Glutaminase Inhibition With CB-839 Enhances Anti-Tumor Activity of PD-1 and PD-L1 Antibodies by Overcoming a Metabolic Checkpoint Blocking T Cell Activation

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Abstract

Recent studies have highlighted the importance of the tumor metabolic environment for controlling immune activation. T-cells activated through the TCR/CD28 receptor switch to a highly glycolytic metabolism and increase their requirement for glucose and glutamine. Consequently, limited availability of glucose or glutamine can block T-cell activation and proliferation. Likewise, immune checkpoints proteins, PD-1 and CTLA-4, suppress T cell metabolism by inhibiting glycolysis, glutamine uptake and glutaminolysis[1]. Chang et al[2] recently demonstrated that glucose consumption by tumors restricts glucose availability and blocks activation of T cells, and that treatment with CTLA-4, PD-1, or PD-L1 antibodies can re-activate T cell glycolysis.

CB-839 is a glutaminase inhibitor currently in Phase 1 trials in patients with solid and hematological malignancies[3,4,5]. CB-839 blocks glutamine consumption in tumors and causes a significant elevation of tumor glutamine levels[6]. Therefore, we hypothesized that CB-839 might enhance the activity of immune checkpoint inhibitors via metabolic modulation of the tumor microenvironment. We first confirmed that T- cell proliferation is dependent on glutamine but is only minimally inhibited by CB-839. In the absence of glutamine, splenic mouse T cells stimulated with anti-CD3/CD28 had reduced glucose consumption and did not proliferate. In contrast, CB-839 treatment did not mimic the effects of glutamine withdrawal on T-cells. CB-839 had no effect on glucose consumption by activated T-cells and only a minimal effect on proliferation. We also confirmed in the OVA vaccinia model that CB-839 had minimal effects on CD4 and CD8 T-cell proliferation in vivo, while the non-specific glutamine inhibitor DON caused a dramatic reduction in the number of CD4 and CD8 T-cells.

To determine if CB-839 could enhance the anti-tumor efficacy of immune checkpoint inhibitors, we treated mice bearing syngeneic CT26 colon carcinoma tumors with anti PD-1 or anti PD-L1 alone or in combination with CB-839. The addition of CB-839 to either anti PD-1 or anti PD-L1 treatment enhanced anti-tumor activity, augmenting tumor regression and promoting survival. Depletion of CD8+ T-cells from CT26 tumors reversed the anti-tumor effects of PD-L1 and CB-839, demonstrating that the combination targets CD8+ T-cells in the immune microenvironment.

These data are the first demonstration that modulation of glutamine metabolism in tumors can enhance the activity of checkpoint inhibitors and provide a rationale for combining CB-839 with immune checkpoint inhibitors in the clinic. Overall, these data highlight a new therapeutic approach to treating cancer by targeting tumor metabolism as a means of enhancing the efficacy of immunotherapy.

Introduction

Metabolic suppression of T cells in tumors

- **Metabolic competition for nutrients in the tumor microenvironment**
- **PD-1 Ligation suppresses T-cell metabolism**

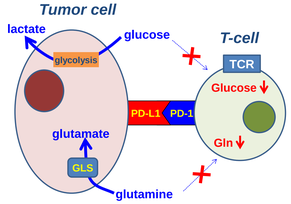
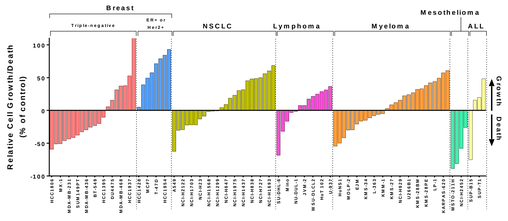


Figure 1: Model of the tumor microenvironment wherein tumor cells out-compete T-cells for limiting nutrients such as glutamine and glucose. Immune checkpoints such as PD-1 ligation with PD-L1 can further restrict T-cell metabolism.

Tumor Cells and T-Cells Require Glutamine for Proliferation and Survival

Many tumor cells are dependent on glutamine for proliferation and survival



T-cell division is dependent on glutamine

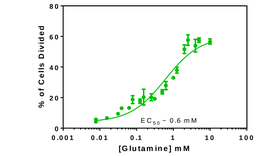
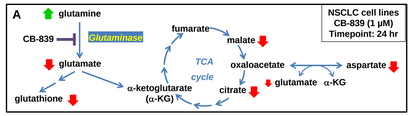


Figure 2: (A) Relative cell growth/death of human cancer cells grown for 72 hrs in the absence of glutamine was assessed with CellTiterGlo. **(B)** Purified mouse splenic T-cells were labeled with CFSE and stimulated with plate-bound CD3 and soluble CD28 in the presence of the indicated concentration of glutamine. Cell division was assessed after 96 hours by CFSE dye-dilution.

CB-839 is a Glutaminase Inhibitor



CB-839 inhibits GLS splice variants: GAC and KGA

B

Enzyme	IC_{50} (nM)
rHuGAC	29
Kidney (KGA)	24
Liver (GLS2)	>5000

CB-839 decreases levels of metabolites downstream of glutamate

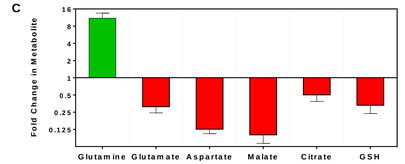


Figure 3: (A) Schematic representation of glutamine metabolism. **(B)** The IC50 of CB-839 on purified recombinant human glutaminase (rHu-GAC) or lysates from mouse kidney (which expresses primarily KGA), or mouse liver (which expresses primarily GLS2) were determined in a biochemical assay[6] **(C)** Metabolite levels were measured in five NSCLC cell lines 24 hours after treatment with 1 μM CB-839.

CB-839 Has Broad Anti-Tumor Activity

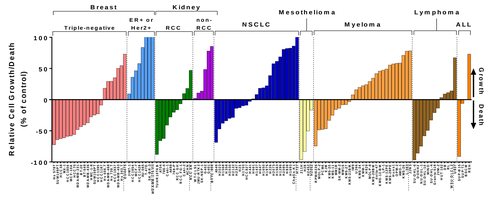
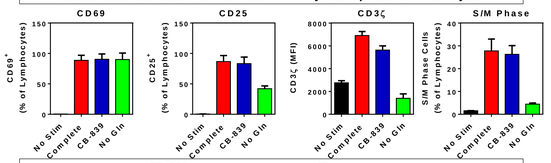


Figure 4: A panel of tumor cells lines were treated with 1μM CB-839 for 72 h in RMPI-1640 media containing 10% fetal bovine serum and 2 mM glutamine. Cell growth and survival was measured using CellTiterGlo reagent (Promega).

CB-839 Has Minimal Impact on T-Cell Activation and Division

CB-839 does not block T-cell activation or entry into S phase of the cell cycle



CB-839 has minimal impact on T-cell division

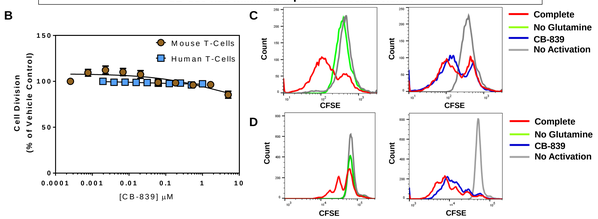


Figure 5: (A) Human T-cells were activated with CD3/CD28 and the expression of T-cell activation markers (CD69, CD25, CD3ζ) and DNA content were quantified by flow cytometry after 48 hrs. CB-839 was present at 1 μM where indicated. **(B)** Mouse or human T-cells were labeled with CFSE and activated in the presence of a dose-titration of CB-839. Cell division was quantified by CFSE dye dilution after 96 hrs. Representative CFSE histograms from **(C)** mouse and **(D)** human T-cell division assays. CB-839 was present at 1 μM where indicated.

In vivo T-cell Response to Antigen is Minimally Impacted by CB-839

CB-839 minimally impacts CD8 and CD4 T-cell proliferation in the OVA vaccinia model.

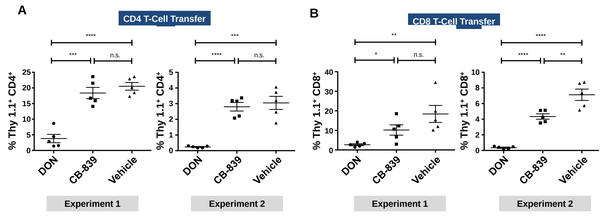


Figure 6: One million OT-I Thy1.1+ CD4+ **(A)** or CD8+ **(B)** T cells were adoptively transferred into WT Thy 1.2+ recipient mice. The hosts were infected with vaccinia-OVA and treated with Vehicle (PBS), 6-Diazo-5-oxo-D-norleucine (DON), or CB-839 for 6 days. Host splenocytes were harvested at day 7 to interrogate proliferation of antigen specific CD4+ or CD8+ T cells. Each symbol represents an individual mouse. Horizontal lines indicate mean ± SEM. n.s., not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (Student's *t* test).

CB-839 Blocks Tumor Cell Glutamine Consumption and Restores T-Cell Division in Vitro

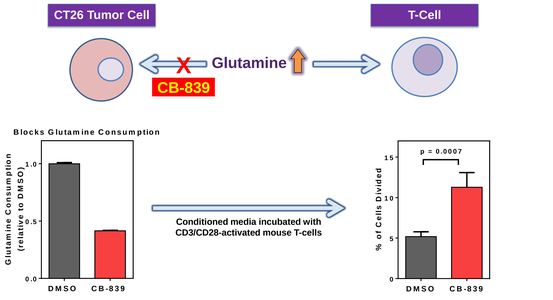


Figure 7: CT26 mouse tumor cells were incubated with DMSO or CB-839 (1 μM) for 72 hrs and conditioned media was harvested and glutamine consumption was quantified. Purified mouse T-cells were then activated with plate-bound CD3 and soluble CD28 in the presence of conditioned media and T-cell division was measured after 96 hrs by CFSE dye dilution.

CB-839 Increases Glutamine in Tumors in Vivo

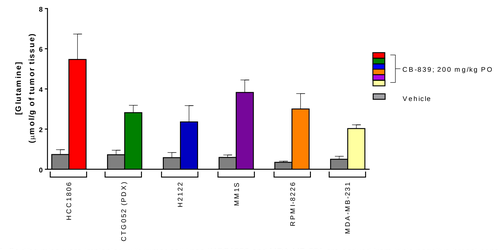
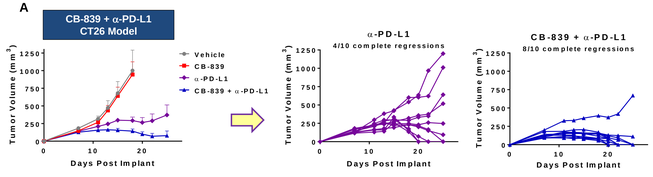
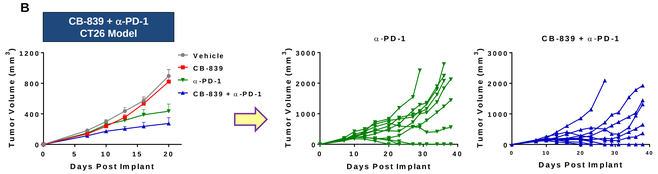


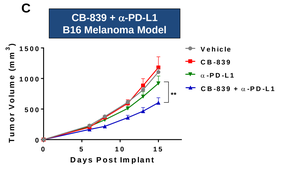
Figure 8: Scid/bg mice were implanted with tumor cell lines (HCC1806 and MDA-MB-231 are derived from triple negative breast cancer; H2122 is derived from non-small cell lung carcinoma; MM1S and RPMI-8226 are derived from multple myeloma tumor cells) or with a triple negative patient derived xenograft tumor [CTG052 (PDX)]. When tumors reached ~100 mm^3, CB-839 was administered as a single oral gavage at 200 mg/kg. Four hours post-dose, tumor samples were collected and flash frozen in liquid nitrogen. Glutamine levels were quantified by LC/MS/MS.

CB-839 Synergized With Immune Checkpoint Inhibition

Combining CB-839 with anti-PD-L1 or anti-PD-1 showed enhanced anti tumor activity in syngeneic models







CD8 depletion reverses the anti-tumor activity of CB-839 + α-PD-L1 in the CT26 model

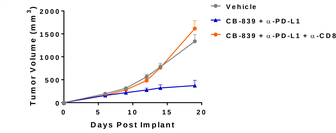
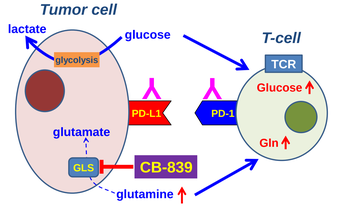


Figure 9: (A) Balb/c mice were implanted subcutaneously with 1 x 10^6 CT-26 colon carcinoma cells. Starting 24 hr post implant, groups of n=10 mice were treated with (i) vehicle dosed orally BID, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) α-PD-L1 at 5 mg/kg dosed IP on days 5, 7, 9, 11, 13, and 15 and (iv) CB-839 and α-PD-L1. Tumor volumes are shown as an average and for individual animals. **(B)** The CT26 syngeneic model was used as in panel A, except that α-PD-1 was used instead of α-PD-L1; α-PD-1 was dosed IP at 5 mg/kg on Days 6, 10 and 14. **(C)** C57.Bl/6 mice were implanted subcutaneously with 1 x 10^6 B16 melanoma cells. Starting 24 hr post implant, groups of n=10 mice were treated with (i) vehicle dosed orally BID, (ii) CB-839 at 200 mg/kg orally BID, (iii) α-PD-L1 at 5 mg/kg dosed orally on days 6, 10 and 14, and (iv) CB-839 and α-PD-1. **(D)** The CT26 model was used as in panel A, except that CD8$^+$ cells were depleted by pre-treatment with an anti-CD8 antibody in one group treated with the combination of CB-839 + α-PD-L1.

Conclusions



- **Competition for glutamine in the tumor microenvironment can potentially limit T- cell proliferation**
- **CB-839 is a glutaminase inhibitor that has broad anti-tumor activity but minimal impact on T-cells**
- **CB-839 elevates glutamine levels in xenografted tumors**
- **Combining CB-839 with anti-PD-1 or anti-PD-L1 shows enhanced anti-tumor activity in syngeneic CT26 mouse tumor model**
- **Ongoing Phase 1 studies with CB-839 have established the safety and tolerability of CB-839 as a monotherapy**
- **Clinical testing of CB-839 with anti-PD-1 will be initiated**

References

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