Targeting Tumor Glutamine Metabolism with CB-839 Enhances the Efficacy of Immune Checkpoint Inhibitors

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Abstract

Background
T-cell activation and proliferation is a metabolically demanding process that requires essential nutrients such as glucose and glutamine. Within the tumor microenvironment, competition between tumor cells and immune cells for limited nutrients can lead to poor T-cell activation and suppression of an anti-tumor immune response. Engagement of immune checkpoints such as PD-1 further suppresses T-cell activation. While therapeutic blockade of immune checkpoints may partially relieve T-cell suppression, low nutrient availability in the tumor microenvironment is expected to limit an optimal immune response.
CB-839 is a glutaminase inhibitor currently in Phase 1 oncology trials. CB-839 blocks glutamine consumption by tumors leading to elevated glutamine levels in the tumor microenvironment. Based on the high demand of T-cells for glutamine, we hypothesized that CB-839 might synergize with immune checkpoint inhibitors to relieve immune suppression and lead to enhanced anti-tumor immune responses.
Materials and methods
Ex-vivo T-cell activation was performed with anti-CD3/CD28 on CD3 cells isolated from human PBMCs. Changes in mRNA expression after T-cell activation was monitored by Nanostring analysis. In vivo efficacy studies were conducted in syngeneic CT-26 or B16 tumor models.
Results
T-cell activation in the absence of glutamine inhibited cell proliferation and the expression of cell surface activation markers. Analysis of mRNA expression also showed suppression of normal activation markers and induction of T-cell exhaustion markers including PD-1, CTLA-4 and BTLA, suggesting that T-cell activation in the absence of glutamine may be sufficient to induce an exhausted phenotype.
Previous work showed that CB-839 blocks glutamine consumption in tumors leading to reduced cell proliferation. Surprisingly, CB-839 had only minimal impact on T-cell proliferation, highlighting differences in glutamine utilization pathways between tumor cells and T-cells. In mouse tumor models, administration of CB-839 elevated tumor glutamine levels, consistent with inhibition of tumor glutaminase. Combination of CB-839 with anti-PD-1 or anti-PD-L1 in the syngeneic CT-26 colon model augmented tumor regressions relative to checkpoint inhibition alone. CB-839 also enhanced the anti-tumor activity of checkpoint inhibitors in the B16 melanoma model. Depletion of CD8+ T-cells from tumor-bearing animals reversed the anti-tumor effects of the combination, confirming an immune-mediated mechanism of action.
Conclusions
These data highlight a novel therapeutic approach to treat cancer by selectively targeting tumor metabolism as a means of enhancing the efficacy of checkpoint blockade. Our data provide a rationale for combining CB-839 with immune checkpoint inhibitors in the clinic.

Introduction

Metabolic suppression of T cells in tumors

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

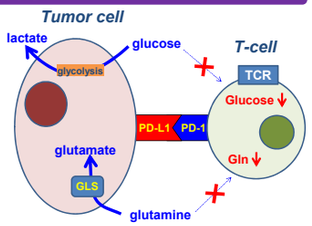
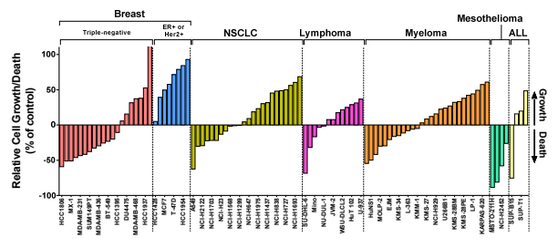


Figure 1: Model of the tumor microenvironment wherein tumor cells out-compete T-cells for limiting nutrients such as glutamine and glucose. Immune checkpoints such as PD-1 ligation with PD-L1 can further restrict T-cell metabolism.

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

Tumor cells are dependent on glutamine for proliferation and survival



T-cell division is dependent on glutamine

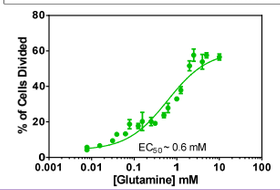
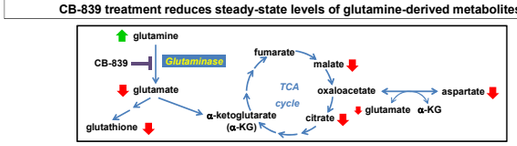


Figure 2: (A) Relative cell growth/death of human cancer cells grown for 72 hrs in the absence of glutamine was assessed with CellTiterGlo. **(B)** Purified mouse splenic T-cells were labeled with CFSE and stimulated with plate-bound αCD3 and soluble αCD28 in the presence of the indicated concentration of glutamine. Cell division was assessed after 96 hours by CFSE dye-dilution.

CB-839 is a Glutaminase Inhibitor with Broad Anti-tumor Activity

CB-839 treatment reduces steady-state levels of glutamine-derived metabolites



CB-839 has anti-proliferative activity across a broad panel of cancer cells

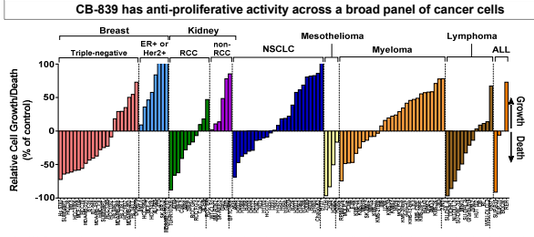
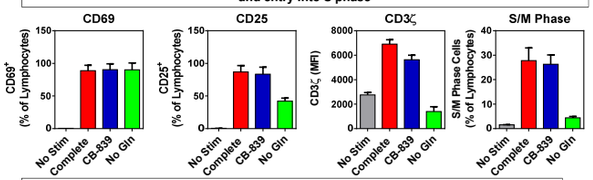


Figure 3: (A) Schematic representation of glutamine metabolism showing experimentally observed changes to levels of glutamine-derived metabolites following treatment with CB-839. **(B)** A panel of tumor cells lines were treated with 1μM CB-839 for 72 hrs in RMPI-1640 media containing 10% fetal bovine 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

Glutamine deprivation but not CB-839 treatment blocks expression of T-cell activation markers and entry into S phase



Glutamine deprivation but not CB-839 treatment blocks T-cell division

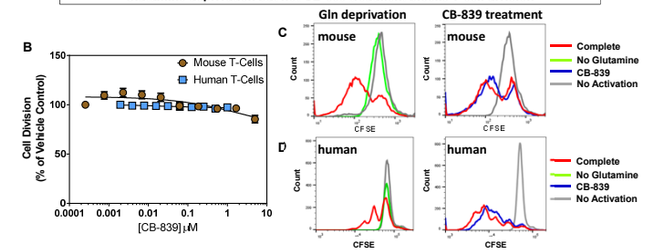
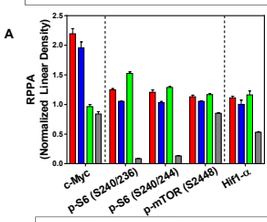


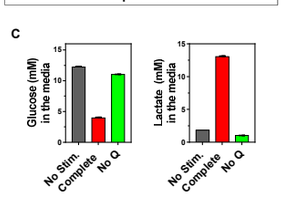
Figure 5: (A) Human T-cells were activated with αCD3/αCD28 and the expression of T-cell activation markers (CD69, CD25, CD3ζ) and DNA content were quantified by flow cytometry after 48 hrs. CB-839 was present at 1 μM where indicated. **(B)** Mouse or human T-cells were labeled with CFSE and activated 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.

Glutamine Deprivation Downregulates Myc and Blocks Metabolic Reprograming in Activated T Cells

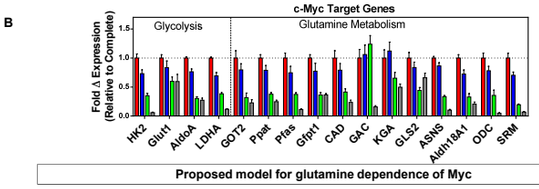
Glutamine deprivation downregulates Myc but not mTOR or Hif1-α



Glycolysis is downregulated by glutamine deprivation



Myc driven metabolic reprogramming is downregulated by glutamine deprivation



Proposed model for glutamine dependence of Myc

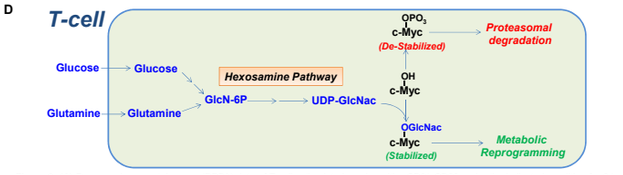


Figure 6: (A) Reverse phase protein array (RPPA) data of T cells stimulated ex vivo with αCD3/αCD28 under the indicated condition for 24 hrs. No Q (glutamine deprivation), No R (arginine deprivation), No Gluc. (glucose deprivation), No Stim. (cells not activated with αCD3/αCD28). **(B)** Nanostring transcriptional profiling of T cells stimulated as in (A) highlighting metabolic genes previously shown to be activated through c-Myc (Immunity 35, 871–882, 2011). **(C)** Measurement of glucose and lactate in media of T cells activated with αCD3/αCD28 under the indicated condition for 96 hrs. **(D)** Proposed mechanism for glutamine-regulated c-Myc stabilization in T cells. See also Nature Immunology 17, 712-720, 2016.

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

More robust T-cell proliferation in conditioned media from CB-839 treated tumor cells

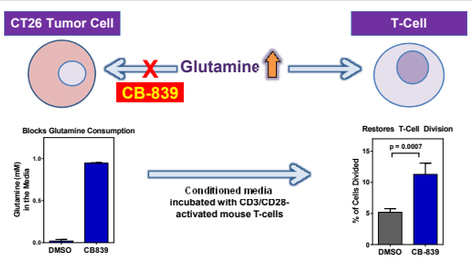


Figure 7: CT26 mouse tumor cells were incubated with DMSO or CB-839 (1 μM) for 72 hrs and conditioned media was harvested and glutamine levels measured. Purified mouse T-cells were then activated with plate-bound αCD3 and soluble αCD28 in the presence of conditioned media and T-cell division was measured after 96 hrs by CFSE dye dilution.

CB-839 Increases Glutamine in Tumors in Vivo

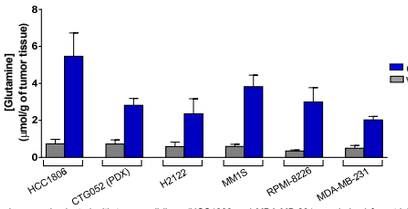
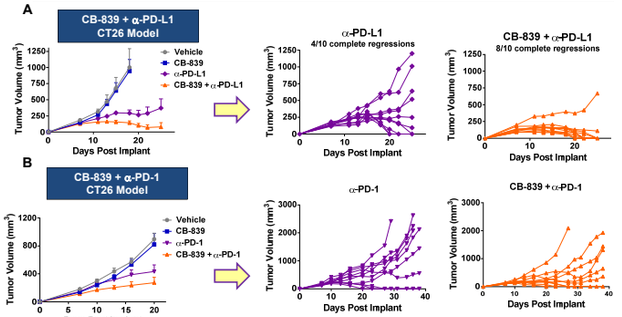


Figure 8: Scid/bg mice were implanted with tumor cell lines (HCC1806 and MDA-MB-231 are derived from triple negative breast cancer; H2122 is derived from non-small cell lung carcinoma; MM1S and RPMI-8226 are derived from multiple myeloma tumor cells) or with a triple negative patient derived xenograft tumor [CTG052 (PDX)]. When tumors reached ~100 mm³, 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 Synergizes 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

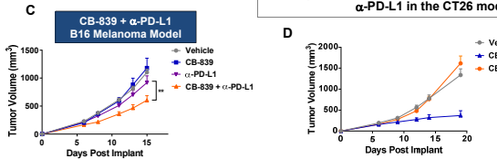


Figure 9: (A) Balb/c mice were implanted subcutaneously with 1 x 10⁶ CT-26 colon carcinoma cells. Starting 24 hrs post implant, groups of n=10 mice were treated with (i) vehicle dosed orally BID, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) α-PD-L1 (clone 10F.9G2, BioXCell) at 5 mg/kg dosed IP on 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-L1 (clone RMP1-14, BioXCell) 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 10⁶ B16 melanoma cells. Starting 24 hrs 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.

Potential Biomarkers For Tumors With Glutamine Deprived T-cells

Hierarchical clustering of gene expression reveals a specific transcriptional signature associated with glutamine deprivation

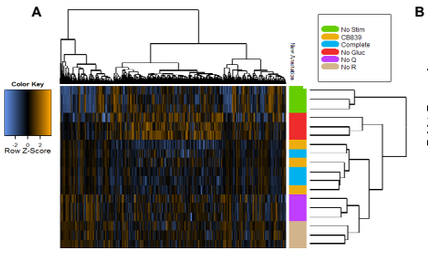
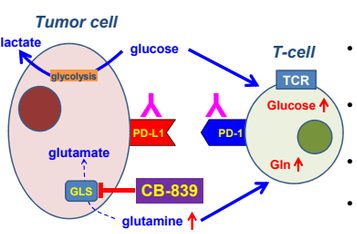


Figure 10: (A) T cells were stimulated ex vivo with αCD3/αCD28 under the indicated condition for 24 hours and transcript levels analyzed by Nanostring. Unsupervised hierarchical clustering analysis of triplicate samples from each treatment group. **(B)** Relative expression level of selected genes involved in suppression of T cell immune responses. No Q (glutamine deprivation), No R (arginine deprivation), No Gluc. (glucose deprivation).

Conclusions



- **Competition for glutamine in the tumor microenvironment can suppress T cell proliferation**
- **Glutamine deprivation blocks Myc expression, Myc-driven metabolic reprogramming, and promotes expression of suppressive markers**
- **CB-839 is a glutaminase inhibitor with broad anti-tumor activity, elevates glutamine levels in tumors thereby potentiating T cell proliferation**
- **CB-839 has minimal impact on T-cell proliferation**
- **Combining CB-839 with anti-PD-1 or anti-PD-L1 shows enhanced anti-tumor activity in syngeneic CT26 and B16 mouse tumor models**
- **Clinical testing of CB-839 with anti-PD-1 has been initiated in cancer patients. See poster 293 for clinical trial design.**