

Review Article

The Role of Intracellular Signaling Pathways in the Pathogenesis of Multiple Myeloma and Novel Therapeutic Approaches

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The introduction of novel agents, such as bortezomib, thalidomide, and lenalidomide, into daily practice has dramatically improved clinical outcomes and prolonged survival of patients with multiple myeloma (MM). However, despite these advanced clinical benefits, MM remains an incurable hematological malignancy. Therefore, development of new agents and novel therapeutic strategies is urgently needed. Recent advances toward understanding the mechanism of myeloma cell growth and drug resistance in the bone marrow milieu have provided clues for the development of next-generation agents aimed at improving patient outcomes. In this review article, we discuss new possible agents for the treatment of MM based on recent advances in the understanding of signaling pathways in myeloma cells. [*J Clin Exp Hematop* 56(1):20-27, 2016]

Keywords: multiple myeloma, cytokines, signaling pathway, molecular-targeted therapy

INTRODUCTION

Multiple myeloma (MM) is a refractory hematological malignancy that occurs in many elderly patients. With the use of proteasome inhibitors, such as bortezomib, and immunomodulatory agents, such as thalidomide and lenalidomide, treatment outcomes have dramatically improved.¹⁻³ In addition, new agents and various monoclonal antibodies that target proteins in MM cells continue to be developed and tested in clinical trials. Reflecting the impact of these novel agents, the current clinical data show continued improvement in survival in patients with MM.⁴ Treatment regimens using these new agents have shown high efficacy rates and have prolonged not only progression-free survival (PFS), but also overall survival.⁵ Such improved treatment outcomes may be attributed to advances in understanding of onset and progression pathogenesis of MM at the molecular level, as well as the ongoing development of new drugs that target molecular abnormalities in myeloma cells. However, MM is a disease with a complex pathogenesis that is characterized by

complex heterogeneous cytogenetic abnormalities together with microenvironmental changes, leading to clinically diverse findings.⁶ Myeloma clones are heterogeneous among individual patients, and the clones have multiple intracellular signals with complex interactions. Myeloma cell adhesion with the bone marrow microenvironment and contact signaling with niches are also involved.⁷

Many myeloma cell proliferation signals have been identified, leading to the development of specific inhibitors as new treatment options. Current treatment regimens include inhibitors of interleukin (IL)-6 downstream signals and molecular complexes, such as nuclear factor (NF)- κ B, which are essential for myeloma cell proliferation. Next-generation drug candidates are also being developed, and are aimed at inhibiting pathways that play a role in myeloma cell proliferation, including the histone deacetylase (HDAC), WNT, PI3K (phosphoinositide 3-kinase)/AKT/mammalian target of rapamycin (mTOR), heat shock protein (HSP), p38/mitogen-activated protein kinase (MAPK), NOTCH, and Hedgehog pathways.⁸

MYELOMA CELL PROLIFERATION AND CYTOKINES

Cytokines produced in the bone marrow microenvironment are directly involved in myeloma cell proliferation (Fig. 1). In addition, interactions between myeloma cells and various components of the bone marrow microenvironment are mediated through cell surface receptors, such as integrins,

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cadherins, and selectins. These cell adhesion molecules increase cell growth, proliferation, migration, adhesion, and drug resistance.⁸ Cytokines that mediate myeloma cell growth and proliferation include IL-6, insulin-like growth factor-1, vascular endothelial growth factor, and B-cell activating factor (BAFF).⁷

BAFF is a key player in the interaction between myeloma cells and the microenvironment.⁹ BAFF is secreted by osteoclasts in the bone marrow microenvironment, and its signaling stimulates myeloma cell proliferation via BAFF receptors, transmembrane activator calcium-modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen. In particular, *TACI* gene expression level plays an important role in the interaction between myeloma cells and the bone marrow microenvironment, because BAFF is a ligand of TACI. Binding of BAFF to TACI receptors on myeloma cells activates the NF- κ B pathway, a crucial pathway in the pathogenesis of MM.⁹ In addition, cytokines such as tumor necrosis factor (TNF)- α , transforming growth

factor- β , macrophage inflammatory protein-1 α , IL-6, IL-15, and IL-21 are important in myeloma cell proliferation.⁷

Among these cytokines, IL-6 plays the most important role in myeloma cell proliferation and survival.^{7,8} The IL-6 receptor (IL-6R) is a complex of IL-6R α and glycoprotein (gp) 130. IL-6 binds directly to IL-6R α , followed by binding with gp130 and hexamer formation, resulting in Janus kinase (JAK) activation. JAK phosphorylates gp130, followed by activation of the JAK/signal transducer and activator of transcription 3 (STAT), p38/MAPK, and PI3K/AKT/mTOR pathways. Increases in IL-6 production, which is NF- κ B dependent, occur at the transcriptional level following adhesion of myeloma cells with cells in the bone marrow microenvironment.⁷⁻¹¹ More recently, IL-6 was reported to activate the JAK/STAT3 pathway and increase sensitivity to HSP inhibitors in myeloma cells.¹² Although, theoretically, blocking any of these pathways should inhibit the signaling that increases myeloma cell proliferation, the pathways involved are multiple and complex (Fig. 1).

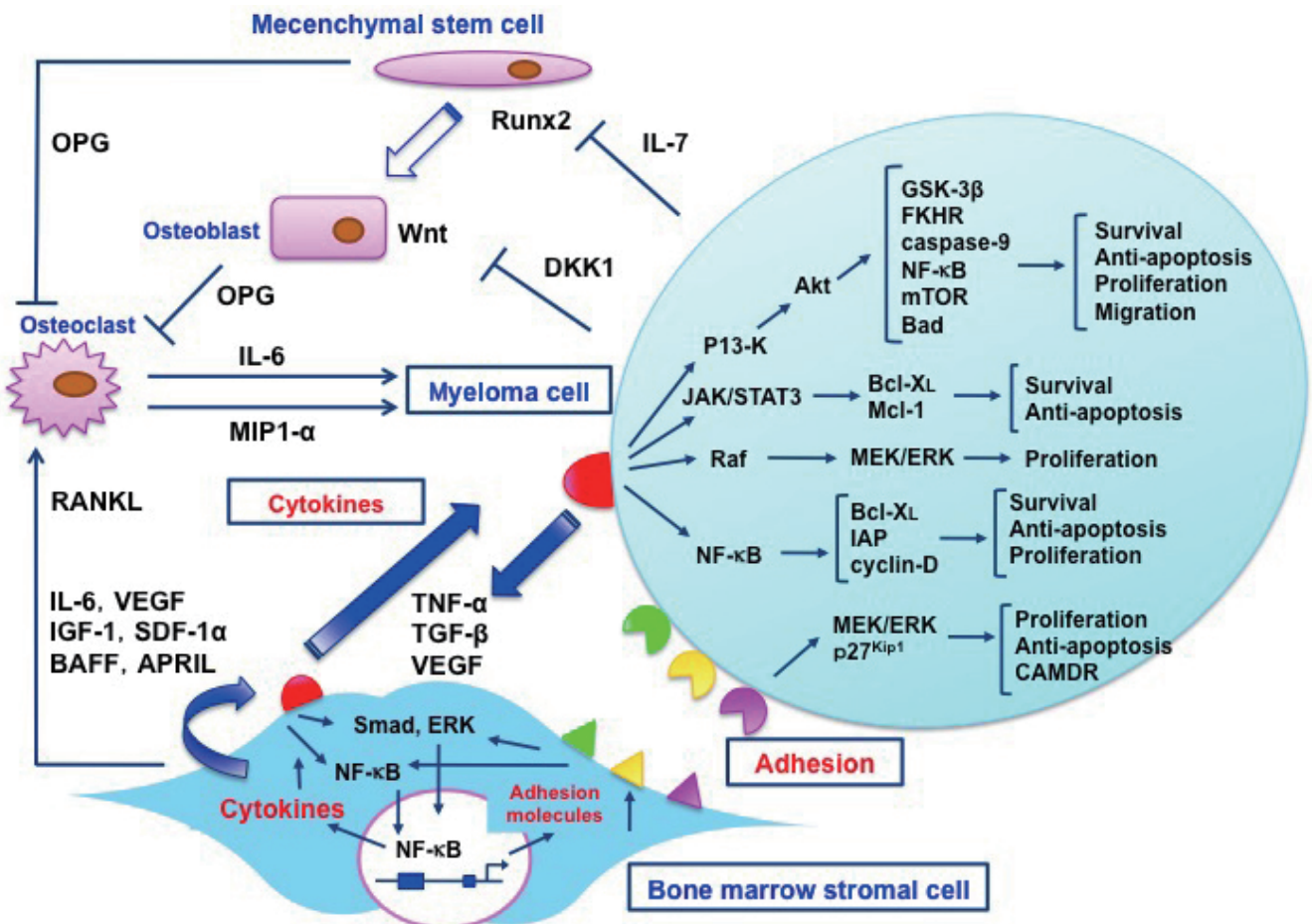


Fig. 1. Signaling interactions between myeloma cells and the bone marrow microenvironment. Adhesion of myeloma cells to components of the bone marrow microenvironment, such as stromal cells, triggers cytokine-mediated cell growth, survival, and drug resistance. OPG, osteoprotegerin; RANXL, receptor activator of nuclear factor κ B ligand; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; TGF, transforming growth factor; DKK, Dickkopf; CAMDR, cytokine adhesion-mediated drug resistance

THE PI3K/AKT/mTOR PATHWAY AND INHIBITORS

PI3K-mediated signals are complex signal transduction systems that are activated by various cytokines. However, stimulation of myeloma cell proliferation can be cytokine dependent or cytokine independent, and this activity correlates with disease activity. *PI3KCA* gene mutations are frequently seen in tumors, such as breast cancer, and due to its oncogenic properties, PI3K is an important molecular target in the treatment of MM.¹³ The PI3K cascade is constitutively activated in myeloma cells, and most primary myeloma samples from patients with MM show activated AKT phosphorylation; therefore, inhibition of the PI3K/AKT pathway has been shown to contribute to the successful treatment of MM.¹⁴

Activated PI3K activates AKT, phosphorylates downstream molecules such as mTOR, and controls cell proliferation and the cell cycle. mTOR is a serine/threonine kinase that has been identified as a target molecule of rapamycin, and mTOR plays a central role in cell survival and division. This series of pathways, which is called the PI3K/AKT/mTOR pathway, is important in cancer cell proliferation and is also involved in myeloma cell proliferation, survival, and drug resistance.¹⁵ AKT activation is observed in about 60% of MM patients; furthermore, phosphorylation of mTOR and its downstream molecules S6K (S6 kinase) 1 and 4E-BP (4E-binding protein) 1 has been reported. In addition, AKT indirectly influences two important signaling pathways in MM for survival, NF- κ B and p53. NF- κ B is indirectly activated by AKT via direct phosphorylation and activation of I κ B kinase, resulting in degradation of I κ B.¹⁶ p53 activity is mediated by the phosphorylation of the p53-binding protein murine double minute-2, resulting in degradation of the proapoptotic tumor suppressor p53.¹⁶ NF- κ B activation and the loss of p53 via activation of the AKT pathway may lead to overexpression of the anti-apoptotic protein, Bcl-2, which is involved in the proliferation of myeloma cells.

Perifosine

Perifosine is an oral synthetic alkylphospholipid that blocks the AKT, NF- κ B, and JNK (Jun-amino-terminal kinase) signaling pathways and induces cell death of myeloma cells.¹⁷ Perifosine inhibits phosphorylation by blocking the translocation of AKT to the cell membrane. In addition, perifosine activates the SAPK (stress-activated protein kinase)/JNK pathway via JNK phosphorylation, and activates extrinsic cell death receptor-dependent cell death pathways.¹⁸

A multicenter phase I/II clinical trial of perifosine (KRX-0401), bortezomib, and dexamethasone was conducted in 84 patients with relapsed/refractory MM. The overall efficacy

rate was 41% (recurrence after bortezomib: 65%, bortezomib resistance: 32%). Most adverse events were gastrointestinal and manageable, including nausea/vomiting (61%) and diarrhea (31%). The median PFS was 25 months.¹⁹ Currently, a phase III randomized trial is ongoing to test the same combination in patients with relapsed/refractory MM previously exposed to bortezomib.

Other PI3K inhibitors

Many PI3K inhibitors are currently in development. GDC-0941, a highly specific inhibitor of class I PI3K, and BYL719, a specific inhibitor of PI3KCA, have been shown to have anti-tumor effects in MM.

mTOR inhibitors

mTOR is an intracellular serine-threonine kinase that regulates cellular proliferation, migration, and motility in many cancer cells. In a phase II clinical trial, temsirolimus showed a 38% response rate in 16 patients with relapsed/refractory MM.²⁰ Everolimus (RAD001) has also been investigated in patients with MM as a single agent and in combination with lenalidomide. However, no encouraging results have been reported.

THE JAK/STAT PATHWAY AND INHIBITORS

IL-6 is the most important cytokine in myeloma cell proliferation. JAK/STAT signaling pathways are specifically activated by cytokines belonging to the gp130 family, such as IL-6. Therefore, inhibitors of this pathway may play an important role in the treatment of patients with MM. The effects of anti-IL-6-related signals in MM have been analyzed using monoclonal antibodies targeting both IL-6 and IL-6 receptors. Siltuximab (CNTO 328), a chimeric human/mouse-neutralizing monoclonal antibody against IL-6, has been shown to be effective in an *in vivo* mouse model of MM in combination with bortezomib and dexamethasone.²¹ Clinical trials for relapsed/refractory MM are now ongoing. The combination of siltuximab and bortezomib may not show promising clinical results for relapsed/refractory MM. However, future therapeutic options involving a combination of siltuximab and other agents will be interesting. Selective inhibitors have previously been unavailable, but low molecular-weight JAK1/2 and STAT3 inhibitors have been reported.^{22,23} However, these drugs have not been actively used in clinical practice. Despite the importance of this signaling pathway in MM, whether these inhibitors prove to be effective for MM remains to be determined.

NF- κ B SIGNALS AND INHIBITORS

NF- κ B is a transcription factor that is activated by many cytokines, such as TNF- α , IL-1 β , and CD40, as well as chemokines and cell adhesion molecules. NF- κ B plays an important role in controlling expression of cytokines, cell adhesion molecules, and molecules involved in resistance to cell death. NF- κ B is activated in many types of cancer, including MM, and has received great attention as a molecular target for treatment. In myeloma cells, many genes that are targets of NF- κ B are highly expressed, suggesting that myeloma cells are dependent on bone marrow stromal cells, and that extrinsic signaling is important in MM.^{24,25} Bone marrow stromal cells produce extrinsic ligands, such as BAFF, and a proliferation-inducing ligand to stimulate BAFF receptors, TACI, and B-cell maturation antigen, which activate the NF- κ B signaling pathway and provide a survival benefit to plasma cells.²⁶ The NF- κ B signaling pathway is affected by both activating and inactivating mutations in genes, such as TNF-receptor-associated factor (*TRAF 2/3*) and NF- κ B-inducing kinase, as regulators of the non-canonical NF- κ B signaling pathway.²⁶ These mutations have been identified in 20% of patients with MM, resulting in activation of NF- κ B signaling without ligands,^{24,25} and may contribute to the progression of the disease.

Agents that inhibit NF- κ B enhance the anti-MM effects of conventional chemotherapeutic agents. Bortezomib, a proteasome inhibitor, has been developed and is now used clinically to treat MM.⁸ Bortezomib is thought to inhibit NF- κ B activation by blocking proteasome degradation of I κ B α (nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor α) and decreasing NF- κ B nuclear translocation (canonical pathway). Bortezomib activates the canonical NF- κ B pathway in myeloma cells, and bortezomib used in combination with an I κ B kinase β inhibitor has been shown to have a synergic effect on the growth inhibition of myeloma cells.²⁷ In addition, other mechanisms involving inhibition of other pathways have also been reported recently.^{27,28} Crosstalk signaling has recently been reported between canonical and non-canonical pathways.²⁹ Subsequently, next-generation proteasome inhibitors with high efficacy, such as carfilzomib, ixazomib, and marizomib, have been developed and recently tested in clinical trials.^{8,30} Carfilzomib was recently approved in the US for the treatment of patients who were previously treated with at least two therapies, including bortezomib and immunomodulatory drugs. Marizomib is an oral proteasome inhibitor with more potent NF- κ B inhibitory activity compared with other proteasome inhibitors.³¹ Distinct from bortezomib, marizomib blocks chymotrypsin-like, trypsin-like, and caspase-like proteolytic activity of proteasomes, thus overcoming bortezomib resistance both *in vitro* and *in vivo*.³¹

THE p38 MAPK PATHWAY AND INHIBITORS

p38 MAPK is a serine/threonine kinase that is phosphorylated and activated by various environmental factors and is stimulated by inflammatory cytokines.³² p38 is structurally activated in myeloma cells, and this activity is involved in osteoclast and osteoblast activity as well as in osteolysis. SCIO-469 [indole-5-carboxamide, adenosine 5'-triphosphate (ATP) competitive inhibitor] is a selective inhibitor of p38, which together with the structural analog, SD-282 (indole-5-carboxamide, ATP competitive inhibitor), reduces myeloma cell proliferation and enhances the activity of dexamethasone.³³ SCIO-469 also inhibits TNF- α -induced adhesion of myeloma cells to bone marrow stromal cells that is mediated by various adhesion molecules.³⁴ LY2228820, a well-known p38 MAPK inhibitor, enhances bortezomib cytotoxicity and improves bone lesions.³⁵

HSP90 SIGNALING AND INHIBITORS

HSP90 is a molecular chaperone that is important for protein stability and maintenance. HSP90 is a chaperon of a wide variety of proteins, so when HSP90 function is blocked, stability of these proteins decrease, leading to protein dysfunction in cancer cells.³⁶ Proteins chaperoned by HSP90 include oncogenic kinases, receptors, and various transcription factors that are involved in cell cycle control and signaling. HSP90 expression is elevated in myeloma cells compared with normal plasma cells. Other proteins chaperoned by HSP90 include AKT and MAPK, which are involved in myeloma cell survival and proliferation. Thus, HSP90 is an important molecular target for treatment of MM.

The HSP90 inhibitor tanespimycin (KOS-953), in combination with bortezomib, reduces myeloma cell proliferation, and a phase I/II trial of both drugs was conducted in patients with relapsed/refractory MM.³⁷ The rationale for this combination was the up-regulation of stress response gene transcripts.³⁸ Little bortezomib-related peripheral neuropathy was reported in this study, and the overall efficacy was 27% (including a molecular response of 12%). Adverse events in this study included diarrhea (60%), fatigue (49%), nausea (49%), thrombocytopenia (40%), and transaminase elevation (29%).³⁷ Peripheral neuropathy of Grades 1/2 was seen in 21% of patients, but no Grades 3/4 PN (peripheral neuropathy) peripheral neuropathy was observed.³⁷

WNT, NOTCH, AND HEDGEHOG PATHWAYS AND THEIR INHIBITORS

The Wnt, Notch, and Hedgehog molecules are involved in the proliferation of normal hematopoietic cells and cancer stem cells.³⁹ Therefore, inhibitors targeting these molecules may provide even more effective treatment for MM.

Research aimed at the clinical use of such drugs is currently being conducted.

Wnt inhibitors

The Wnt proteins are ligand glycoproteins that bind to the Frizzled (Fz) transmembrane receptor, and abnormal Wnt signaling has been reported in MM. AV-65, a novel Wnt/ β -catenin inhibitor, inhibits myeloma cell proliferation. Myeloma cells secrete proteins such as Dickkopf and soluble frizzled receptor-like proteins that block Wnt signaling. Inhibition of osteoblast differentiation has also been reported. Wnt signals also play an important role in myeloma cell proliferation and the formation of bone lesions.⁴⁰

Notch inhibitors

In many cells, Notch signaling, which occurs via intercellular contact, is involved in apoptosis, cell proliferation, and the regulation of cell differentiation. Notch signaling is also important in hematopoietic cell proliferation and differentiation, as well as in the pathogenesis of hematopoietic tumors. Notch signaling is activated in MM and plays a role in cell proliferation, drug resistance, and osteolysis. The Notch inhibitor MRK003 has been shown to inhibit myeloma cell proliferation *in vitro*.⁴¹ In addition, the Notch inhibitor DAPT increases bortezomib sensitivity.

Hedgehog inhibitors

Hedgehog signaling is involved in cell proliferation and differentiation, and in determining the fate of fetal cells. Hedgehog signal activation occurs in many cancer cells, including MM.⁴² LDE225, a newly synthesized smoothed antagonist, inhibits myeloma cell proliferation by blocking Hedgehog signaling. This inhibitor may be promising for clinical use.

HDAC INHIBITORS

Histone acetylation affects DNA/chromatin structure, resulting in transcriptional activation of genes that are epigenetically silenced by chromatin condensation.⁴³ Histones control the activity of transcription factors, and this activation requires histone acetylation. In other words, gene expression is regulated by controlling histone proteins. Control is mediated by histone acetyl transferase and HDAC, thereby controlling cell proliferation and differentiation. HDAC inhibition can reverse epigenetic silencing of genes that regulate cellular proliferation and survival. Histone proteins, which are the main structural component of chromatin, are divided into four classes. Inhibition of HDAC activity arrests the myeloma cell cycle and induces cell differ-

entiation and cell death.⁴⁴ HDACs are also divided into four classes; however, inhibitors are only available for class I and II HDACs.^{45,46} HDAC inhibition, by preserving or increasing histone acetylation, results in amplification of transcription factor activity. In cancer cells, HDACs target not only histones, but also non-histone proteins, such as HSP90, STAT3, β -catenin, p53, murine double minute-2, c-Myc, Rel-A, and phosphatase and tensin homolog (PTEN), the last of which is involved in the pathogenesis of MM.^{47,48} Following HDAC inhibition, many molecules involved in the cell cycle and DNA repair are acetylated.

Panobinostat (LBH589)

Panobinostat is a nonselective HDAC inhibitor that inhibits class I, II, and IV HDACs at very low concentrations.^{49,50} In an early phase II trial of 38 patients with relapsed/refractory MM who were previously treated with at least two drugs, including bortezomib, lenalidomide, or thalidomide, panobinostat alone produced a partial response (PR) in one patient and a minimal response in one patient.⁵¹

A phase I/II study of panobinostat with melpharan for relapsed/refractory patients with MM was performed. The maximal tolerance dose of panobinostat was 20 mg, and the overall response rate in this trial was 7.5% with Grade 3/4 adverse effects, including thrombocytopenia (33.9%), neutropenia (30.5%), lymphopenia (22.5%), anemia (15%), hyponatremia (7.5%), increased creatinine (2.5%), and deep-vein thrombosis (2.5%).⁵² Subsequently, clinical trials were conducted with combined use of panobinostat and other drugs. The PANORAMA 2 trial was a single-arm, phase II study of panobinostat with bortezomib and dexamethasone for patients who are refractory to bortezomib and who have received more than two prior therapies.⁵³ Responses were a near complete response in one patient (1.8%), partial PR in 18 patients (32.7%), and a minimal response in 10 patients (18.2%). The median duration of response was 6 months, and median PFS was 5.4 months. In a phase III trial (PANORAMA 1) using a combination of panobinostat and bortezomib for relapsed/refractory MM, no previously unknown adverse events were reported, and good clinical responses were achieved.⁵⁴ In this trial, panobinostat plus bortezomib and dexamethasone significantly extended PFS compared with placebo plus bortezomib and dexamethasone (12.0 months vs. 8.1 months, $p < 0.0001$). In another clinical trial, a combination of panobinostat/ dexamethasone plus either bortezomib or lenalidomide was effective in reducing tumors and preventing the progression of bone lesions. From these results, panobinostat was recently approved in the US, EU, and Japan.

Vorinostat (SAHA)

Vorinostat, an inhibitor of class I and II HDACs, induces myeloma cell death and reduces IL-6 production by bone marrow stromal cells.⁵⁵ Vorinostat induces expression of p21 and p53 in myeloma cells, inhibits molecules with caspase inhibitory activity, inhibits proteasome activity, and induces cell death.⁵⁵ In addition, vorinostat enhances the effectiveness of dexamethasone and immunomodulatory agents, such as thalidomide and lenalidomide. In a phase I trial with vorinostat, lenalidomide, bortezomib, and dexamethasone, excellent results were achieved, with an efficacy (\geq PR) of 52%, and in particular, a complete response rate of 28%. The main adverse events were anemia, thrombocytopenia, diarrhea, fatigue, and cough.⁵⁶ A global phase III study (VANTAGE 088) was performed in which relapsed/refractory patients with MM were randomized to receive bortezomib and dexamethasone plus either vorinostat or a matching placebo.⁵⁷ Vorinostat and bortezomib/dexamethasone produced a more potent response than placebo and bortezomib/dexamethasone in terms of median PFS (7.6 months vs. 6.8 months, $p = 0.01$). In an international, multicenter, open-label trial of vorinostat in combination with bortezomib in previously treated patients (VANTAGE 095), the combination of vorinostat and bortezomib produced an overall response rate of 17%.⁵⁸

CONCLUSION

The complex signal transduction pathways required for myeloma cell proliferation are gradually being elucidated, and novel drugs that target these pathways are being developed for clinical use. These strategies include treatments that target the JAK/ STAT, NF- κ B, HDAC, PI3K/AKT/mTOR, p38MAPK, HSP90, and Wnt/Notch/ Hedgehog pathways. In addition, treatments that target the bone marrow microenvironment, hypoxia, angiogenesis, CD44, CXCR4, and integrins are also being investigated. With the advent of novel drugs, such as bortezomib, lenalidomide, and thalidomide, survival in MM patients compared to the era of melpharan/prednisolone treatment has been prolonged from approximately 3 years to more than 5 years. Development of other new drugs that target diverse signaling pathways in myeloma cells is expected in the future. More novel agents will target affected signaling pathways to provide new treatment options for patients with MM, and are expected to further improve treatment outcomes.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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