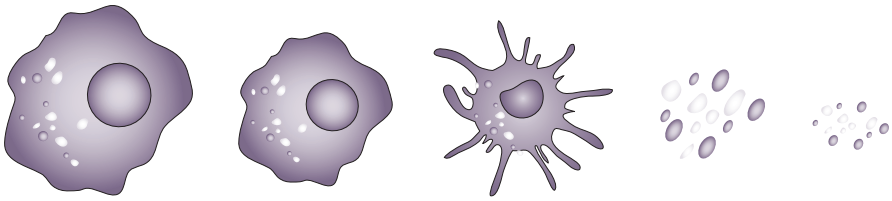
HEMATOLOGY

NPI-0052 and lenalidomide trigger apoptosis in multiple myeloma cells



Recent evidence indicates that the novel 20S proteasome inhibitor NPI-0052 (Marizomib) triggers apoptosis in multiple myeloma (MM) cells, when given in combination with the immunomodulatory agent lenalidomide. This is significant because NPI-0052 might offer a novel therapeutic option for patients resistant to bortezomib and lenalidomide. As a bicyclic β-lactone γ-lactam, NPI-0052 is structurally and functionally different to bortezomib. "Importantly, some patients cannot tolerate bortezomib or are resistant to this agent and therefore this new combination of NPI-0052 and lenalidomide may be of clinical utility," explains Dharminder Chauhan, lead investigator of the study.

The researchers tested the effects of NPI-0052 (2 nM) and lenalidomide (3 μM) on human MM cell lines and primary tumor cells from seven patients with MM who had relapsed following a variety of treatment regimens. They observed a significant decrease in cell viability only when both agents were used in combination. This result was due to an increase in apoptosis, as indicated by a marked increase in the annexin V^+/propidium $iodide^-$ apoptotic cell population. Crucially, no increase in apoptosis was observed in normal peripheral blood mononuclear cells from the MM patients following exposure to the combined treatment.

Chauhan's team next investigated the mechanism by which NPI-0052 triggers apoptosis in MM cell lines, and through the use of caspase inhibitors they identified an important role for caspase-8-dependent signaling (and to a lesser extent signaling pathways involving caspase-9 and caspase-3). Specifically, by inhibiting caspase-8, the researchers were able to significantly decrease the extent of NPI-0052–lenalidomide-mediated MM cell death. Furthermore, the investigators observed increased levels of the BH3-only Bcl-2 family protein BIM in MM cells treated with combined NPI-0052 and lenalidomide, but not in cells treated with either agent alone. BIM is known to have an important role in apoptotic pathways and, accordingly, when the researchers knocked down BIM expression in the MM cells with siRNA, they noted a significant reduction in NPI-0052–lenalidomide-dependent apoptosis. The importance of BIM in this context was highlighted by NPI-0052–lenalidomide-induced accumulation of the BIM_{EL} isoform in the endoplasmic reticulum of MM cells—an event known to trigger apoptosis.

In addition, the investigators noted that low doses of this combination regimen blocked both MM cell migration and angiogenesis, although these effects seem to be unconnected to the NPI-0052–lenalidomide-induced apoptosis.

Finally, Chauhan's research group observed prolonged survival and a significant reduction in tumor growth in human plasmacytoma (MM1S)-xenografted mice treated orally with NPI-0052 and lenalidomide in comparison with control mice. Similar to the *in vitro* studies, increased accumulation of BIM_{EL} was noted in treated mice, a further indication of the central role of this protein in NPI-0052–lenalidomide-induced apoptosis in MM.

"The experimental strategies used here are similar to those that validated the efficacy of bortezomib and lenalidomide, leading to phase I clinical trials and FDA approval," says Chauhan, who comments that the results reported by his team provide the preclinical framework for a phase I/II clinical trial of NPI-0052 and lenalidomide combination therapy to overcome drug resistance and improve patient outcome in MM.

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Original article Chauhan, D. *et al*. Combination of novel proteasome inhibitor NPI-0052 and lenalidomide trigger *in vitro* and *in vivo* synergistic cytotoxicty in multiple myeloma. *Blood* **115**, 834–845 (2010)