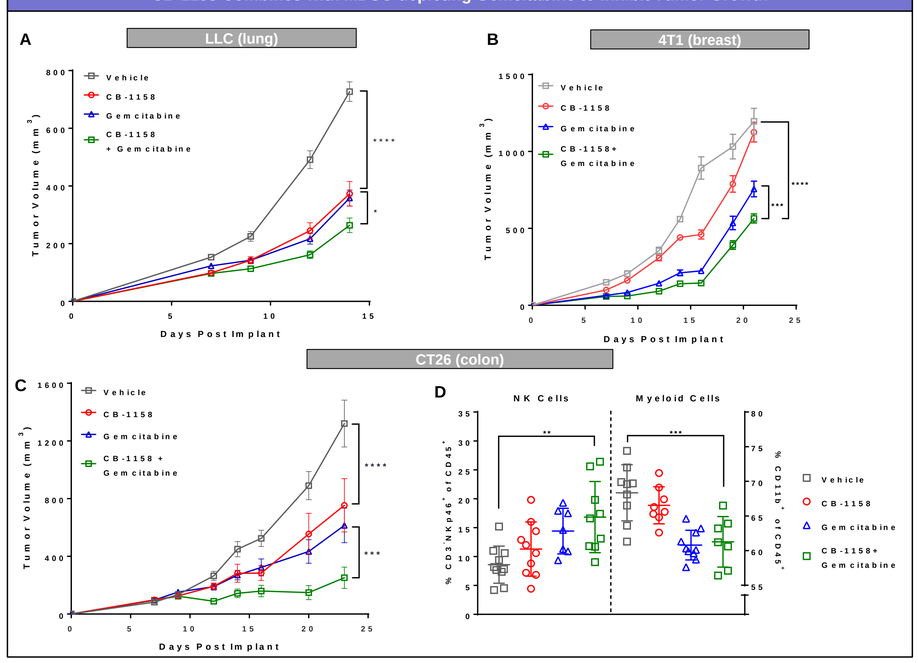


Figure 7: (A) LLC cells were implanted in C57.Bl/6 mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. Gemcitabine was dosed at 60 mg/kg IP on days 6 and 10. **(B)** 4T1 cells were implanted orthotopically into female Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. Gemcitabine was dosed at 30 mg/kg IP on day 5. **(C)** 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. **(D)** Levels of immune cell subsets determined by flow cytometry in CT26 tumors from mice treated as in **(C)** for 13 days.
(N = 10 per group; **** P < 0.0001; *** P < 0.001; ** P < 0.01; * P < 0.05).

Arginase 1 is Expressed in Human Cancer

Arginase 1 is Expressed in Tumor-Infiltrating Leukocytes in Human Tumors

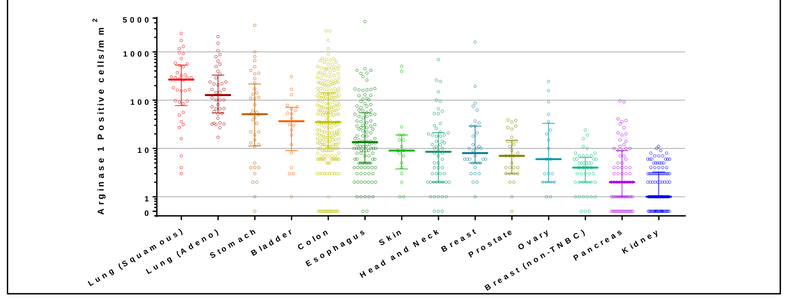


Figure 8: Human tumor tissue microarrays were stained with anti-Arginase 1 antibody by immunohistochemistry. Arginase 1-positive infiltrating granulocytes were quantified by digital histopathology.

Arginase 1 Co-localizes with Tumor-Infiltrating Granulocytes

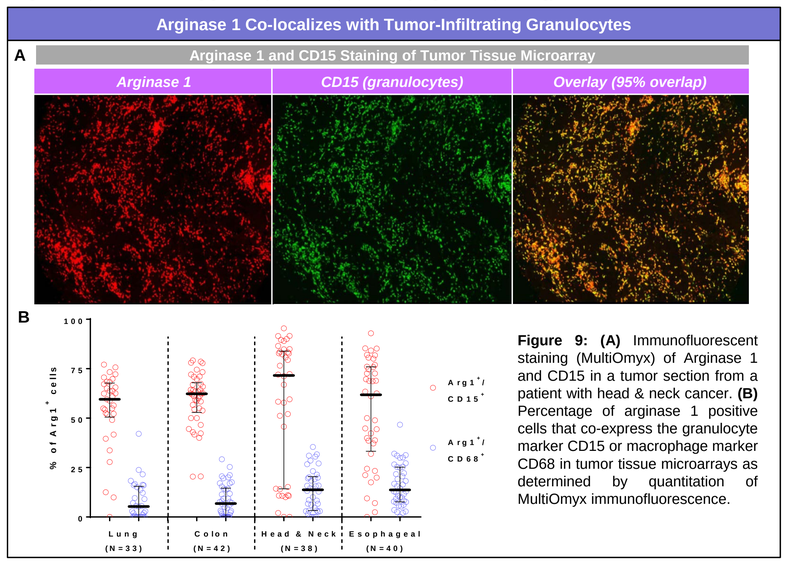


Figure 9: (A) Immunofluorescent staining (MultiOmyx) of Arginase 1 and CD15 in a tumor section from a patient with head & neck cancer. **(B)** Percentage of arginase 1 positive cells that co-express the granulocyte marker CD15 or macrophage marker CD68 in tumor tissue microarrays as determined by quantitation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

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

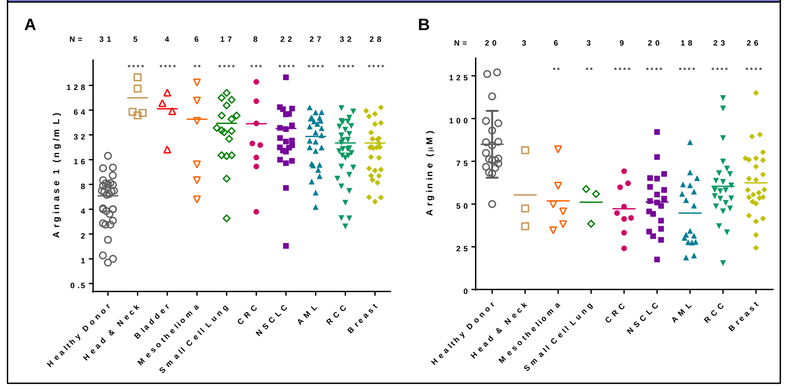


Figure 10: (A) Arginase 1 protein levels in plasma from healthy donors and cancer patients as determined by ELISA. **(B)** Plasma arginine concentrations in healthy donor and cancer patient samples as determined by LC/MS. (**** P < 0.0001; *** P < 0.001; ** P < 0.01; vs. healthy).

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

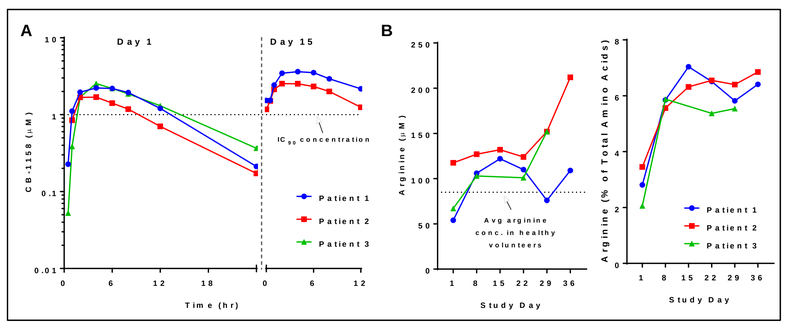


Figure 11: (A) Plasma levels of CB-1158 in the first dose cohort (50 mg BID) of clinical trial CX-1158-101 (ClinicalTrials.gov #NCT02903914) evaluated after the first dose (Day 1) and at steady state (Day 15). **(B)** Fasted plasma arginine levels (absolute concentration or normalized to total plasma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

Conclusions

- **CB-1158 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 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)

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