Our prior studies showed that the proteasome inhibitor marizomib, distinct from bortezomib, triggers apoptosis in multiple myeloma (MM) cells resistant to bortezomib, and induces synergistic anti-MM activity in combination with immunomodulatory agent pomalidomide. Like lenalidomide, pomalidomide is an analogue of thalidomide, has immunomodulatory properties, and has been approved by FDA for treatment of MM. The approved indication for pomalidomide is for MM patients who have received at least two prior therapies including lenalidomide and bortezomib and have demonstrated disease progression on or within 60 days of completion of the last therapy. Approval of treatment is based on response rate. Clinical benefit, such as improvement in survival or symptoms, has not been verified. Here, we examined the anti-MM activity of low dose combination of marizomib and pomalidomide using in vitro and in vivo models of MM. MM cells were pretreated with DMSO control or with pomalidomide for 24h; marizomib was then added for an additional 24h, followed by analysis of cell viability. A significant decrease in viability of all cell lines was observed in response to treatment with combined low doses of marizomib and pomalidomide, compared with either agent alone. Isobologram analysis confirmed the synergistic anti-MM activity of these agents. The cytotoxicity of combination therapy was observed in MM cells resistant to bortezomib therapies. Marizomib plus pomalidomide-induced apoptosis was associated with: 1) activation of caspase-8, -9, caspase-3, and PARP; 2) downregulation of IRF4, c-Myc, and Mcl-1; 3) inhibition of migration of MM cells and angiogenesis; and 4) suppression of proteasome activity. Low doses of marizomib and pomalidomide overcomes the cytoprotective effects of MM-host BM microenvironment. Finally, in animal tumor model studies, combination of marizomib and pomalidomide is well tolerated, and significantly inhibits tumor growth.

Marizomib was obtained from Triphase Accelerator, San Diego, CA and Pomalidomide was purchased from Selleck chemicals, USA.

Material and Methods

MM cell lines, patient MM cells and peripheral blood mononuclear cells (PBMCs) from normal healthy donors were utilized to assess the synergistic anti-MM activity of marizomib and pomalidomide. All studies involving human samples and animal models were performed under approved protocols at Dana-Farber. Cell viability was assessed by MTT assay: MM.1S cells were pretreated with pomalidomide for 12h, and marizomib was then added for an additional 12h. Isobologram analysis was performed under approved protocols at Dana-Farber. Cell viability was assessed by MTT

Abstract

Combination of proteasome inhibitor marizomib and immunomodulatory agent pomalidomide trigger synergistic cytotoxicity in multiple myeloma

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Results

1. Combination of marizomib and pomalidomide trigger synergistic cytotoxicity in MM cells. (A) MM.1S cell lines were pretreated with or without pomalidomide for 24h, and marizomib was then added for an additional 24h. Isobologram analysis shown in the Table. Combination index (CI) of CI = 1 indicates synergy. (B) Pooled patient MM cells were pretreated with marizomib, pomalidomide or marizomib plus pomalidomide for 48h and then analyzed for viability. The percent cell viability was normalized to DMSO control (100%) (mean ± SD of triplicate cultures)

2. Mechanisms underlying marizomib plus pomalidomide-induced apoptosis. MM.1S cells were pretreated with or without pomalidomide for 24h and then treated for additional 24h and cells were harvested. Protein lysates were subjected to immunoblot analysis using antibodies against PARP, caspase-3, caspase-6, caspase-9, IRF4, c-Myc, Mcl-1, and GAPDH. FL indicates full length, and CF, cleaved fragment

3. Combination of low doses of marizomib and pomalidomide block migration and tubule formation. (A) Transwell migration assays, angiogenesis using capillary-like tube structure formation assays. Cells migrating to bottom face of the membrane were stained with crystal violet and images were taken. (B) MM.1S cells were treated with drugs as indicated for 48h and then assessed for in vitro angiogenesis using capillary-like tube structure formation assays.

4. Combination of low doses of marizomib and pomalidomide reduces human myeloma xenograft growth in CB17-SCID mice. Mice were treated with vehicle control, marizomib, pomalidomide, or marizomib plus pomalidomide (pomalidomide at the indicated doses for 21 days on a twice-weekly schedule for marizomib and a consecutive days weekly for pomalidomide. Cells presented are mean ± SD (n=5, p<0.05)

Conclusions

Our preclinical studies demonstrate potent in vitro and in vivo anti-MM activity of marizomib combined with pomalidomide. These findings provide the framework for clinical trials of low dose combination of marizomib and pomalidomide to increase response, overcome drug resistance and improve patient outcome in MM.