Glutaminase Inhibitor CB-839 Synergizes with Pomalidomide in Preclinical Multiple Myeloma Models

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Abstract #4720
Many hematological tumor cells are dependent on glutamine for growth and survival. Glutamine is the most abundant amino acid in plasma and can be utilized by tumor cells for production of energy and generation of building blocks for the synthesis of macromolecules. Small molecule CB-839 inhibits glutaminase (GLS) activity thereby blocking cellular glutamine utilization resulting in an anti-tumor effect in several hematological tumor types including multiple myeloma (MM), acute lymphocytic leukemia, and several types of non-Hodgkin’s lymphoma [Parlati et al. Blood 2013 122:4226]. Phase 1 clinical trials have been initiated to test the safety, pharmacokinetics, pharmacodynamics, and clinical activity of single agent CB-839 in several hematological malignancies. In anticipation of potential combinations of CB-839 with standard of care agents in future MM clinical trials, we tested the effects of CB-839 in combination with the IMID, pomalidomide (POM). POM caused complete growth inhibition in MM.1S cells with an EC$_{50}$ of 16 nM as opposed to partial growth inhibition in RPMI-8226 cells, with an EC$_{50}$ of 130 nM. CB-839 caused complete growth inhibition in MM.1S cells with an EC$_{50}$ value of 26 nM and produced a cytotoxic effect in RPMI-8226 cells with an EC$_{50}$ of 160 nM. When combined, CB-839 enhanced the antiproliferative activity of POM in both POM-sensitive MM.1S and POM-resistant RPMI-8226 cells resulting in a synergistic anti-tumor effect as demonstrated by combination index values between 0.18-0.62 (mean= 0.36) for the MM.1S and 0.25-0.72 (mean= 0.38) for the RPMI-8226 cells. To investigate the mechanism that underlies the observed synergy, RPMI-8226 cells were treated for 24 hours and changes in proteins and metabolites were measured by reverse-phase-protein array and CE/MS, respectively. When treated with CB-839 alone, RPMI-8226 cells respond by decreasing mTOR pathway signaling proteins (e.g. phospho-mTOR, phospho-p70S6K, phospho-PRAS40, phospho-S6), decreasing the amount of oncogenic proteins (c-Myc and c-Kit), and increasing programmed cell death pathway proteins (e.g. cleaved caspase 7, cleaved PARP), consistent with the cytotoxic activity observed for CB-839. Several of these changes were further enhanced in the presence of POM (e.g. phospho-p70S6K, phospho-S6, phospho-PRAS40, c-kit, c-Myc), however only the enhanced decrease in c-Myc reached statistical significance. Metabolite analysis showed changes with CB-839 consistent with GLS inhibition (e.g. decreases in glutamate, aspartate, succinate and malate and increases in glutamine). On the other hand, single agent POM caused very modest changes in the metabolite profile. When the two agents were combined, metabolite levels were consistent with those observed with single agent CB-839, with the notable exception of carbamoyl-aspartate where lower levels were measured in the combination group in comparison to cells treated with either agent alone. Carbamoyl-aspartate is an intermediate in the pyrimidine biosynthesis pathway and is synthesized by the multi-catalytic enzyme CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, dihydroorotase), an enzyme that is regulated by mTOR [Ben-Sahra et al. (2013) Science 339: 1323-8]. These observations suggest that CB-839 dampens mTOR signaling and POM may further attenuate this response, possibly contributing to the synergistic anti-tumor effect. These data motivated testing the anti-tumor effect of the combination of CB-839 and POM in mice bearing RPMI-8226 xenografts. Oral dosing with single agent CB-839 and POM resulted in tumor growth inhibition (TGI) of 64% and 48%, respectively, whereas the combination of the two agents resulted in a TGI of 97%. Efficacious doses of CB-839 and POM alone or in combination were well tolerated with no effect on animal body weight. These promising results indicate that GLS inhibition with CB-839 in combination with POM may provide therapeutic benefit in MM and provide motivation for future clinical studies.
Altered Glutamine Metabolism in Cancer

Many tumors rely on the catabolism of glucose and glutamine to produce metabolic intermediates that fuel bioenergetic and biosynthetic demands (Ref 1-4). Glutaminase initiates this process by converting glutamine to glutamate. CB-839 is an orally-bioavailable glutaminase inhibitor that decreases levels of glutamate and other downstream metabolites (e.g. aspartate, TCA cycle intermediates, GSH) thereby producing an anti-tumor effect in several in vitro and in vivo preclinical models (Ref 5-7). CB-839 is currently being tested in Phase 1 clinical trials for the treatment of cancer.
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Figure 1: CB-839 treatment of mice bearing RPMI-8226 tumor xenografts causes an increase in glutamine and decreases in glutamate and downstream metabolites. Mice bearing RPMI-8226 myeloma xenografts were dosed with CB-839 (200 mg/kg) or vehicle. Mice were sacrificed 4h after the last dose and tumors were harvested and analyzed for metabolite levels. (A) Schematic representation of metabolic pathways downstream of glutamate. Metabolites with significant decreases following CB-839 administration are noted with a downward red arrow. (B) CB-839 causes an increase in glutamine and decreases in glutamate, aspartate, glutathione, fumarate, malate and citrate in RPMI 8226 tumors. Significant changes in metabolites are noted by: * (p < 0.05); ** (p < 0.01); *** (p < 0.001); and **** (p < 0.0001).
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**Antiproliferative Activity of CB-839**

*Glutaminase inhibition blocks growth in the majority of multiple myeloma cell lines*

![Graph A](image)

**Figure 2:** CB-839 has broad antiproliferative activity across a panel of hematological tumor cell lines representing multiple myeloma, lymphoma and acute lymphocytic leukemia (ALL). (A) Antiproliferative activity of CB-839 treatment for 72 h across a panel of cell lines. EC_{50} values were calculated and graphed in ascending order of resistance. (B) Cell proliferation and cell loss was measured after 72 h of 1μM CB-839 treatment.
Synergistic Antiproliferative Activity of CB-839 and Pomalidomide

CB-839 combines with pomalidomide to produce synergistic antiproliferative activity

**Figure 3:** CB-839 in combination with pomalidomide acts synergistically to produce an antiproliferative effect in multiple myeloma cells *in vitro*. (A) EC$_{50}$ values for multiple myeloma cell lines MM.1S (blue circles) and RPMI-8226 (red squares) treated with pomalidomide (left panel) or CB-839 (right panel) for 72 h. (B) RPMI-8226 cells are resistant to pomalidomide. The calculated EC$_{50}$ values for pomalidomide and CB-839 as well as the response type is noted. (C-D) Combinations of CB-839 with pomalidomide act synergistically to produce an antiproliferative effect in multiple myeloma cells. MM.1S cells (C) and RPMI-8226 cells (D) were treated with either CB-839, pomalidomide, or a mixture thereof for 72 h and cell viability was measured. Cell survival ratios for all compound treatments are represented as bar graphs. Combination indices were calculated using the CalcuSyn program. (E) Combination indices from multiple antiproliferative experiments for CB-839/pomalidomide mixtures are represented as scatter plots and mean combination index value for MM1.S and RPMI-8226 cells are noted.
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Altered Metabolism with CB-839 and Pomalidomide Combination

Inhibition of tumor metabolism by CB-839 is enhanced by pomalidomide

Figure 4: CB-839 combines with pomalidomide to further decrease metabolite levels in multiple myeloma RPMI-8226 cells. RPMI-8226 cells were treated with either DMSO, pomalidomide (1 µM), CB-839 (50 nM) or a mixture of both agents for 24 h. Targeted quantitative analysis was performed and a total of 116 metabolites were quantitated by Human Metabolome Technologies, Inc. (A-C) Bivariate plots of metabolite levels in compound-treated versus DMSO-treated cells are shown. Metabolites whose levels change significantly are highlighted. (D) Levels of select metabolites in response to pomalidomide, CB-839, and pomalidomide/CB-839 treatment compared to DMSO-treatment are shown. Significant changes in metabolites between CB-839-treated and CB-839/pomalidomide-treated cells are noted.
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**CB-839 and Pomalidomide Combine to Inhibit Signal Transduction**

Enhanced suppression of signal transduction pathways by the addition of pomalidomide to CB-839

**A**

Median Centered Unsupervised Hierarchical Clustering

**B**

Cytokines showing inhibition with CB-839 and Pomalidomide combination

- p70S6K pThr189
- S6 pSer235
- S6 pSer240
- PRAS40 pThr246
- Rictor pThr1135
- mTOR pSer2448

**C**

- 4E-BP1 pSer65
- c-myc
- c-kit
- PARP Cleaved
- Caspase-7 Cleaved

Figure 5: CB-839 combines with pomalidomide to decrease phosphorylated proteins in the mTORC1 pathway in multiple myeloma RPMI-8226 cells. RPMI-8226 cells were treated with either DMSO, pomalidomide (1 μM), CB-839 (50 nM) or a combination of both agents for 4h or 24h. Changes in cellular signaling pathways were measured by reverse phase protein array (RPPA) performed by the MD Anderson RPPA Core Facility. (A) The heatmap was generated in Cluster 3.0 as a hierarchical cluster using Pearson Correlation and a center metric. (B) Levels of select proteins and phosphorylated proteins in response to pomalidomide, CB-839- and pomalidomide/CB-839-treatment compared to DMSO-treatment are shown. Significant changes in proteins and phosphorylated proteins between CB-839-treated and CB-839/pomalidomide-treated cells are noted. (C) Western blot analysis of lysates prepared from RPMI-8226 cells treated with either DMSO, CB-839, pomalidomide or a combination of CB-839 and pomalidomide.
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**In Vivo Anti-tumor Activity**

*Combinations of CB-839 and pomalidomide produce strong anti-tumor activity in a myeloma xenograft model*

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**Figure 6:** CB-839 shows significant single-agent activity and combines with pomalidomide to produce strong anti-tumor activity in the IMID-resistant RPMI-8226 xenograft model.  
(A) Mice were implanted subcutaneously with RPMI-8226 myeloma cells. When tumor volume had increased in three consecutive measurements, mice were randomized into the following four treatment groups: (i) vehicle, (ii) CB-839 at 200 mg/kg and dosed orally BID, (iii) pomalidomide at 1 mg/kg dosed orally QD, and (iv) CB-839 and pomalidomide.  
(B) Scatter plot of tumor volumes at the end of study.  
(C) Efficacious doses of CB-839 have no impact on body weight gain either as a single agent or in combination with pomalidomide in mice from xenograft efficacy studies.
Summary and Conclusions

• CB-839 is a novel, potent and selective glutaminase inhibitor that is currently in Phase 1 clinical trials for the treatment of multiple myeloma, lymphoma, leukemia and solid tumors.

• CB-839 targets metabolic pathways critical for tumor growth and survival and shows potent in vitro and in vivo antiproliferative activity in preclinical models of multiple myeloma.

• CB-839 combines with pomalidomide, an approved agent for the treatment of relapsed/refractory multiple myeloma, to produce a synergistic antiproliferative effect in IMiD-resistant cells.

• The combination of CB-839 and pomalidomide produces enhanced effects on several metabolic and signal transduction pathways likely contributing to the synergistic antiproliferative activity.

• Synergistic decreases in the metabolite carbamoyl-aspartate indicate that the CB-839/pomalidomide combination may result in a critical deficiency in nucleotide biosynthesis.

• In a multiple myeloma xenograft model, CB-839 showed significant single agent anti-tumor efficacy and displayed enhanced anti-tumor activity when combined with pomalidomide.

• This study provides a preclinical rationale for testing the combination of CB-839 and pomalidomide in multiple myeloma patients.

References

5. Gross et al. (2014) Mol Cancer Ther 13:890-901