**Abstract**

Glutaminase is a rate-limiting enzyme that converts glutamine to glutamate to support several metabolic processes including amino acid and nucleotide synthesis, cellular ATP production, and the maintenance of redox homeostasis. Genetic and pharmacological inhibition of glutaminase further affects translation, proteome, lysosomal, and mitochondrial activities. Across a panel of twenty-nine myeloma cell lines, we found that glutaminase inhibition with CB-839 at 5 μM concentrations resulted in growth inhibition and a lack of cell death. To identify biomarkers that predict sensitivity to CB-839 in multiple myeloma cells, we profiled cellular metabolites, mitochondrial activity, and signaling pathways in sensitive and resistant cell lines (ρCB-839 vs ωCB-839).

Biomarker analysis showed that CB-839 treatment suppressed the activity of the α-keto acid-sensitive kinase mTORC1 in CB-839-sensitive cells, leading to downregulation of protein synthesis and expression of amino acids. Analysis of steady state levels of mTORC1 signaling substrate pS6K and pPRAS40 demonstrated that CB-839 treatment led to a dose-dependent decrease in these proteins in sensitive cell lines. These proteins were highly correlated with sensitivity to CB-839 across multiple myeloma cell types, with pPRAS40 acting as a biomarker.

**Conclusion**

Sensitive myeloma cells show varying antiproliferative responses to glutaminase inhibition by CB-839; CB-839-resistant cell lines do not. These results highlight that glutaminase inhibition should be considered as a novel therapeutic approach to overcome resistance to existing glutaminase inhibitors. Further studies are needed to evaluate the potential clinical utility of CB-839 in myeloma patients resistant to existing inhibitors.

**Discovery of Biomarkers That Predict CB-839 Sensitivity**

**CB-839 Causes a Pharmacodynamic Decrease in Nucleotides in a Tumor Xenograft**

**Summary and Conclusions**

- CB-839, a selective and potent inhibitor of glutaminase, blocks proliferation and induces apoptosis in a sub-set of multiple myeloma cells.
- To discover biomarkers for CB-839 sensitivity in multiple myeloma cells, a comprehensive metabolomic and proteomic analysis was performed.
- CB-839 suppressed mTORC1 pathway in myeloma sensitive cells which led to lower nucleotide levels.
- Low pyruvate carbamoylase expression was correlated with sensitivity to CB-839.
- Knockdown of PC sensitive resistant cells to CB-839.
- AVT inhibition decreased glucose metabolism and sensitive resistant cells to CB-839.