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, peripheral neuropathy and drug resistance. Our earlier studies showed that a novel proteasome inhibitor marizomib is distinct from bortezomib in its chemical structure, mechanisms of action, and effects on proteasome activities (Chauhan et al., Blood 2010, 115:834-45). Pomalidomide, like lenalidomide, is an analogue of thalidomide with potent immunomodulatory activity, and has been approved by FDA for treatment of RRMM patients who have received at least two prior therapies including lenalidomide and bortezomib and showed disease progression on or within 60 days of completion of the last therapy. Approval of treatment is based on progression-free survival. Here we utilized in vitro and in vivo models of MM to examine the anti-MM activity of combined marizomib and pomalidomide.

Methods: MM cell lines, patient tumor cells, and peripheral blood mononuclear cells (PBMCs) from normal donors were utilized to assess the anti-MM activity of marizomib and pomalidomide. Cell viability, apoptosis, and migration assays were performed using WST-8MTT, and Transwell Inserts, respectively. Synergistic/additive anti-MM activity was measured by isobologram analysis using "Calcusyn" software program. Proteasome activity was measured, as previously described (Chauhan et al., Cancer Cell 2005, 8:407-419). In vitro angiogenesis was assessed using matrigel capillary-like tube structure formation assays. MM.1S-tumor-bearing mice were treated with vehicle control, marizomib, pomalidomide or marizomib plus pomalidomide at the indicated doses for 21 days on a twice-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, USA; and marizomib was obtained from Triphase Inc., USA.

Results

Figure 1 Combination of marizomib and pomalidomide triggers synergistic cytotoxicity in MM cells. MM.1S MM cells were pretreated with or without pomalidomide for 12h and then treated with low doses of marizomib. MM.1S-tumor-bearing mice were treated with vehicle control, marizomib, pomalidomide or marizomib plus pomalidomide at the indicated doses for 21 days on a twice-weekly schedule for marizomib and 4 consecutive days weekly for pomalidomide.

Figure 2 Mechanism(s) of marizomib plus pomalidomide- induced MM cell apoptosis. MM.1S cells were pretreated with or without pomalidomide for 12h and then treated with low doses of marizomib. T-L proteasome activities. Data presented are mean ± SE (n=3, p<0.05).

Figure 3 Combination of low doses of marizomib and pomalidomide overcomes synergistic effect of BM stromal cells. MM.1S cells were treated with or without BMSCs in the presence of drugs for 48h, followed by BrdU incorporation assay for analysis of cell proliferation.

Figure 4 Combination of low doses of marizomib and pomalidomide induces synergistic migration inhibition. MM.1S-tumor-bearing mice were treated with vehicle control, marizomib, pomalidomide or marizomib plus pomalidomide at the indicated doses for 21 days on a twice-weekly schedule for marizomib and 4 consecutive days weekly for pomalidomide.

Conclusions

Our preclinical studies in MM disease models support a clinical trial of combined marizomib and pomalidomide to improve outcome in patients with relapsed and refractory MM.

Abstract

Synergistic Anti-Myeloma Activity of a Proteasome Inhibitor Marizomib and IMiD® Immunomodulatory Drug Pomalidomide

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Materials and Methods

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