The Glutaminase Inhibitor CB-839 Synergizes with CDK4/6 and PARP Inhibitors in Pre-Clinical Tumor Models

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Abstract Number: 3509

Abstract

Many tumor cells utilize the amino acid 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 nucleotides, and balance cellular oxidative stress. We developed CB-839, a potent and orally bioavailable small molecule inhibitor of glutaminase, that blocks production of glutamate and generation of downstream metabolites glutathione, malate and aspartate. Mice treated with CB-839 had decreased levels of nucleotides in their tumors, likely due to glutamine-derived aspartate being required for nucleotide biosynthesis. Consistent with our finding that CB-839 decreases nucleotide pools, CB-839 treatment delayed cancer cells from either entry into S-phase or progression through S-phase. Based on this observation, the ability of CB-839 to synergize with therapies that block cell cycle progression was tested. CB-839 synergized with the CDK4/6 inhibitor palbociclib in colorectal carcinoma (CRC), triple negative breast cancer (TNBC) and ER+ breast cancer cell lines resulting in anti-proliferative activity. The combination of CB-839 with palbociclib also led to decreased cell cycle progression through S-phase and caused an accumulation of cells in G1. In vivo, the combination of CB-839 with palbociclib resulted in enhanced anti-tumor activity in both an ER+ breast cancer and CRC xenograft tumor model. We next investigated whether CB-839 treatment would enhance the anti-tumor effects of DNA repair inhibitors, given the ability of CB-839 treatment to decrease nucleotide pools. CB-839 treatment in combination with the PARP inhibitors niraparib and talazoparib led to synergistic anti-proliferative activity in TNBC, CRC, non-small cell lung carcinoma, ovarian and prostate cancer cells. In vivo, the combination of CB-839 with PARP inhibitors showed enhanced anti-tumor activity compared to single agent treatment in a CRC tumor xenograft model. CB-839 is currently undergoing evaluation for efficacy in the treatment of cancer in several phase I/II clinical trials. These encouraging pre-clinical results support the testing of CB-839 with PARP or CDK4/6 inhibitors in cancer patients.

Glutaminolysis is Required for Nucleotide Biosynthesis

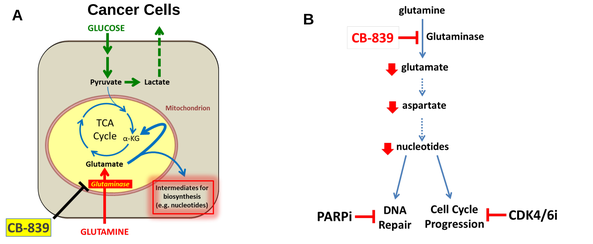

Figure 1. CB-839 inhibits tumor cell metabolism. **(A)** Tumors rely on the catabolism of glucose and glutamine to produce metabolic intermediates that fuel bioenergetic and biosynthetic demands. Glutaminase initiates this process by converting glutamine to glutamate that is used in multiple reactions to support tumor cell growth including nucleotide biosynthesis. CB-839 is an orally-bioavailable glutaminase inhibitor that decreases levels of glutamate and other downstream metabolites producing an anti-tumor effect. **(B)** Inhibition of glutaminase results in decreased levels of aspartate and downstream nucleotide metabolites. Due to decreased nucleotide biosynthesis by CB-839, we hypothesized that CB-839 treatment may limit DNA repair and cell cycle progression. This unique mechanistic rationale provides justification for testing the combination of CB-839 with agents that target PARP enzymes and cell cycle regulating CDK4/6.

CB-839 Reduces Nucleotides in Tumor Cells

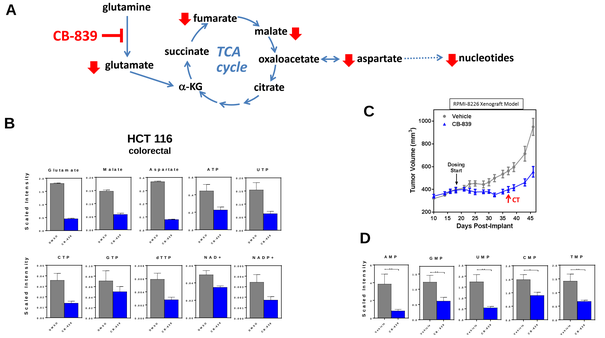

Figure 2. Steady state metabolites needed for nucleotide biosynthesis decrease in response to CB-839. **(A)** Schematic representation of glutamine metabolism showing experimentally observed changes to levels to glutamine-derived metabolites following treatment with CB-839. **(B)** HCT 116 cells were treated with DMSO or CB-839 at 1 μM for 24 hours. Cells were collected and analyzed for nucleotide metabolites. CB-839 promotes a consistent metabolic response that includes a suppression of glutamate and downstream metabolites, such as amino acids, TCA cycle intermediates and nucleotides. **(C)** Groups of n=10 scid/bg mice were implanted with RPMI-8226 myeloma cells and dosed with CB-839 (200 mg/kg BID) or vehicle. **(D)** A separate cohort of xenografted animals from **(C)** (n=5 per group) were harvested at the indicated collection time (CT) for metabolic profiling. Significant changes are noted by; ** (p<0.01); * (p<0.05). LC-MS metabolic profiling was performed by Human Metabolome Technologies.

CB-839 + PARP Inhibitor Synergize To Inhibit Cell Growth

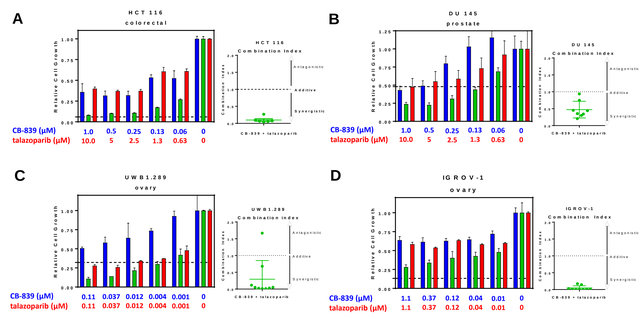

Figure 3. CB-839 synergizes with talazoparib to inhibit cancer cell proliferation. Viability of cells treated with a dose titration of CB-839, talazoparib, or a mixture of CB-839 and talazoparib were measured using CellTiter-Glo (Promega) after 72 hours for **(A), (B)** and **(D)**, and 7 days for **(C)**. Relative cell growth was normalized to DMSO control. Combination indices were calculated using CalcuSyn software (Biosoft) and reported for individual mixtures of CB-839 and talazoparib. Dotted lines on treatment graphs represents biomass plating density at Day 0. Mutational status of cell lines used: **(A)** HCT 116 are heterozygous mutant for BRCA2. **(B)** DU 145 are homozygous mutant for BRCA2 and heterozygous mutant for BRCA1. **(C)** UWB1.289 are homozygous mutant for BRCA1. **(D)** IGROV-1 are heterozygous mutant for BRCA1 and BRCA2.

CB-839 + PARP Inhibitor Cause DNA Damage

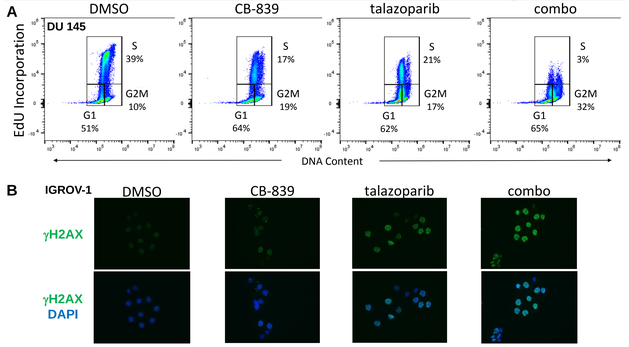

Figure 4. CB-839 combines with talazoparib to inhibit DNA synthesis and elicit a DNA damage response. **(A)** DU 145 cells were treated with CB-839 (1 μM), talazoparib (10 μM) or in combination for 24 hours. At the end of incubation, cells were treated with EdU (Thermo Fisher Scientific) and stained for DNA content. Data was collected on the Attune flow cytometer (Thermo Fisher Scientific). Gating and analysis were performed using FlowJo software (flowjo.com). **(B)** IGROV-1 cells were grown on glass coverslips and treated with CB-839 (1 μM), talazoparib (1 μM), or in combination for 24 hours. At the end of incubation, cells were fixed, permeabilized, and stained with anti-γH2AX antibody (Cell Signaling Technology) and DAPI. Images were acquired with a 40X objective on a Zeiss AxioImager microscope using equivalent exposure times. A representative image is shown for each treatment group.

CB-839 + PARP Inhibitors Combine To Inhibit Tumor Growth

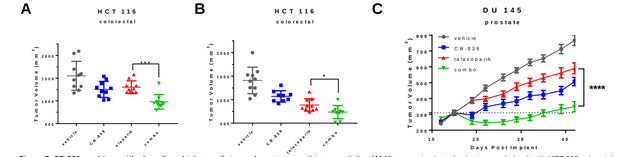

Figure 5. CB-839 combines with olaparib and talazoparib to produce strong anti-tumor activity. **(A)** Mice were implanted subcutaneously (s.c.) with HCT 116 colorectal cancer cells. When tumors reached ~250 mm³ (day 7), mice were randomized into 4 treatment groups: (i) vehicle, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) olaparib at 50 mg/kg dosed orally QD, and (iv) CB-839 and olaparib. **(B)** Same as in **(A)** except the 4 treatment groups were: (i) vehicle, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) talazoparib at 0.25 mg/kg dosed orally QD, and (iv) CB-839 and talazoparib. Mann-Whitney analysis of tumor volumes on Day 32 for **(A)** *** $p = 0.0007$, **(B)** * $p = 0.0115$. **(C)** Mice were implanted subcutaneously (s.c.) with DU 145 prostate cancer cells. When tumors reached ~300 mm³ (day 15), mice were randomized into 4 treatment groups: (i) vehicle, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) talazoparib at 0.25 mg/kg dosed orally QD, and (iv) CB-839 and talazoparib. Two-way ANOVA; **** $p < 0.0001$ for combo vs. talazoparib.

CB-839 + CDK4/6 Inhibition Synergize To Inhibit Cell Growth

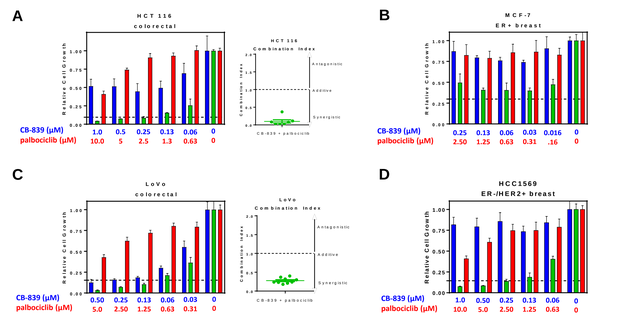

Figure 6. CB-839 synergizes with palbociclib to inhibit cancer cell proliferation. **(A)-(D)** Viability of cells treated with a dose titration of CB-839, palbociclib, or a mixture of CB-839 and palbociclib was measured using CellTiter-Glo (Promega) after 72 hours. Relative cell growth was normalized to DMSO control. Combination indices for **(B)** and **(D)** were calculated using CalcuSyn software (Biosoft) and reported for individual mixtures of CB-839 and palbociclib. Combination Index could not be calculated for MCF-7 and HCC1569 due to absence of single agent dose response. Dotted lines on treatment graphs represents biomass plating density at Day 0.

CB-839 + CDK4/6 Inhibitor Block Cell Cycle Progression

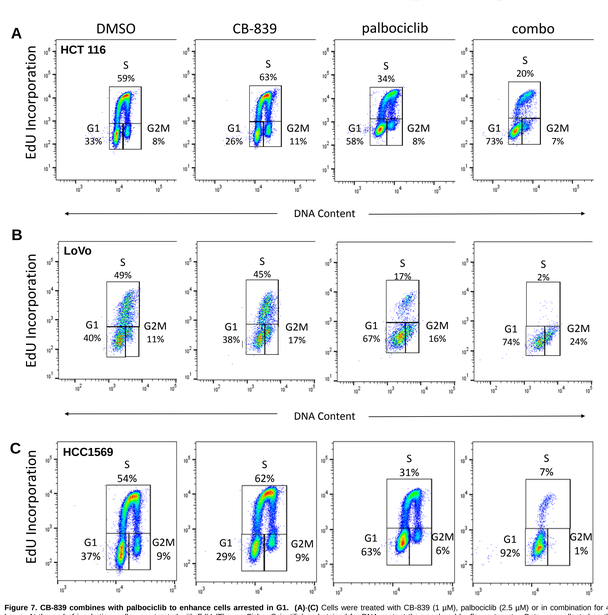

Figure 7. CB-839 combines with palbociclib to enhance cells arrested in G1. **(A)-(C)** Cells were treated with CB-839 (1 μM), palbociclib (2.5 μM) or in combination for 48 hours. At the end of incubation, cells were treated with EdU (Thermo Fisher Scientific) and stained for DNA content then analyzed by flow cytometry. Data was collected on the Attune flow cytometer (Thermo Fisher Scientific). Gating and analysis were performed using FlowJo software (flowjo.com). Cell cycle analysis for all compound treatments are presented as scatter plots, where DNA content is plotted against EdU incorporation.

CB-839 + CDK4/6 Inhibition Enhance Cell Cycle Arrest In G1

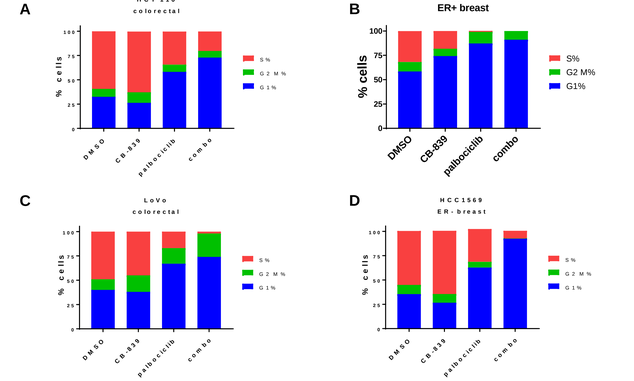

Figure 8. Combining CB-839 with palbociclib enriches for cells in G1 for all cell lines tested. Graphical representation of data from Figure 7 for **(A), (C) and (D).** **(B)** Cells were treated with CB-839 (1 μM), palbociclib (0.3 μM) or in combination for 48 hours. Data collected and analyzed as described in Figure 7.

CB-839 + CDK4/6 Inhibitor Combine To Inhibit Tumor Growth

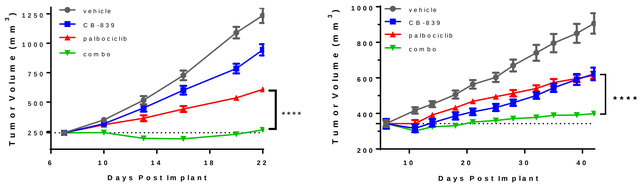

Figure 9. CB-839 combines with palbociclib to produce strong anti-tumor activity. **(A)** Mice were implanted subcutaneously (s.c.) with HCT 116 colorectal cancer cells. When tumors reached ~250 mm³ (day 7), mice were randomized into 4 treatment groups: (i) vehicle, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) palbociclib at 100 mg/kg dosed orally QD, and (iv) CB-839 and palbociclib. The dosing schedule for the combo group was courses of 6-day dosing followed by 4-day drug holidays. **(B)** Mice were implanted s.c. with estradiol pellets 1 day before s.c. implantation of MCF-7 breast cancer cells. When tumors reached ~350 mm³ (day 6), mice were randomized into 4 treatment groups: (i) vehicle, (ii) CB-839 at 200 mg/kg dosed orally BID, (iii) palbociclib at 50 mg/kg dosed orally QD, and (iv) CB-839 and palbociclib. Two-way ANOVA; **** $p < 0.0001$ for combo vs. palbociclib.

Conclusions

- CB-839 decreases nucleotides in tumor cells, impairing DNA synthesis, and cell cycle progression.
- CB-839 synergizes with PARP inhibitors to impair DNA synthesis, enhance DNA damage, and block cell proliferation.
- In vivo, CB-839 in combination with PARP inhibitors demonstrates enhanced anti-tumor activity in colorectal and prostate tumor models.
- CB-839 synergizes with a CDK4/6 inhibitor to block cell cycle progression and cell proliferation.
- In vivo, CB-839 in combination with a CDK4/6 inhibitor demonstrates enhanced anti-tumor activity in ER+ breast and colorectal tumor models.