Oral Proteasome Inhibitor Marizomib and IMiD® Imunomodulatory Drug Pomalidomide Trigger Synergistic Anti-Myeloma Activity and Enhanced Proteasome Inhibition In Vitro and In Vivo

Deepika Sharma Das1, Arghya Ray1, Yan Song1, Paul G. Richardson2, Mohit Trikula2, Dharminder Chauhan2 and Kenneth C. Anderson3, *Joint Senior Authors
1The Icahn Institute for Myeloma Therapeutics and Jerome Lipper Myeloma Center, Department of Medical Oncology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA; 2Triphase Accelerator, San Diego, CA

Abstract

Proteasome inhibitor bortezomib is an effective therapy for the treatment of relapsed and refractory multiple myeloma (RRMM); however, prolonged treatment can be associated with toxicity and drug resistance. A novel proteasome inhibitor marizomib is distinct from bortezomib in its chemical structure, mechanisms of action, and effects on proteasomal activities (Chauhan et al., Cancer Cell 2005, 8:407-419). Pomalidomide is an analogue of thalidomide with potent immunomodulatory activity. Based on increased progression-free survival, pomalidomide has been approved by the FDA for the treatment of patients with RRMM who have received at least two prior therapies, including lenalidomide and bortezomib, and who showed disease progression on or within 60 days of completion of the most recent therapy. Here we utilized in vitro and in vivo models of MM to examine the anti-MM activity of combined marizomib and pomalidomide. Animal model studies examined the efficacy of marizomib (PO) using both single weekly and twice weekly schedule either alone or together with pomalidomide (PO).

Materials and Methods

Methods: Human MM cell lines MM.1S, desamethasone-resistant (MM.1R), INA-6, ARP-1, RPMI-8226, doxorubicin-resistant (DOX-40), melphalan-resistant (LR5), ANBL-6.WT (wild type), and bortezomib-resistant (ANBL-6.BR) were used. Cell viability assays were performed using WST/MTT. Synergistic anti-MM activity was determined with CalcuSyn software program. Proteasome activity was measured as in prior study (Chauhan et al., Cancer Cell 2005). MM.1S-tumor-bearing mice were treated with vehicle control, marizomib (PO) pomalidomide (PO), or marizomib plus pomalidomide at the indicated doses for 21 days on a twice-weekly or once weekly schedule for marizomib and 4 consecutive days weekly for pomalidomide. Statistical significance was determined using a Student’s  \( t \) test. Pomalidomide was purchased from Selleck chemicals; marizomib was obtained from Triphase Accelerator, USA.

Results

Figure 1 Combination of low doses of marizomib and pomalidomide inhibits human plasmacytoma growth and promotes survival in CB-17 SCID mice. (A) MM.1S tumor-bearing mice were treated with vehicle control, marizomib, pomalidomide, or marizomib plus pomalidomide (orally) at the indicated doses for 21 days on a twice-weekly schedule for marizomib and 4 consecutive days weekly for pomalidomide. Data presented are mean ± SE (n=5, P<0.05). (B) Kaplan-Meier plots showing survival for mice treated with marizomib, pomalidomide, or marizomib plus pomalidomide (as in panel A). Marizomib plus pomalidomide treated mice showed significantly increased survival (P<0.05) compared with the untreated group.

Figure 2 Mechanisms of marizomib plus pomalidomide-induced MM cell apoptosis. MM.1S cells were pretreated with or without marizomib for 12h and then treated with or without pomalidomide for 12h. The cells were then analyzed for apoptosis using antibodies against PARP, caspase-3, caspase-8, IRF-4, c-Myc, Mcl-1 and GAPDH. FL indicates full length and GF, cleaved fragment. Combination index (CI) of < 1 indicates synergy.

Figure 3 CRBN siRNA attenuates marizomib plus pomalidomide-induced MM cell death. MM.1S cells were transfected with scr-siRNA or CRBN-siRNA. 48h, followed by WST-1 assay. Immunoblot shows CRBN expression in cells transfected with scr-siRNA or CRBN-siRNA.

Figure 4 Combination of low doses of marizomib and pomalidomide overcomes cytotoxicity effects of BM stromal cells. MM.1S cells were cultured with or without BMSCs in the presence of drugs for 48h, followed by Brdu incorporation assay for analysis of cell proliferation.

Conclusions

Our preliminary data from in vitro-studies and in vivo MM transgraft models demonstrate that oral marizomib plus pomalidomide trigger synergistic anti-MM activity, enhance proteasome inhibition, and overcome drug resistance. These studies report the combination of clinical trials of combined marizomib and pomalidomide to improve outcome in patients with RRMM.