CB-839, a Selective Glutaminase Inhibitor, has Anti-Tumor Activity in Renal Cell Carcinoma and Synergizes with Cabozantinib and Everolimus

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Abstract

Many tumor cells utilize glutamine to meet the elevated bioenergetic and biosynthetic demands of rapid cell growth. The enzyme glutaminase converts glutamine to glutamate, which is used to fuel the TCA cycle, synthesize amino acids and balance cellular oxidative stress. Since glutaminase is highly expressed in RCC tumors, we tested the anti-tumor effect of the glutaminase inhibitor CB-839 in preclinical models. CB-839 had a cytotoxic effect in 18 out of 23 tested RCC cell lines. Kidney tumor cells that were CB-839-sensitive exhibited more pronounced decreases in aspartate and malate levels compared to resistant cells, indicating that sensitivity to glutaminase inhibition may be dependent on the intensity of TCA cycle flux. CB-839-sensitive cells showed stronger pharmacodynamic decreases in mTOR signaling, suggesting that nutrient deprivation by CB-839 treatment is sensed by the mTOR pathway. We tested synergy between CB-839 and the signal transduction inhibitors cabozantinib and everolimus and found that CB-839 synergized with either agent. The CB-839/cabozantinib combination showed enhanced reduction in signaling via AKT and ERK, and TCA cycle activity as compared to single agent treatment. In the Caki-1 xenograft model, the CB-839/cabozantinib combination enhanced tumor growth inhibition compared to either monotherapy. Similarly, the combination of CB-839/everolimus inhibited both glucose and glutamine consumption, leading to decreased glycolytic and TCA cycle function and enhanced anti-proliferative activity. The CB-839/everolimus combination showed greater anti-tumor activity and tumor metabolomics showed evidence of oxidative stress in tumors treated with this combination. The combination of CB-839 and everolimus is currently being tested in patients with advanced RCC and is showing encouraging clinical activity.

CB-839 is a Potent and Selective GLS Inhibitor

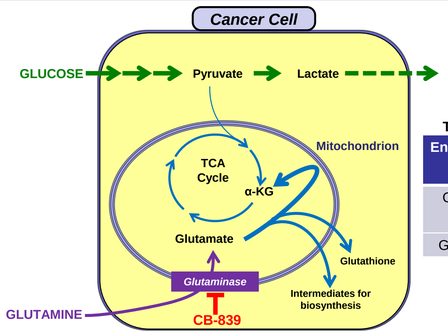


Table 1: IC_{50} determination for CB-839

Enzyme	Splice Variant	Tissue Distribution	IC_{50} (nM)
GLS	GAC	Tumor	24
	KGA	Broad	29
GLS2	-	Liver	>5000

Figure 1. CB-839 inhibits tumor cell metabolism. Tumors rely on the catabolism of glucose and glutamine to produce metabolic intermediates that fuel bioenergetic and biosynthetic demands. Glutaminase initiates this process by converting glutamine to glutamate that is subsequently used in multiple reactions that support tumor cell growth. CB-839 is an orally-bioavailable glutaminase inhibitor that decreases levels of glutamate and other downstream metabolites thereby producing an anti-tumor effect in several in vitro and in vivo preclinical models. CB-839 is currently in Ph.1 clinical testing in RCC patients.

CB-839 Potently Inhibits Glutaminase in RCC Cell Lines

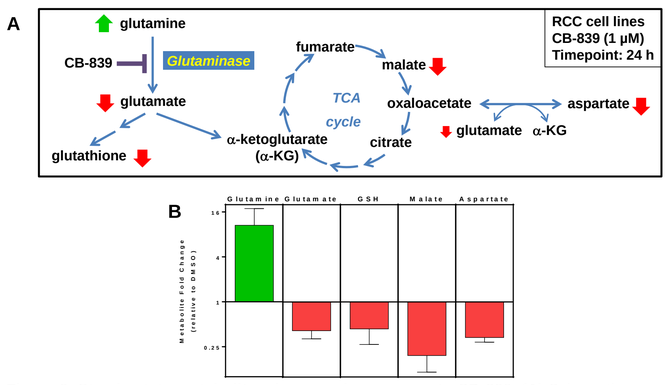


Figure 2. Steady state metabolites decrease in response to CB-839. (A) Schematic representation of glutamine metabolism showing experimentally observed changes to levels of glutamine-derived metabolites following treatment with CB-839. **(B)** CB-839 promotes a consistent metabolic response that includes a suppression of glutamate and downstream metabolites (amino acids, TCA cycle intermediates).

RCC Cells are Sensitive to CB-839

A RCC cell lines are sensitive to CB-839

B Sensitivity to CB-839 correlates with glutamine requirement

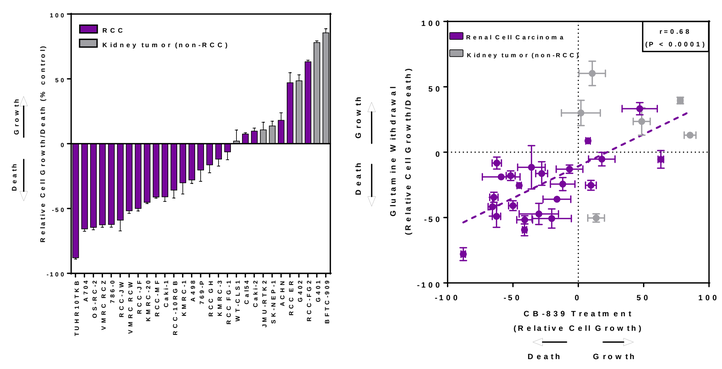


Figure 3. CB-839 has potent anti-proliferative activity in RCC cells that correlates with glutamine dependency. (A) Relative cell growth or cell death across a panel of kidney tumor-derived cell lines following a 72 h treatment with CB-839 (1 μM). **(B)** Correlation between CB-839 sensitivity (1 μM) and glutamine withdrawal. Each data point on the bivariate plots depicts an individual cell line (response to glutamine withdrawal on the y-axis and response to CB-839 on the x-axis).

Cross Talk between Signal Transduction and Metabolism

Rationale for combining glutaminase inhibitor CB-839 with signal transduction inhibitors

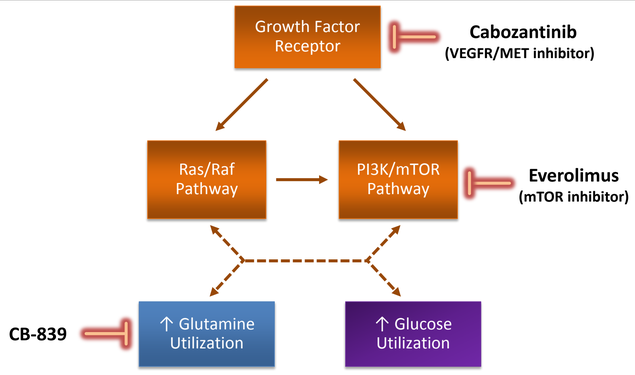
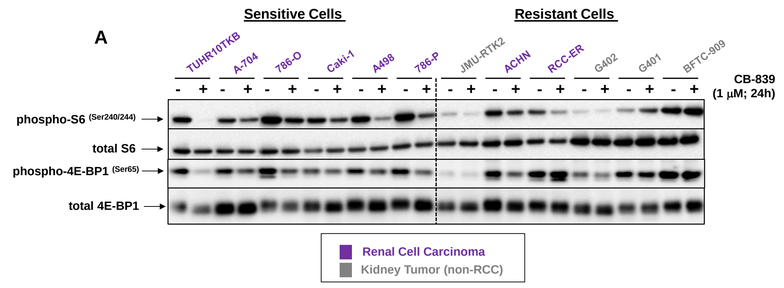


Figure 4. Combination strategy between glutaminase inhibitor CB-839 and signal transduction inhibitors everolimus and cabozantinib. Signal transduction inhibition has been shown to reduce glucose and glutamine utilization, and in combination with CB-839, can synergize to kill cancer cells.

CB-839 Treatment Suppresses the mTOR Pathway

Pharmacodynamic decrease in mTORC1 pathway signaling upon CB-839 treatment in sensitive cells



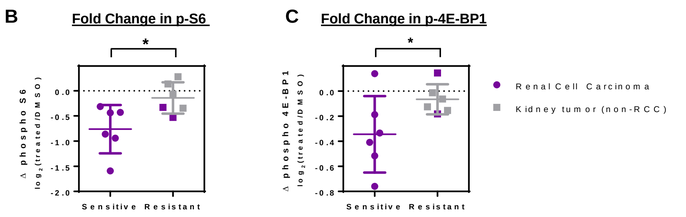


Figure 5. CB-839 inhibits the mTORC1 pathway in sensitive RCC cells. (A) Western blots of the mTORC1 pathway signaling following 24 h treatment with CB-839 (1 μM) or DMSO. **(B, C)** Fold change in phospho-S6 and phospho-4E-BP1 were determined using densitometry by comparing protein levels in CB-839-treated to DMSO treated cells. Each point represents a single CB-839 resistant or sensitive cell line. * (p = 0.05 – 0.01)

CB-839 Synergizes with Cabozantinib in RCC cells

Synergistic anti-proliferative activity | **Combination inhibits signal transduction** | **Combination inhibits TCA cycle activity**

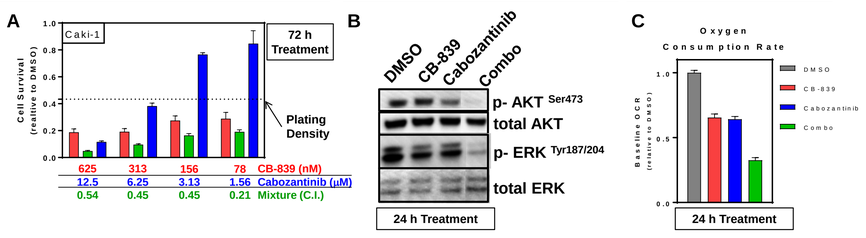


Figure 6. CB-839 synergizes with cabozantinib to decrease proliferation, signal transduction and TCA cycle activity in RCC cell line Caki-1. (A) Viability of Caki-1 cells treated with CB-839, cabozantinib or a combination of both inhibitors for 72 h. Combination Index (C.I.) was calculated using the Calcusyn Software (BioSoft). **(B)** Measurement of signal transduction in cells treated with DMSO, CB-839 (1 μM), cabozantinib (6 μM) or a combination of both drugs for 24 h and cell lysates were probed with anti-phospho-AKT and anti-phospho-ERK antibodies. **(C)** Determination of oxygen consumption rate using the Seahorse Metabolic Analyzer in cells treated for 24 hours as outlined in *(B)*.

Enhanced In Vivo Efficacy of CB-839 plus Cabozantinib

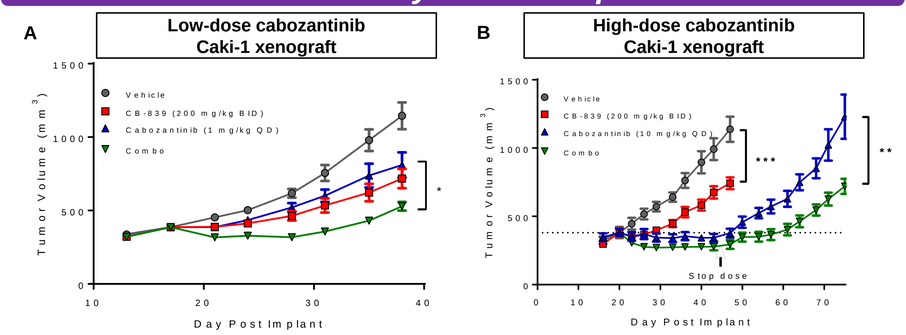
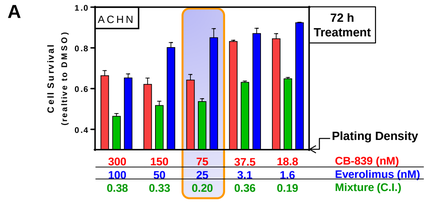


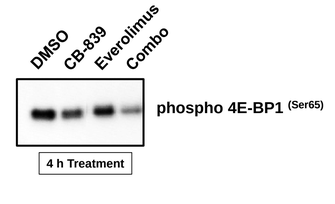
Figure 7. CB-839 shows significant single-agent activity and combines with cabozantinib to produce strong anti-tumor activity. (A) Mice were implanted subcutaneously with Caki-1 RCC cells. When tumor volumes reached ~400 mm^3, mice were randomized into the following four treatment groups: (i) vehicle, (ii) CB-839 at 200 mg/kg and dosed orally BID, (iii) cabozantinib at 1 mg/kg dosed orally QD, and (iv) CB-839 and cabozantinib. **(B)** Same as in (A) except: (i) cabozantinib was dosed at 10 mg/kg QD orally and (ii) dosing was stopped in the cabozantinib and combination groups after day 47.
* (p=.05-.01), ** (p=.01-.001), *** (p=.001-.0001)

CB-839 Synergizes with Everolimus in RCC cells

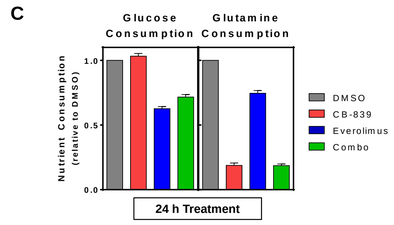
Synergistic anti-proliferative activity



Combination inhibits mTORC1 signal transduction



Combination inhibits glucose and glutamine consumption



Combination inhibits glycolysis and TCA cycle activity

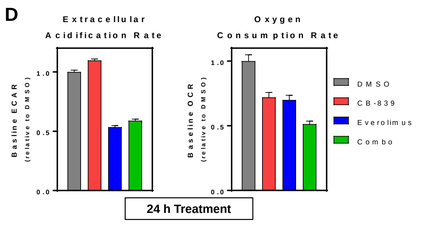
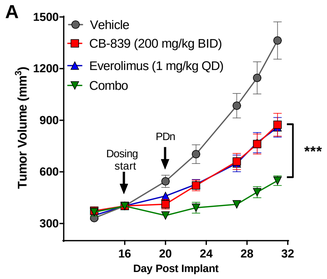


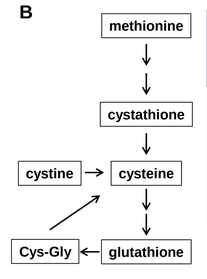
Figure 8. CB-839 synergizes with everolimus to decrease proliferation, mTORC1 signaling and metabolism in RCC cell line ACHN. (A) Viability of ACHN cells treated with CB-839, everolimus or a combination of both inhibitors for 72 h. Combination Index (C.I.) was calculated using the Calcusyn Software (BioSoft). **(B)** Measurement of mTORC1 signaling in cells treated with DMSO, CB-839 (75 nM), everlolimus (25 nM) or a combination of both drugs for 4 h and cell lysates were probed with anti-phospho-4E-BP1 antibody. **(C)** Measurements of glucose or glutamine consumption using the YSI analyzer in cells treated for 24 h as outlined in *(B)*. **(D)** Determination of extracellular acidification rate and oxygen consumption rate using the Seahorse Metabolic Analyzer in cells treated for 24 h as outlined in *(B)*.

Enhanced In Vivo Efficacy of CB-839 plus Everolimus

Anti-tumor efficacy in Caki-1 xenograft



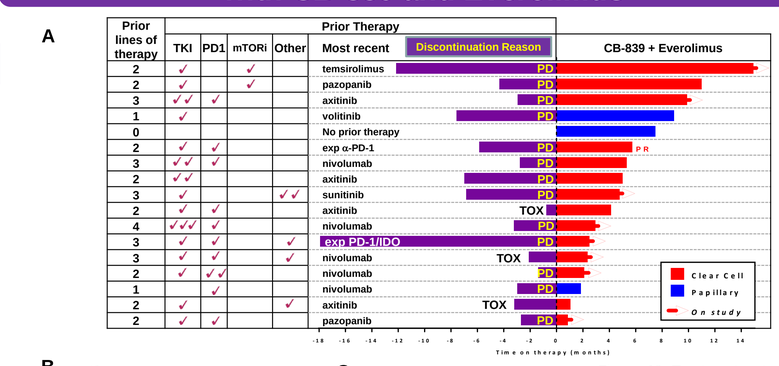
Evidence for oxidative stress in Caki-1 xenograft



Metabolite	Fold Change (compared to vehicle)		
	CB-839	Everolimus	Combo
methionine	1.28	**1.63**	**1.54**
cystathione	1.69	**1.67**	**2.33**
cysteine	1.15	1.04	**1.55**
cystine	**1.74**	**1.78**	**2.13**
Cys-Gly	1.01	0.91	**1.9**
Glutathione	1.0	0.8	0.93
		n.s.	**p <0.05**

Figure 9. CB-839 combines with everolimus to produce strong anti-tumor activity. (A) Mice were implanted subcutaneously with Caki-1 RCC cells. When tumors reached ~400 mm^3 (day 16), mice were randomized into the following four treatment groups : (i) vehicle, (ii) CB-839 at 200 mg/kg and dosed orally BID, (iii) everolimus at 1 mg/kg dosed orally QD, and (iv) CB-839 and everolimus *** (p=.001-.0001). **(B)** Caki-1 xenografted tumors were harvested following 4 consecutive days of dosing (PDn, day 20), and metabolites were measured by LC/MS (Metabolon Inc.). Metabolites involved in glutathione synthesis were upregulated in treatment groups compared to vehicle control. Fold changes that are statistically significant than vehicle treated groups are highlighted in yellow.

Clinical Outcome for Patients Treated with CB-839 and Everolimus



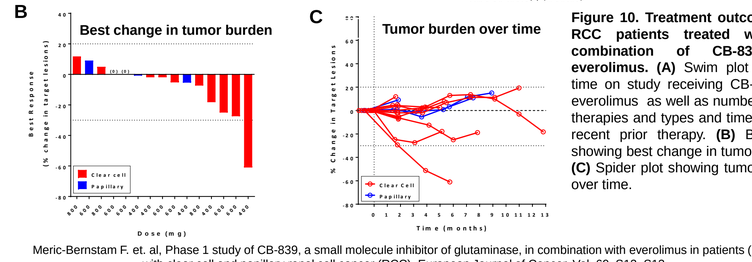


Figure 10. Treatment outcomes for RCC patients treated with the combination of CB-839 and everolimus. (A) Swim plot showing time on study receiving CB-839 and everolimus as well as number of prior therapies and types and time on most recent prior therapy. **(B)** Bar chart showing best change in tumor burden. **(C)** Spider plot showing tumor burden over time.

Meric-Bernstam F. et. al, Phase 1 study of CB-839, a small molecule inhibitor of glutaminase, in combination with everolimus in patients (pts) with clear cell and papillary renal cell cancer (RCC). European Journal of Cancer, Vol. 69, S12–S13

Conclusions

- **CB-839 is a potent and selective glutaminase inhibitor**
- **CB-839 treatment of RCC cells causes decreases in glutamine derived metabolites, mTOR pathway signaling and cellular proliferation**
- **CB-839 combines with everolimus and cabozantinib to produce synergistic anti-proliferative activity and to decrease signal transduction and metabolism in RCC cells**
- **CB-839 in combination with everolimus or cabozantinib enhances anti-tumor activity in xenograft mouse models**
- **CB-839 in combination with everolimus or cabozantinib is currently being tested in RCC patients**
- **The combination of CB-839 with everolimus has encouraging efficacy in clear cell and papillary cell carcinoma**

Keystone Symposia, Tumor Metabolism: Mechanisms and Targets, Whistler Canada, March 5-9, 2017