CB-708, an orally bioavailable small molecule inhibitor of CD73 with immunostimulatory and anti-tumor activity

Clarissa Lee, Deepthi Bhupathi, Roland Billedeau, Jason Chen, Lijing Chen, Rosalyn Dang, Tahereh Ejtehadian, Matthew Gross, Tony Huang, Weiqun Li, Yong Ma, Andrew MacKinnon, Gisele Marguier, Silinda Neou, Francesco Parlati, Natalija Sotirovska, Sandra Spurlock, Timothy Stanton, Susanne Steggerda, Jing Zhang, Winter Zhang, Jim Li

Calithera Biosciences Inc., South San Francisco, CA

Abstract

BACKGROUND

High adenosine (ADO) in the tumor microenvironment suppresses the immune response against cancer cells by inhibiting immune effector functions and promoting the development of immunosuppressive cells. Extracellular ADO can be generated from ATP released by cells undergoing stress or death through the combined actions of the ecto-nucleotidases CD39 (ATP to AMP) and CD73 (AMP to ADO). Inhibition of ADO production via CD73 is a promising therapeutic approach for the treatment of cancer.

METHODS

We developed CB-708, a potent and selective small molecule inhibitor of CD73. The potency of CB-708 was evaluated against recombinant CD73 and CD73-expressing cells using a malachite green assay. Selectivity against related ectonucleotidases was also assessed. Inhibition of CD73 in plasma was measured using LC/MS to assess conversion of $^{15}N_5$-AMP into $^{15}N_5$-ADO. Reversal of AMP-mediated immune suppression of human CD8+ T cells was determined by measuring T cell activation in the presence of exogenous AMP. T cell proliferation was assayed by flow cytometry and cytokine levels were measured by ELISA. The EG7 and CT26 syngeneic tumor models were used to assess the therapeutic effect of CB-708.

RESULTS

CB-708 potently and completely inhibited soluble human CD73 (IC_{50} = 170 pM) and cell-bound human CD73 (IC_{50} = 210 pM), but did not inhibit human CD39, ENTPD2, or ENTPD3. CB-708 retained high potency in the presence of whole human plasma (IC_{50} = 380 pM) and reversed AMP-mediated suppression of human CD8+ T cell proliferation and production of IFNγ and granzyme B in vitro. Oral administration of CB-708 was well-tolerated in tumor-bearing mice, resulted in sustained exposure above mouse plasma IC50, and exhibited single-agent tumor growth inhibition in syngeneic tumor models including established EG7 tumors. Efficacy in the EG7 model was dependent on CD8+ T cells and was correlated with pharmacodynamic inhibition of CD73. Enhanced tumor growth inhibition was observed when CB-708 was combined with checkpoint inhibition (anti-PD-L1) or with chemotherapy (oxaliplatin, doxorubicin, docetaxel) in the EG7 model.

CONCLUSIONS

CB-708 is an orally bioavailable and highly potent small molecule inhibitor of CD73. CB-708 reverses the immunosuppressive effects of AMP-derived ADO in vitro and in vivo and has anti-tumor activity.

CD73 Generates Immunosuppressive Adenosine

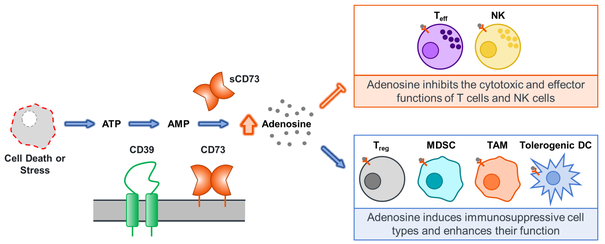


Figure 1. CD73 generates immunosuppressive adenosine. Extracellular ATP can be generated from cells undergoing death or stress. Extracellular ATP is converted to AMP by CD39. Membrane-bound CD73 or soluble CD73 (sCD73) metabolize AMP into adenosine which can bind to adenosine receptors on immune cells leading to immunosuppression.

CB-708 is a Potent CD73 Inhibitor in Full Human Plasma

A

Assay	IC_{50} (nM)
Human Recombinant CD73	0.17
Human Plasma CD73	0.38
Human Cell Surface CD73	0.21
Mouse Recombinant CD73	1.3
Mouse Plasma CD73	1.0

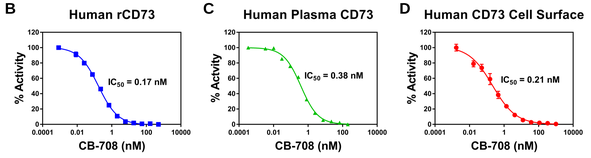


Figure 2. CB-708 is a potent CD73 inhibitor. (A, B, D) The potency of CB-708 was evaluated against recombinant CD73 and CD73-expressing SK-MEL-28 cells using a malachite green assay. **(A, C)** Inhibition of CD73 in plasma was measured using LC/MS to assess conversion of $^{15}N_5$-AMP into $^{15}N_5$-ADO.

CB-708 is Selective for CD73

A

Enzyme	IC_{50} (nM)
CD39	>10,000
ENTPD2	>10,000
ENTPD3	>10,000

B

	No. of Targets Inhibited >50% at 10 μM CB-708
Safety Panel	1/87*
Kinase Panel	0/45

*PDE3 59% inhibited

CB-708 does not have anti-proliferative effects on cells

C

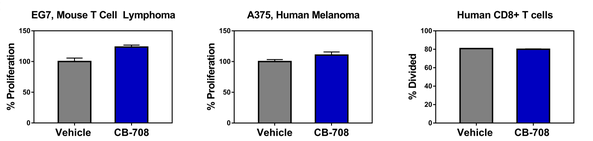
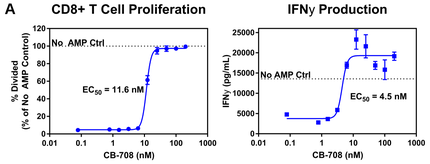


Figure 3. CB-708 is selective for CD73 and does not have anti-proliferative effects. (A) Activity of cell surface CD39 was assessed using K562 cells expressing human CD39. Activity of recombinant human ENTPD2 and ENTPD3 was assessed. **(B)** CB-708 was screened in the Eurofins Safety Screen Panel and the Eurofins Express Diversity Kinase Profile Panel. **(C)** Proliferation of EG7 and A375 cells treated with 100 μM CB-708 was measured after 3 days. Proliferation of human CD8+ T cells was measured by flow cytometry after 4 days of treatment with 100 μM CB-708.

CB-708 Reverses Adenosine-Mediated Immunosuppression

A



B

Functional Human CD8+ T Cell Read-out	IC_{50} (nM)
Proliferation	11.6
CD25 Expression	9.6
IFNγ	4.5
Granzyme B	5.6

Figure 4. CB-708 reverses AMP-mediated suppression of human CD8+ T cells. (A, B) Human CD8+ T cells were labeled with CFSE and then pre-incubated with an adenosine deaminase inhibitor and CB-708 or vehicle before addition of AMP. T cells were activated with α-CD3, α-CD28, and hIL2. After 4 days, proliferation and CD25 expression were assessed by flow cytometry and cytokine levels in the supernatant were measured by ELISA. **(B)** EC50s determined using a four-parameter dose-response curve equation.

CB-708 has Oral Exposure and Pharmacodynamic Effect

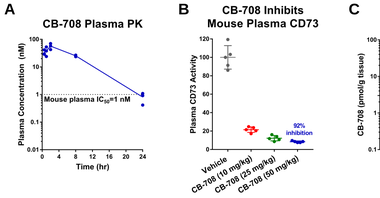


Figure 5. CB-708 has oral exposure and pharmacodynamic effect. (A) Single dose CB-708 (50 mg/kg) was orally administered to mice and plasma was collected at indicated time points. CB-708 levels were measured by LC/MS. **(B)** CB-708 was orally administered BID to EG7 tumor bearing mice for 20 days. Plasma was harvested 2 hours after the last dose and spiked with $^{15}N_5$-AMP and a TNAP inhibitor. $^{15}N_5$-ADO levels were measured by LC/MS. **(C)** Tumor tissues from the 50 mg/kg group in **(B)** were collected 2 or 16 hours after the last dose. CB-708 levels were measured by LC/MS. pmol/g tissue ≈ nM.

Orally Dosed CB-708 has Single-Agent Efficacy

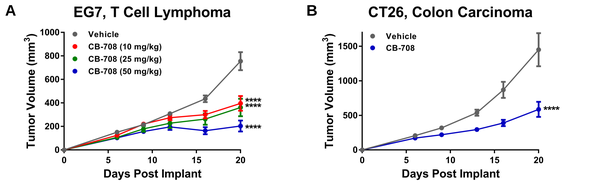


Figure 6. CB-708 has single-agent efficacy. (A) EG7 cells were implanted subcutaneously into C57BL/6 mice. CB-708 or vehicle was orally administered BID starting on day 1 (N=10 per group). **(B)** CT26 cells were implanted subcutaneously into Balb/c mice. 100 mg/kg CB-708 or vehicle was orally administered BID starting on day 1 (N=10 per group). ns indicates not significant; * indicates p<0.5; ** indicates p<0.01; *** indicates p<0.001; **** indicates p<0.0001.

CB-708 Monotherapy Inhibits Established Tumors

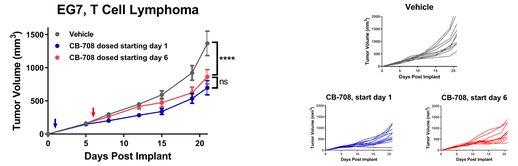


Figure 7. CB-708 is efficacious against established tumors. EG7 cells were implanted subcutaneously into C57BL/6 mice. CB-708 (100 mg/kg) was orally dosed BID starting one or six days post-implant. Vehicle was orally dosed BID starting one day post-implant. N=10 per group.

CB-708 Activates a Tumor-Directed Immune Response

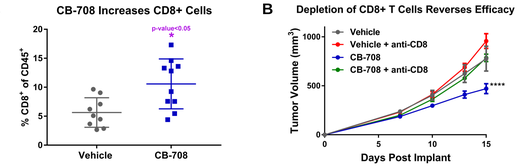


Figure 8. CB-708 activates a tumor-directed immune response. (A-B) EG7 cells were implanted subcutaneously into C57BL/6 mice. CB-708 (50 mg/kg) or vehicle was orally administered BID starting day one post implant (N=10 per group). **(A)** Tumors were excised on day 14 and analyzed by flow cytometry. **(B)** Anti-CD8 antibody was dosed i.p. on days -1, 0, 5, and 10.

CB-708 Enhances the Efficacy of IO and Chemotherapy Agents

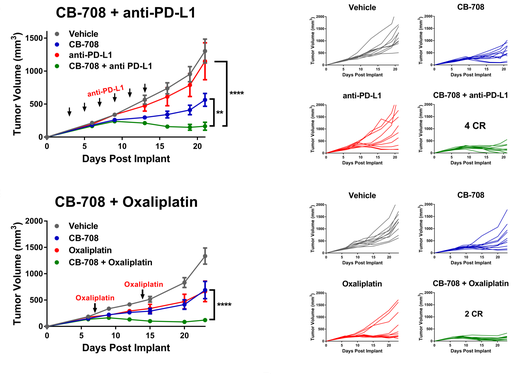


Figure 9. CB-708 combines with checkpoint blockade and chemotherapy treatment. (A-D) EG7 cells were implanted subcutaneously into C57BL/6 mice (N=10 per group). **(A, B, D)** CB-708 (100 mg/kg) or vehicle was orally dosed BID starting on day 1. **(A)** anti-PD-L1 antibody was dosed i.p. 5 mg/kg. **(B)** Oxaliplatin was dosed i.p. 6 mg/kg. **(C)** CB-708 (50 mg/kg) or vehicle was orally dosed BID starting on day 1. Doxorubicin was dosed i.v. 2.5 mg/kg. **(D)** Docetaxel was dosed i.p. 5 mg/kg.

CB-708 is More Efficacious than a Clinical CD73 Antibody

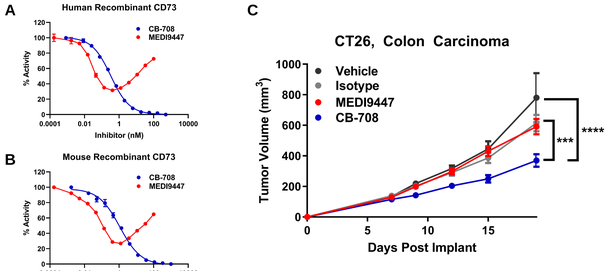
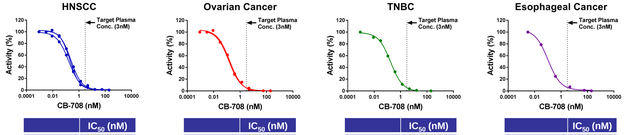


Figure 10. CB-708 is more efficacious than MEDI9447. (A-B) A MEDI9447 biosimilar was generated using sequences from US Patent Application US 2016/0129108. The potency was evaluated against recombinant CD73 using a malachite green assay. **(C)** For the in vivo efficacy study, a version of MEDI9447 was used in which a mouse IgG1 Fc domain was substituted for the human IgG1 Fc domain. CB-708 (100 mg/kg) or vehicle was orally dosed BID starting two days post-implant. MEDI9447 (10 mg/kg) or isotype (40 mg/kg) was dosed twice weekly starting two days post-implant. N=10 per group.

CB-708 Inhibits CD73 in Patient Serum



	IC_{50} (nM)
HNSCC #1	0.21
HNSCC #2	0.24
HNSCC #3	0.17

	IC_{50} (nM)
Ovarian #1	0.14
Ovarian #2	0.16

	IC_{50} (nM)
TNBC #1	0.18

	IC_{50} (nM)
Esophageal #1	0.10

Figure 11. CB-708 inhibits CD73 in patient serum. Serum was procured from Discovery Life Sciences. Serum from head and neck squamous cell carcinoma (HNSCC), ovarian cancer, triple-negative breast cancer (TNBC), and esophageal cancer patients were incubated with a serial dilution of CB-708 in the presence of a TNAP inhibitor. Conversion of $^{15}N_5$-AMP to $^{15}N_5$-ADO was measured by LC/MS.

CD73 is Highly Expressed in Human Tumors

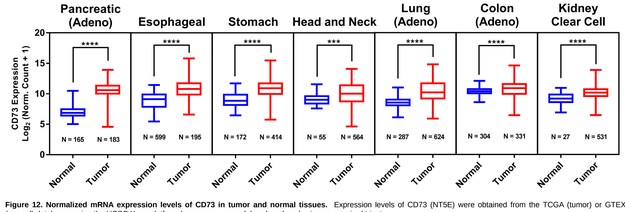


Figure 12. Normalized mRNA expression levels of CD73 in tumor and normal tissues. Expression levels of CD73 (NTSE) were obtained from the TCGA (tumor) or GTEx (normal) databases using the UCSC Xena platform (www.xena.ucsc.edu) and analyzed using an unpaired t-test.

Conclusions

- CB-708 is an orally bioavailable and selective CD73 inhibitor with picomolar potency.
- Adenosine-mediated suppression of CD8+ T cell function and proliferation is reversed by CB-708.
- CB-708 has good exposure in animals, is well-tolerated, and shows pharmacodynamic effect.
- Anti-tumor single-agent activity of CB-708 is immune-mediated.
- CB-708 enhances the anti-tumor effect of checkpoint blockade and chemotherapy.
- Preclinically, CB-708 has superior activity compared to a clinical CD73 antibody biosimilar.
- In GLP toxicology studies, no CB-708-related toxicity was identified at high exposure.

SITC, November 6-10, 2019, National Harbor, Maryland