



Pilgrim boats, Perfume Pagoda outside Hanoi, Vietnam. Photograph courtesy of J. Bryan Murphy, MD, Clearwater, Florida.

*A new class of compounds with
a novel mechanism of action
has shown clinical efficacy for
the treatment of multiple myeloma.*

Targeted Therapy in Multiple Myeloma

Wee Joo Chng, MD, Lee Gong Lau, MD, Noorainun Yusof, MD, and Benjamin M. F. Mow, MD

Background: Multiple myeloma (MM) is an incurable malignancy. Recent insights into its biology has allowed the use of novel therapies targeting not only the deregulated intracellular signaling in MM cells but also its interaction with the bone marrow microenvironment that confers drug resistance, growth, and survival advantage to the malignant cells.

Methods: We review and summarize the recent advances in our knowledge of myeloma biology as well as the mechanism of action and clinical efficacy for novel therapeutic agents in clinical trials.

Results: Several novel therapeutic agents are currently in clinical trials. Thalidomide is already established for both initial and salvage treatment. Bortezomib is being tested alone and in combination with conventional chemotherapy in various settings. Other agents are less effective in producing response but have been able to stabilize disease in patients with relapsed and/or refractory disease, such as arsenic trioxide, farnesyltransferase inhibitors, 2-methoxyestradiol, and vascular endothelial growth factor receptor inhibitors. Insights into drug resistance mechanism have also led to the development of novel agents that sensitize myeloma cells to chemotherapy (Bcl-2 antisense). Gene expression studies have in many instances identified pathways other than the intended target of the drug and have provided insights into the therapeutic mechanisms.

Conclusions: In the future, patients with MM will have more therapeutic options available than ever before. The challenge will be to identify patient subgroups that will benefit most from the different therapies and then determine how these biologically based therapies could be combined and incorporated into the overall management of patients.

From the Department of Hematology-Oncology, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074, SINGAPORE
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Address correspondence to Benjamin M. F. Mow, MD, Department of Hematology-Oncology, National University Hospital, 5 Lower Kent Ridge Road, 119074, Singapore. E-mail: mowmf@nuh.com.sg

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Abbreviations used in this paper: MM = multiple myeloma, BMSCs = bone marrow stromal cells, TNF- α = tumor necrosis factor alpha, VEGF = vascular endothelial growth factor.

Introduction

Multiple myeloma (MM) is a malignancy arising from post-germinal mature B cells characterized by an excess of monotypic plasma cells in the bone marrow secreting monoclonal immunoglobulins in the serum and/or urine, with a concomitant decrease in normal immunoglobulins and lytic bone lesions.¹ In the United States, the estimated incidence of MM in 2005 is 16,000, and it accounts for 10% of hematologic malignancies and 1% of all malignancies.² Despite therapeutic advances, the disease is essen-

tially incurable. The current standard of treatment with high-dose therapy followed by autologous stem cell transplant^{3,4} prolongs survival to a median of 4 to 5 years compared to 3 years with conventional chemotherapy.^{5,6} Attempts at improving autografting with a tandem procedure has improved outcome,^{7,8} but despite a small subgroup of patients surviving more than 8 years, there is currently no plateau in the overall survival curves. Allogeneic stem cell transplant offers a potential for cure due to the well-documented graft-vs-myeloma effect⁹ but is associated with a high transplant-related mortality.¹⁰ Recent efforts to reduce the transplant-related mortality with non-myeloablative conditioning have shown encouraging results, but longer follow-up is needed to determine the role of this treatment modality.¹¹⁻¹³ New insights into the biology of MM have provided a framework in the development of drugs that target not only specific intracellular pathways but also the myeloma-bone marrow interaction. These agents have shown promising results in clinical trials and are reviewed in this paper.

Genetics of Multiple Myeloma

Multiple myeloma is a neoplastic disease of either (1) transformed plasmablasts that have successfully completed

somatic hypermutation and immunoglobulin H (IgH) switching in the germinal center before migrating to the bone marrow or (2) transformed terminally differentiated long-lived plasma cells in the bone marrow (Fig 1).¹⁴ It is usually preceded by the premalignant monoclonal gammopathy of unknown significance (MGUS). The karyotypes of MM are complex, often with a mixture of numerical and structural chromosomal changes.^{1,15} Recent reports suggest continued genomic instability throughout the course of the disease and a stepwise progression of karyotypic complexity vis a vis clinical progression from MGUS to frank MM (Fig 2).^{1,14,16} Understanding these genetic events is important as there is correlation between frequency and extent of karyotypic abnormalities with plasma cell labeling index, disease stage, and prognosis.^{14,16}

By conventional analyses, cytogenetic abnormalities are detected in only 30% to 40% of patients in large studies of MM.^{17,18} This gross underestimation is due to the low mitotic index of malignant plasma cells, the telomeric locations of some of the chromosomal changes, and the variable degree of bone marrow infiltration. Abnormal karyotypes are generally in the form of numerous numerical and structural aberrations.¹⁷ Patients with abnormal karyotypes are more likely to show hyperdiploidy rather than hypodiploidy, the latter generally correlating with a poorer prognosis.¹⁸

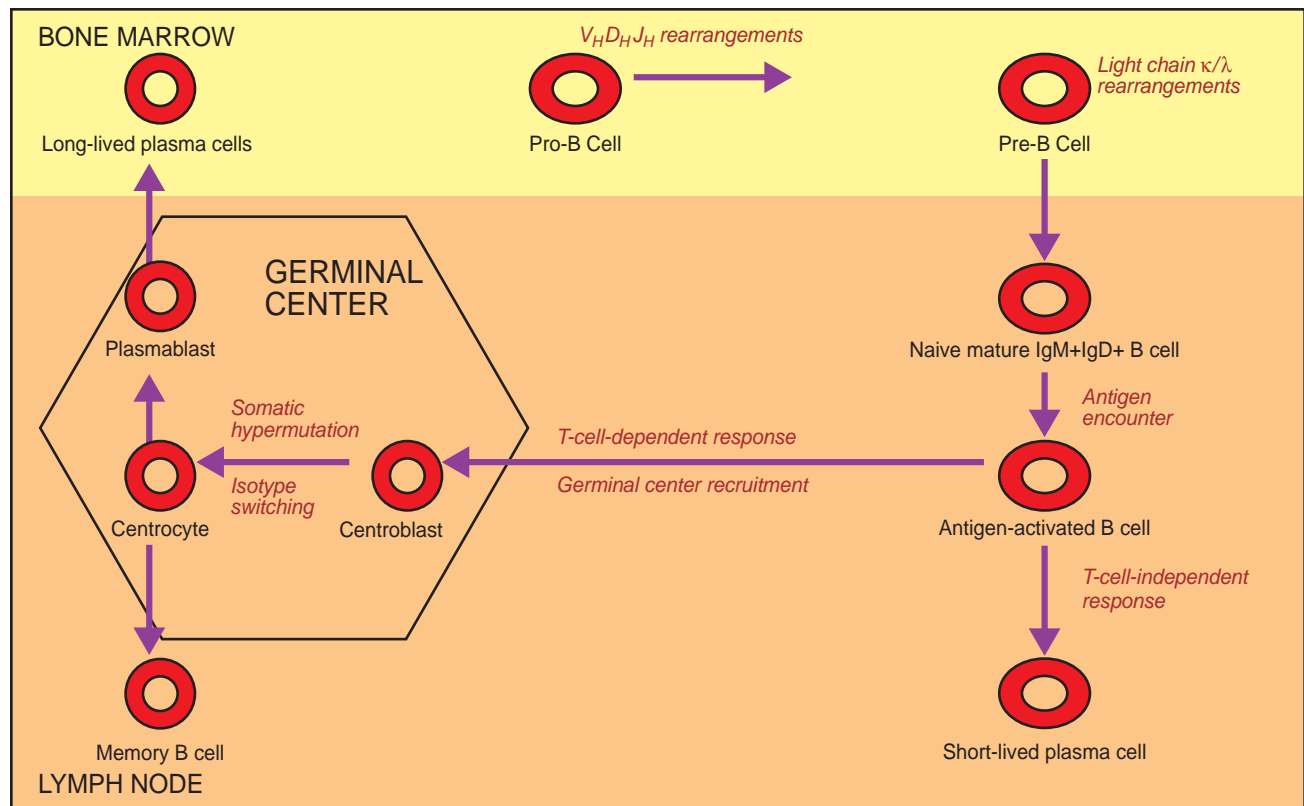


Fig 1. — The development of normal plasma cells. Committed precursor B cells (pro-B cells) undergo functional VDJ rearrangement of the IgH and IgL genes in the bone marrow before exiting as naive mature B cells. After antigen encounter, these cells differentiate into short-lived plasma cells during early immune response and secrete mainly IgM. Later in the response, some antigen-activated B cells enter a germinal center in a T-cell–dependent immune process and undergo somatic hypermutation and IgH isotype switching. These result in plasma cells that are able to secrete all the different classes of immunoglobulins. Subsequently, the B cells develop into either long-lived plasma cells or memory B cells.

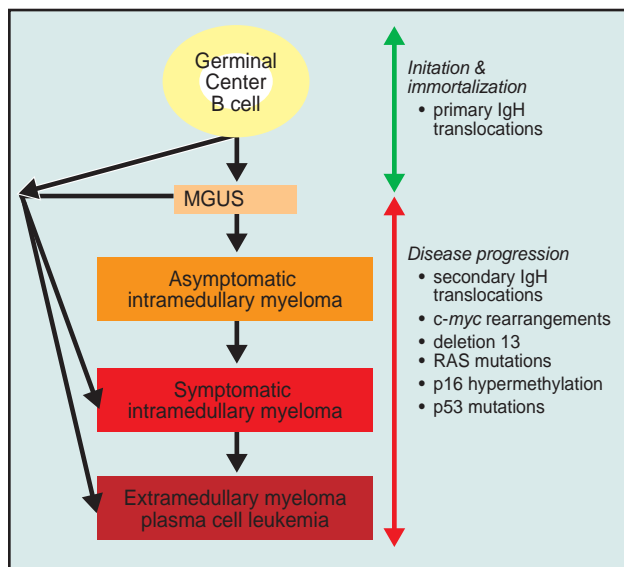


Fig 2. — Genetics and disease progression in multiple myeloma. An initiation genetic event, which is often a primary IgH translocation, causes immortalization of either a long-lived plasma cell or a memory B cell and formation of a malignant plasma cell clone. Further genetic events result in a stepwise progression of karyotypic complexity vis a vis clinical progression from monoclonal gammopathy of unknown significance to frank multiple myeloma (MGUS = monoclonal gammopathy of unknown significance).

Molecular techniques with increased resolution have provided a significantly improved detection of cytogenetic abnormalities in MM. The use of fluorescent in situ hybridization (FISH) analysis for example has revealed aneuploidy in up to 90% of cases.¹⁹ Using the same technique, chromosome 13 abnormality is now the most common anomaly in up to 86% of cases and is associated with an adverse prognosis.²⁰ The similar frequency of MGUS patients having this chromosomal abnormality suggests that this is an early event and additional events are required for the transition from stable MGUS to progressive MM.^{21,22} Comparative genomic hybridization and spectral karyotyping studies have shown that numerical and unbalanced structural chromosomal changes are present in most patients with MM, with the most frequent being deletion of 13q.^{23,24} Hence, with the advent of molecular techniques, 95% to 100% of patients with MM are cytogenetically abnormal.²⁵

It has been proposed that there are two types of translocations involved in the pathogenesis of MM: primary (initiation) and secondary (progression).^{1,14} The primary translocations mostly involve chromosome 14q32 (IgH). They provide an early immortalization event and are mediated by errors in IgH switch recombination and somatic hypermutation during B-cell development in the germinal centers. With conventional cytogenetics, these translocations have been identified at a variable frequency of 10% to 60%.¹⁷ With FISH, the incidence of 14q23 translocations is much higher and increases with the stage of disease: 50% in MGUS, 60% to 65% in intramedullary MM, 70% to 80% in extramedullary MM, and more than 90% in MM cell lines.²⁶ Reciprocal translocations occur

ring between IgH and five partner genes — 11q13 (cyclin D1), 6p21 (cyclin D3), 4p16 (FGFR3 and MMSET), 16q23 (c-MAF), and 20q11 (MAFB) — have been identified. These account for a combined prevalence of about 40% to 50% of MM and result in the dysregulation of a variety of oncogenes.^{1,14} Such translocations have also been found to have prognostic importance, with t(4;14) and t(14;16) having a significantly shorter median survival.²⁷

Secondary translocations, on the other hand, seldom involve IgH, are more complex, and are not mediated by errors in class switch recombination or somatic hypermutation. They involve other chromosomes (eg, *c-myc*), usually in the form of complex translocation and insertions that are nonreciprocal and are associated with enhanced proliferation and disease progression.^{1,14} Recently, *c-myc* rearrangement were identified in 15% of patients with MM and correlate with severity of disease.²⁸ Besides *c-myc*, other genetic and epigenetic events that are associated with disease progression include loss of chromosome 13, hypermethylation of p16^{INK4A} gene promoter, activating mutations of NRAS and KRAS2 oncogenes, inactivating mutations or deletion of p53, and inactivation of PTEN.¹

Biology of Multiple Myeloma

The MM microenvironment consists of clonal myeloma cells, extracellular matrix proteins, bone marrow stromal cells (BMSCs), osteoblasts, and osteoclasts. The interaction of MM cells with BMSCs and extracellular protein matrix via adhesion molecules and/or cytokines is crucial in the pathogenesis of MM and its associated bone disease.^{29,30}

Interleukin-6 (IL-6) is the most important cytokine in MM biology and is predominantly produced in a paracrine fashion by MM cells and BMSCs.³¹ Under normal circumstances, IL-6 causes B-cell differentiation, but in MM, it causes proliferation and inhibits apoptosis of myeloma cells.^{31,32} The interactions between MM cells and BMSCs augment its secretion via nuclear factor- κ B (NF- κ B)-dependent pathways^{31,32} (the role of NF- κ B is discussed in the proteasome inhibitors section) and leads to the following downstream effects of IL-6 on MM cells:

- Promoting cell proliferation via the RAS-MAPK pathway³³
- Promoting cell survival via the JAK-STAT pathway³⁴
- Preventing dexamethasone-induced apoptosis via the PI3K-AKT pathway³⁵
- Blocking differentiation of monocytes to dendritic cells, thus impairing host immune functions³⁶
- Inducing VEGF (vascular endothelial growth factor) secretion, which promotes angiogenesis, stimulates growth and migration of MM cells, further augments IL-6 secretion, and prevents antigen presentation by dendritic cells.^{37,38}

The interactions of MM cells with the bone marrow microenvironment via integrins increase production of

several cytokines: VEGF, IL-1 β , IL-10, tumor necrosis factor α (TNF- α), PTHrP, TGF- β , matrix metalloproteinase 1, MIP-1 α , basic fibroblast growth factor (bFGF), insulin-like growth factor I (IGF-1), and hepatocyte growth factor, all of which play important roles in MM cell growth, angiogenesis, osteoclast activation, and MM-related immunodeficiencies. For example, TNF- α secreted by both MM cells and BMSCs increases expression of certain adhesion molecules (such as VLA4, LFA1, VCAM1, and ICAM1) that further promote the interactions between the cells and the environment and confer protection against apoptotic stimuli. It also increases IL-6 secretion via the NF- κ B pathway in BMSCs.³⁹ Another important cytokine is IGF-1, which promotes MM cell proliferation via the RAS-MAPK pathway and inhibits apoptosis by activation of the PI3K/AKT signaling cascade.⁴⁰ Others such as stromal cell-derived factor-1 α , produced by BMSCs, promote proliferation and induction of resistance to dexamethasone-induced apoptosis and MM cell migration (Fig 3).⁴¹

Of interest are the pathways or mechanisms that confer resistance to therapy. The drugs currently in use appear to induce cell death by activating both apoptotic pathways — the death receptor pathway mediated by caspase-8 and the mitochondrial pathway mediated by caspase-9.⁴² Failure to induce apoptosis is currently thought to be due to the interactions of the MM cells with its environment. For example, MM cells binding to fibronectin via integrins α 4 β 1 (VLA4) and α 5 β 1 (VLA5) induce release and accumulation of cellular FLIP_L in the cytoplasm. Cytoplasmic FLIP_L then competes with procas-

pase-8 for FADD, hence preventing apoptosis of MM cells and contributing to what is currently known as cell adhesion-mediated drug resistance.^{29,43} Other interactions, eg, binding of VLA4 and LFA-1 on MM cells to VCAM1 and ICAM1 on BMSCs, induce secretion of cytokines such as IL-6, IGF-1, and VEGF. These cytokines then contribute to resistance via a number of pathways, including the activation of the JAK/STAT pathway, PI3K/AKT pathway, NF- κ B activation, and upregulation of antiapoptotic proteins such as BCL-X_L and survivin.^{31,35,37,44,45}

The lytic bone lesions observed in MM are the consequence of the uncoupling of the normal process of bone remodeling (bone formation and resorption) leading to increased osteoclastic bone destruction. During normal homeostasis, RANKL (receptor activator of NF- κ B ligand) is expressed by both BMSCs and osteoblasts in the local bone marrow microenvironment, where it then binds to its receptor, RANK (receptor activator of NF- κ B) on the surface of osteoclast precursors. The binding of RANKL to RANK plays an important role in promoting osteoclast differentiation and maturation. It also activates mature osteoclasts and increases bone resorption.⁴⁶ A soluble decoy receptor known as osteoprotegerin (OPG) has been identified that binds RANKL, inhibiting its interaction with RANK and preventing osteoclast formation and activation.⁴⁷ In other words, the osteoclast-activating action of RANKL can be blocked by OPG so that in healthy people, a delicate balance between RANKL and OPG⁷³ regulates osteoclastic activity.

Adhesion of MM cells to BMSCs induces the latter cells to secrete osteoclast-activating factors such as IL-1 β , IL-6, bFGF, IGF-1, and TNF- α . These factors upregulate RANKL secretion and result in greater osteolysis.⁴⁸ Moreover, MM cells express and shed syndecan-1 (CD138), which inactivates OPG, further disturbing the RANKL/OPG balance.⁴⁹ In a vicious cycle, the destruction of the bony matrix is accompanied by further release of the above cytokines, thus stimulating MM cell growth and the release of PTHrP, which further induces RANKL secretion.^{47,50}

In summary, we now have a better understanding of the profile of the pathways that are deregulated in the biology of MM. The potential ability to better target component pathways in crippling this incurable disease is now greater than in the past, as illustrated in the next part of this review.^{29,30,42}

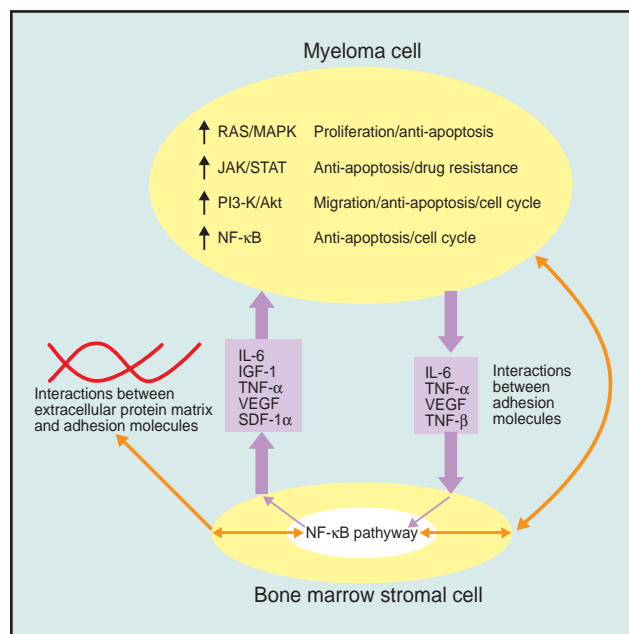


Fig 3. — Interaction of myeloma cell and the bone marrow microenvironment. Binding of myeloma cells to bone marrow stromal cells via adhesion molecules induces secretion of cytokines, which mediate myeloma cell proliferation, survival, drug resistance and migration by activating an array of intracellular signaling pathways. Interactions between adhesion molecules and extracellular protein matrix upregulate the NF- κ B pathway, further enhancing adhesion molecule expression, antiapoptotic pathways, and cytokine secretion.

Targeted Therapies

Thalidomide and Its Analogs

Thalidomide is an agent with significant activity in MM.⁵¹ It was first introduced in 1952 as a sedative agent but was subsequently withdrawn because of teratogenicity. However, interest in thalidomide was renewed when it was found serendipitously to have immunomodulatory activity in treatment of erythema nodosum associated with lep-

rosy. It has since been used in other autoimmune conditions such as Behcet's disease and in chronic graft-vs-host-disease, among others. These conditions are associated with high levels of TNF- α both in the blood and in their lesions, and thalidomide was shown to lower its levels. The increasing number of clinical indications for thalidomide led quickly to its use in various untreatable ailments including cancer. Its astonishing success in myeloma led to attempts to synthesize analogs with greater immunologic/anticancer properties but with fewer toxicities of the parent compound. Using the structural backbone of thalidomide as a template, two promising compounds have emerged: CC-5013 (Revimid) and CC-4047 (Actimid). These drugs are more potent than thalidomide in inhibiting TNF- α and are far more potent in co-stimulating T cells without some of its side effects.⁵²

Mechanism of Action: The immunomodulatory effects of CC-5013 and CC-4047 are believed to be due to their ability to (1) decrease the levels of TNF- α by enhancing the degradation of its mRNA or by inhibiting its synthesis by monocytes,⁵³ (2) stimulate the activation and expansion of T cells by providing the costimulatory "second signal" to naive T cell,⁵³ (3) augment NK-cell-mediated cytotoxicity through its direct effect on T cells with a resultant increase in IL-2 and IFN- γ secretion,⁵⁴ and (4) interfere with NF- κ B activity by blocking its ability to bind to DNA or suppress I κ B kinase activity, thus abrogating normal inflammatory cytokine production.⁵⁵ In myeloma, thalidomide also disrupts the host marrow-MM cell interaction by selective modulation of the density of cell surface adhesion molecules.⁵⁶

Another important action of thalidomide is inhibition of angiogenesis.⁵⁷ This was first demonstrated by using a rabbit cornea micropocket assay showing that thalidomide inhibited bFGF.⁵⁷ With an increase in microvessel density in the bone marrow, together with an increase in plasma levels of bFGF and VEGF in active MM patients, thalidomide was expected to be highly active in this disease.^{58,59} It was but, remarkably, had no effect on the microvessel density of the patients who responded and thus raised the question of its exact effect on angiogenesis.^{60,62}

Other postulated mechanisms of action of thalidomide include its direct induction of caspase-dependent apoptosis and G₁ growth arrest of MM cells.⁵² In particular, the thalidomide analogs revealed that they directly induced apoptosis by downregulation of NF- κ B transcriptional activity.^{54,56,63} Furthermore, this inhibition of NF- κ B potentiated induction of apoptosis by dexamethasone through caspase activation and overcame the resistance of myeloma cells to the drug.^{55,56} Another proposed mechanism is thought to be through its inhibition of IL-6.⁵²

Clinical Studies: The pivotal study of thalidomide in MM was a phase II study by Singhal et al⁶⁰ that involved 84 previously treated patients with refractory disease. The initial dose was 200 mg/day to a maximum dose of 800 mg/day. A majority of these patients had previously been treated with an autologous stem cell transplantation. Remarkably, a response rate of 32% was observed, with 10% showing a complete or near complete response. Side effects in the majority were considered mild to moderate and included constipation, weakness or fatigue and somnolence. Less than 5% had grade 1 or 2 leucopenia. After 12 months of follow up, the event-free survival rate was 22% and the 12-month overall survival rate was 58%. An update involving a total of 169 patients confirmed the earlier report with superior outcomes being noted in those having a normal karyotype, low plasma cell labeling index, and a β -2 microglobulin of less than 3 mg/L.⁶¹ Others have made the same observation (Table 1).^{55,64-72}

Encouraged by its effectiveness in refractory cases, thalidomide was next evaluated in untreated, asymptomatic patients. Rajkumar and colleagues^{73,74} reported significant activity in the treatment of newly diagnosed smoldering MM, with an overall response rate of 38%⁷³ and an estimated progression-free survival rate of 80% at 1 year and 63% at 2 years.⁷⁴ Weber and colleagues⁷⁵ also conducted a trial of single-agent thalidomide in patients with asymptomatic MM at high risk of progression. However, it was combined with dexamethasone for symptomatic untreated patients. The overall response rates were 36% and 73%, respectively, and supported its efficacy in these patient groups. Of note was the doubling of response seen in the combination with dexamethasone.⁷⁵ Others have also noted this synergy with some reporting a response as high as 57% in refractory patients

Table 1. — Thalidomide as a Single Agent in Multiple Myeloma

Trial	No. of Patients	Dose (mg/day)	Overall Response Rate (%)
Singhal et al ⁶⁰	84	200-800	32
Barlogie et al ⁶¹	169	200-800	30
Juliusson et al ⁵⁵	23	200-800	43
Kneller et al ⁶⁴	17	200-800	64
Rajkumar et al ⁶⁵	16	200-800	25
Blade et al ⁶⁶	23	200-800	52
Yakoub-Agha et al ⁶⁷	83	50-800	48
Neben et al ⁶⁸	83	100-400	20
Tosi et al ⁶⁹	65 *	100-800	28
Mileshkin et al ⁷⁰	75 **	200-800	28
Schey et al ⁷¹	69	100-600	49
Richardson et al ⁷²	30	200-600	43

* 60 patients were evaluable for response.

** After 12 weeks of single-agent thalidomide therapy, 19 patients continued thalidomide with the addition of interferon- α 2b.

Table 2. — Thalidomide Plus Dexamethasone for Advanced Refractory Multiple Myeloma

Trial	No. of Patients	Regimen	Efficacy
Palumbo et al ⁷⁶	77	Thalidomide 50-100/mg/day Dexamethasone 40 mg on days 1-4, monthly	ORR: 52% Time to response: 4.2 months Median survival: 27 months 2-yr OS: 53%
Dimopoulos et al ⁷⁷	44	Thalidomide 200-400 mg/day Dexamethasone 20 mg/m ² /day on days 1-4, 9-12, 17-20	ORR: 55% Median survival: 2.6 months
Alexanian et al ⁷⁸	47	Thalidomide 200-600 mg/day Dexamethasone 20 mg/m ² /day on days 1-5 every 15 days	ORR: 47% Time to response: 2 months
Anagnostopoulos et al ⁷⁹	47	Thalidomide 200-600 mg/day Dexamethasone 20 mg/m ² /day on days 1-5 every 15 days	ORR: 47% Time to response: 2 months Median survival: 38 months

ORR = overall response rate
OS = overall survival rate

who were previously unresponsive to either agent alone (Table 2).⁷⁶⁻⁷⁹

This thalidomide/dexamethasone regimen is significant for its nonmyelosuppressive effect and, together with the fact that it is administered orally, encouraged its evaluation in newly diagnosed patients (Table 3). Rajkumar and colleagues⁸⁰ reported in their phase II trial a response rate of 64%, which was comparable to other combination chemotherapy regimens such as VAD (vincristine, doxorubicin, and dexamethasone). Adequate numbers of stem cells were collected with no associated problems. Subsequently Weber et al⁷⁵ reported a similar response rate of 72% with a notably rapid onset of remission (in 0.7 months). In another trial that compared thalidomide plus dexamethasone with dexamethasone alone, a superior response was again noted for the thalidomide-dexamethasone arm (80% vs 53%).⁸¹ These results have led to many centers using this combination as first-line therapy.

Thalidomide has also been combined with chemotherapy (Table 4).⁸²⁻⁸⁵ In the largest study to date, 236

previously treated MM patients were treated with two cycles of DT-PACE (comprising of dexamethasone and thalidomide with infusional cisplatin, doxorubicin, cyclophosphamide, and etoposide) followed by stem cell collection in all patients.⁸⁵ Subsequently patients were randomized to either tandem autologous stem cell transplantation or further cycles of DT-PACE. The 2-year event-free survival rates were 63% and 81% in the tandem transplant and DT-PACE groups, respectively. The overall survival rates were 65% and 79%, respectively, for these two groups. Even though the outcome was similar, it is difficult to draw any firm conclusion from this study because only 40% of patients were randomized and 58% patients in the further DT-PACE group crossed over to the tandem transplant group because of failure to achieve predefined level of response. Others have evaluated the efficacy of thalidomide/dexamethasone with bortezomib in advanced MM and thalidomide-melphalan-prednisolone in newly diagnosed patients, and both have shown promising results.⁸⁵⁻⁸⁷

Table 3. — Thalidomide With or Without Dexamethasone for Early-Stage or Previously Untreated Myeloma

Trial	No. of Patients	Regimen	Efficacy
Rajkumar et al ⁷³	16	Thalidomide 200-800 mg/day	ORR: 38% PFS: 13% at 12 months
Rajkumar et al ⁷⁴	31	Thalidomide 200-800 mg/day	ORR: 34% Time to response: 5 months PFS: 80% at 12 months OS: 63% at 24 months OS: 93% at 12 months
Weber et al ⁷⁵	40	Thalidomide 100-400 mg/day Dexamethasone 40 mg/day on days 1-5, 9-12, 17-22, then days 1-4 monthly	ORR: 72% Time to response: 0.7 months PSF: 3% at 9 months OS: 8% at 9 months
Rajkumar et al ⁸⁰	50	Thalidomide 200 mg/day Dexamethasone 40 mg/day on days 1-5, 9-12, 17-22, then days 1-4 monthly	ORR: 64%

ORR = overall response rate
PFS = progression-free survival rate
OS = overall survival rate

Table 4. — Thalidomide in Combination With Chemotherapy for Refractory and Relapsed Myeloma

Trial	No. of Patients	Regimen	Efficacy
Moehler et al ⁸²	56	Thalidomide 400 mg/day Dexamethasone 40 mg/day on days 1-4 Cyclophosphamide 400 mg/m ² /day for 4 days Etoposide 40 mg/m ² for 4 days	ORR: 68% 1-yr PFS: 60% 1-yr OS: 63%
Srkalovic et al ⁸³	20	Thalidomide 400 mg/day Dexamethasone 40 mg/day on days 1-4 monthly	ORR: 70% PFS: 50% at 9 months OS: 50% at 13 months
Kropff et al ⁸⁴	60	Thalidomide 100-400 mg/day Dexamethasone 20 mg/m ² /day on days 1-4, 9-12, 17-20 each month, optional to days 1-4 monthly Cyclophosphamide 300 mg/m ² on days 1-3	ORR: 68% PFS: 50% at 11 months OS: 50% at 19 months
Lee et al ⁸⁵	236	Thalidomide 400 mg/day Dexamethasone 40 mg/day for 4 days Cisplatin 10 mg/day for 4 days Doxorubicin 10 mg/m ² /day for 4 days Cyclophosphamide 400 mg/m ² /day for 4 days Etoposide 40 mg/m ² /day for 4 days	ORR: 32%

ORR = overall response rate
PFS = progression-free survival rate
OS = overall survival rate

With the increased use of thalidomide in MM, cases of venous⁸⁸ and arterial thrombosis⁸⁹ have been reported. Multiple myeloma itself is associated with a prothrombotic state; however, a clear relationship between the use of thalidomide in nonmalignant conditions has not yet been made.

In view of the potent antitumor activity of the analogue CC-5013 *in vitro*, a phase I clinical trial was conducted to evaluate the efficacy of this drug in patients with refractory and relapsed MM. Of 24 patients treated with 50 mg/day, 17 patients (71%) showed a best response of at least a 25% reduction in paraprotein. Two other patients (8%) showed stable disease. The drug was well tolerated and no somnolence, constipation, or neuropathy was observed. However, granulocytopenia and thrombocytopenia were noted in some patients; these were the major dose-limiting toxic effects.⁹⁰ A multicenter phase II study involving 83 patients with progressive disease has also shown encouraging results, with 85% of evaluable patients experiencing a reduction or stabilization of their M-protein levels.⁹¹ The FDA granted fast-track status to CC-5013 for the treatment of relapsed or refractory MM in February 2003 based on these initial reports of efficacy. The results of several phase II and III trials on CC-5013 either alone or in combination with other compounds are eagerly awaited.

Proteasome Inhibitors

Bortezomib, previously known as PS-341, is a first-in-class peptide boronate proteasome inhibitor that is highly specific and has high affinity for the catalytic site of the 26S proteasome,⁹² a protein complex present in eukaryotic cells that degrades ubiquitin-tagged proteins and hence regulates the turnover of a vast repertoire of intracellular

proteins. Among these are proteins involved in cell cycle, apoptosis, transcription, and regulation of chemotaxis, cell adhesion, and angiogenesis.⁹²

Mechanism of Action: NF- κ B is a transcription factor and a member of the Rel family of proteins; it is a heterodimer composed of p50 and p65 subunits. It is constitutively activated in MM as well as many other hematologic malignancies.⁹³ In MM, NF- κ B has been shown to be involved in the upregulation of IL-6 transcription.³² TNF- α would activate NF- κ B in both the BMSCs and MM cells, mediating further IL-6 secretion and expression of adhesion molecules (ICAM-1, VCAM-1) and resulting in increased MM cell-BMSC interaction (see above).¹⁴ NF- κ B has also been shown to activate the expression of various antiapoptotic molecules such as Bcl-2, X chromosome-linked inhibitor of apoptosis protein (XIAP), and survivin, and downregulate the pro-apoptotic molecule BAX.³⁹ From these *in vitro* experiments, NF- κ B activation promotes the growth, survival, and drug resistance of the MM cells, and it is an important therapeutic target (Fig 4).

NF- κ B is inactivated by its association with I κ B α .⁹³ Various stimuli including TNF- α and chemotherapy trigger I κ B protein phosphorylation by the I κ B kinase complex. Once phosphorylated, I κ B is targeted for ubiquitination and degraded by the 26S proteasome. NF- κ B then translocates into the cell nucleus where it binds to specific DNA sequences in the promoters of target genes and stimulates their transcription. I κ B degradation and hence NF- κ B activation are blocked by inhibition of the 26S proteasome (Fig 4). This was initially thought to be the main target for bortezomib, but specific blockade of NF- κ B using direct inhibitors of I κ B α phosphorylation was insufficient to completely inhibit the proliferation of MM cells, suggesting that bortezomib does not act through NF- κ B

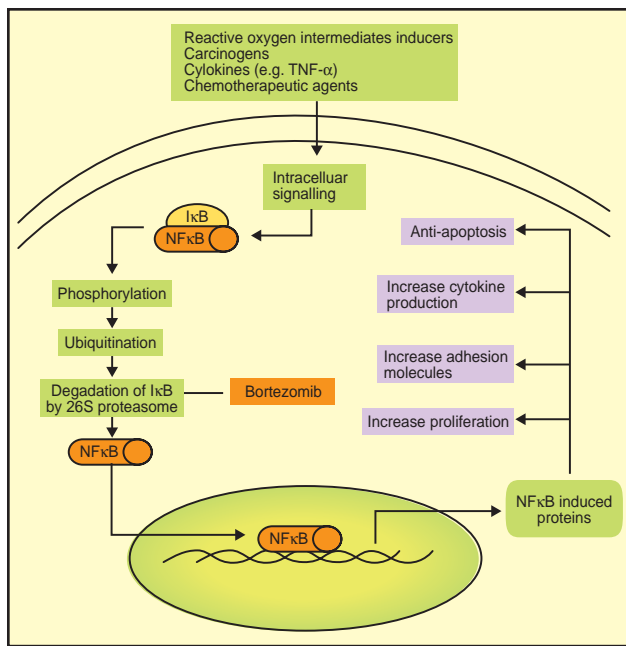


Fig 4. — NF-κB as a therapeutic target of bortezomib. Various factors including TNF-α and chemotherapy induce the degradation of IκB via the ubiquitin-proteasome pathway, resulting in the activation of NF-κB and its subsequent translocation in the cell nucleus where it transcribes genes that contribute to the growth and survival of myeloma cell, their resistance to chemotherapy, and enhances their interaction with the bone marrow microenvironment. Bortezomib blocks the degradation of IκB by the 26S proteasome and hence abrogates the oncogenic effects of NF-κB activation.

blockade alone.⁴⁴ In fact, gene expression profiling has shown that bortezomib not only downregulated genes that encode for growth like IGF-1, but also induced apoptotic, ubiquitin/proteasome and stress response genes in myeloma cells.⁹⁴

Bortezomib was also able to directly induce apoptosis in resistant MM cells by both the mitochondrial and Fas/caspase-8-dependent apoptotic pathways.^{94,95} It also inhibited DNA repair and activated p53 by phosphorylating and degrading MDM2.⁹⁶ Through its inhibitory effect on TNF-α, it overcame the resistance to apoptosis conferred by IL-6 in MM cells and decreased their binding to BMSCs by 50%.⁹⁵

In vitro experiments have also established the synergies between bortezomib and dexamethasone, melphalan, or doxorubicin in either sensitive or resistant MM cells.^{95,97} In addition, bortezomib has shown remarkable in vivo antimyeloma activity in a murine model; it inhibited tumor growth, decreased angiogenesis, and prolonged the survival of severe combined immunodeficient (SCID) mice bearing human MM cells.⁹⁸

Clinical Studies: In the phase I trial using a twice-weekly schedule for 4 weeks followed by a 2-week rest, 4 different doses of bortezomib were evaluated in 27 patients with refractory hematologic malignancies. The maximum tolerated dose was 1.04 mg/m². Among these patients were 9 with refractory MM. All 9 achieved some response, with 1 complete responder.⁹⁹ From this study, a phase II study in relapsed or refractory MM used a dose of

1.3 mg/m² by intravenous push twice-weekly schedule for the first 2 weeks of a 3-week cycle. The addition of dexamethasone was permitted in patients with progressive or stable disease after 2 or 4 cycles, respectively.¹⁰⁰ Of the 202 enrolled patients, 91% were refractory to their most recent treatment, and 92% had been treated with three or more drugs. The overall response rate to bortezomib was 35%, of which 27.5% was for partial remission and 7.5% was for minimal response. A complete response was achieved in 4%, with another 6% positive for M-spike by immunofixation only. The median overall survival in this heavily pretreated patient group was 16 months with a median duration of response of 12 months. Remarkably, time to progression was increased by 2 to 4 times with bortezomib compared to the last course of chemotherapy given before enrollment. The addition of dexamethasone was associated with an improvement in suboptimal responders on bortezomib alone. Response was independent of both the number of prior therapies and chromosome 13 status.¹⁰⁰ The treatment was well tolerated with no grade 4 adverse events reported. The most common grade 3 adverse events included thrombocytopenia and dose-related peripheral neuropathy.¹⁰⁰ Based on these results, bortezomib received accelerated FDA approval in May 2003 for the treatment of MM patients who have received at least 2 prior therapies and have demonstrated disease progression on their last therapy.

The interim result of a large international phase III randomized study comparing bortezomib to dexamethasone in relapsed MM patients has been reported. With more than 300 patients randomized to each arm and using the same dosing as in the phase II trial, there was significant improvement in the primary end point of time to progression from 3.6 months to 5.7 months ($P < .0001$, log-rank test).¹⁰¹

Trials using bortezomib either alone or in combination with chemotherapeutic agents for untreated MM are now underway. Preliminary results from these phase II studies suggest good response rates. Although complete responses were not seen with bortezomib alone, it continued to have a favorable safety profile with no adverse effect on peripheral blood stem cell collection and subsequent engraftment.^{102,103}

Arsenic Trioxide

Arsenic trioxide (ATO) has been used as a medical treatment in Asia for thousands of years.¹⁰⁴ ATO is now approved for the treatment of relapsed acute promyelocytic leukemia based on positive results from a multicenter US trial.¹⁰⁵

Mechanism of Action: ATO inhibits MM cell proliferation possibly through the induction of p21 cyclin-dependent kinase inhibitor protein. It also induces apoptosis in MM cells through an increase in caspase-3 activity.¹⁰⁶ Recently the apoptotic mechanism has been further refined. Two distinct pathways are involved in ATO-induced apoptosis depending on the p53 status of

the cell. In MM cells with functional p53, ATO induces apoptosis through the intrinsic pathway involving release of cytochrome C from the mitochondria and activation of caspase-9. In MM cells with mutated p53, apoptosis is triggered by the activation of caspase-8 and -10.¹⁰⁷

ATO may also induce antitumor activity through an immunologic mechanism by increasing lymphokine-activated killer cells.¹⁰⁸ ATO also acts in the bone marrow microenvironment to decrease MM cell binding to BMSCs and inhibit IL-6 and VEGF secretion.¹⁰⁹ Another potential antitumor effect is via VEGF inhibition,¹¹⁰ although this has yet to be demonstrated in myeloma.

In vitro studies show that pharmacologic concentration of ATO preferentially triggers MM cell death (while sparing normal myeloid cells) and IL-6 could not protect the MM cells from apoptosis.¹¹¹ ATO-induced apoptosis is enhanced by reactive oxygen species and reduced by glutathione. The addition of butathione sulfoximine and ascorbic acid, two glutathione-depleting agent, increased the cytotoxic effect of ATO on MM cells.^{112,113} Significant response following treatment with ATO, including an apparent complete remission lasting up to 5 months, has been observed in SCID mice transplanted with human myeloma cells.¹¹⁴

Clinical Studies: Results of three phase II studies using ATO as a single agent in patients with relapsed MM refractory to conventional chemotherapy have been reported (Table 5).¹¹⁵⁻¹¹⁷ Continuous and intermittent dosing schedules have been used. The combined results of the three trials showed only modest efficacy of ATO as a single agent in this group of patients, with only 2 of the 48 patients achieving greater than 50% reduction in paraprotein levels. Most of the responses were minor (12 of 48 patients, and a 25% to 49% reduction in paraproteins). Most patients experienced grade 3 or higher neutropenia and were unable to tolerate more than 2 months of treatment. It is also unclear from the published data what is

the best dosing schedule; a continuous dosing is associated with better response but higher toxicity, while the intermittent dosing appears to be better tolerated but produces poorer responses.

As a follow-up to their preclinical observation that ascorbic acid accentuated the activity of ATO, Bahlis et al¹¹⁸ reported results from a small NCI-sponsored phase I study using ATO at 0.25 mg/kg per day plus 1,000 mg per day of ascorbic acid. This regimen appeared to be well tolerated and produced response in 2 out of 6 refractory MM patients. Combinations with conventional therapies have also been tested. In one study, combination of low-dose ATO (0.25 mg/kg twice weekly), oral melphalan, and intravenous ascorbic acid produced 4 responses in 10 relapsed patients.¹¹⁹ In another study, a regimen combining ATO (0.25 mg/kg 5 days in week 1 and 2 times a week for weeks 2 to 10), dexamethasone, and ascorbic acid was used in 16 relapsed and/or refractory patients and produced 1 solitary response.¹²⁰ Overall, it seems that ATO would have limited efficacy in patients in whom multiple prior treatment has failed.

Farnesyltransferase Inhibitors and Lovastatin

Farnesyltransferase catalyzes the transfer of farnesyl moiety to the cysteine terminal residue of substrate proteins. One of these substrate proteins is Ras, which is a G-protein signal transducer that requires prenyl lipid modification and membrane association for signal transduction.^{121,122} The modification involves the covalent addition of either farnesyl or geranylgeranyl groups catalyzed by farnesyltransferase or geranylgeranyl transferase, respectively. The former process is inhibited by farnesyltransferase inhibitors (FTIs). Both processes are inhibited by lovastatin by inhibiting the production of mevalonate and depriving the cells of isoprenoids.¹²³

Mechanism of Action: Ras is a valid target in MM as the *ras* gene is mutated in 39% of newly diagnosed MM

Table 5. — Phase II Studies of Arsenic Trioxide in Multiple Myeloma

Study	No. of Patients	Patient Group	Dosing	Completion of Allocated Treatment	Response	Toxicity
Munshi et al ¹¹⁵	14	Relapsed/refractory High risk Multiple prior therapy	0.15 mg/kg/day daily for up to 60 days	5 patients completed 60 days	>75% reduction: 1 50-74% reduction: 1 25-49% reduction: 1	≥ grade 3 neutropenia: 11 Infection: 5 Deep vein thrombosis: 3
Rousselot et al ¹¹⁶	10	Relapsed/refractory High-dose therapy (n = 8) ≥3 therapies (n = 8)	0.15 mg/kg/day daily for up to 56 days (n = 8) 0.20 mg/kg/day for 2 weeks in 4-weekly cycles for up to 3 cycles (n = 2)	Patients completed a median of 35 days of treatment. Both patients completed 3 cycles	>75% reduction: 0 50-74% reduction: 0 25-49% reduction: 3	≥ grade 3 neutropenia: 4 ≥ grade 3 thrombocytopenia: 4 Transaminitis: 10
Hussein et al ¹¹⁷	24	Relapsed/refractory High-dose therapy (29%) ≥3 therapies (50%)	0.25 mg/kg/day for 5 days/week during 1st 2 weeks of 4-weekly cycles	6 completed 2 cycles 2 completed 3 cycles 2 completed 4 cycles 2 completed 5 cycles 3 completed 6 cycles 1 completed >6 cycles	>75% reduction: 0 50-74% reduction: 0 25-49% reduction: 8	≥ grade 3 neutropenia: 17 ≥ grade 3 thrombocytopenia: 5 Infection: 1

and is associated with poorer clinical outcome.¹⁴ The percentage of patients with mutations increases to 81% at the time of relapse, making ras mutation the most prevalent mutation in MM.¹²¹ Furthermore, IL-6 triggers myeloma cell growth via the Ras-dependent MAPK pathway.³³

Originally developed to block Ras activity,^{122,123} the antitumor effect of FTI is now believed to be mediated through other mechanisms, as the antitumor effect does not correlate with mutated Ras status.^{124,125} Furthermore, Ras can also be prenylated by geranylgeranyl transferase; therefore, it is unlikely that Ras is the dominant target.¹²² The exact mechanism is still unclear at present.

Lovastatin is an attractive option for targeting Ras as it could potentially inhibit the isoprenylation process completely. In myeloma cell lines, lovastatin depleted membrane-localized Ras due to the inhibition of isoprenylation.¹²⁶ A more recent study showed that lovastatin induced apoptosis through inhibition of geranylgeranylation rather than farnesylation possibly via regulation of Mcl-1, which is a critical survival factor for myeloma cells.¹²⁷ Lovastatin has also been shown to overcome cell adhesion-mediated drug resistance in cell line studies.¹²⁸

Several compounds including FTI-277 and R115777 have been shown to induce apoptosis in myeloma cell lines that were resistant to conventional cytotoxics (including doxorubicin and melphalan) in a dose- and time-dependent manner.¹²⁹⁻¹³¹ For lovastatin, an in vitro study using myeloma cell lines and primary myeloma cells from patients showed that induction of cell death was possible at concentrations achievable in vivo (0.1 to 4 μ M corresponding to therapeutic dose of 1 mg/kg per day used in treatment of hypercholesterolemia).¹²⁶

Clinical Studies: A phase II study in patients with relapsed or refractory myeloma using R115777 (tipifarnib) at 300 mg orally twice a day on a 3-weeks-on, 1-week-off schedule has been reported.¹³² In this heavily pretreated population where more than 50% of enrolled patients failed previous high-dose chemotherapy and thalidomide, tipifarnib alone did not produce any objective response, although 4 patients achieved minimal responses (defined as 25% to 49% decrease in serum paraprotein concentration) that persisted for 26, 14, 7, and 3 cycles of treatment. However, disease stabilization (defined as 0% to 25% decrease in serum paraprotein concentration) was achieved in 64% of the 43 patients enrolled. The median time to progression from the start of treatment for these patients is 4 months (range 2 to 26 months). The treatment was well tolerated, with fatigue being the most common complaint. Hematologic toxicity was not a significant problem. The dose used was also shown to be sufficient to inhibit the biochemical target farnesyltransferase, protein farnesylation, and oncogenic survival pathways.¹³²

Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitor

Patients with MM have increased level of VEGF^{37,38} and

this correlates with increased angiogenesis and a high plasma cell labeling index.¹³³ Binding of MM cells to BMSCs markedly upregulates VEGF secretion.¹⁴ The VEGF secreted triggers IL-6 production from BMSCs and augments paracrine MM growth. VEGF can also act directly on MM cells, stimulating proliferation and migration.³⁷ Therefore, VEGF appears to play an important role in both autocrine and paracrine growth of MM cells.

In a study using PTK787, which is specific for VEGF receptor 2 and with some activity against VEGF receptor 1, the drug directly inhibited the proliferation and migration of MM cells. The agent also enhanced the anti-MM activity of dexamethasone and overcame the protective effect of IL-6. It also inhibited the secretion of IL-6 induced by MM cell binding to BMSCs.¹³⁴

Clinical Studies: In a phase II study using a small molecule VEGF receptor 2 inhibitor, SU5416 at 145 mg/m² twice weekly, no objective response was seen in 27 heavily pretreated patients with MM (median of 4 lines of prior treatment, 14 with prior thalidomide therapy).¹³⁵ Disease stabilization for longer than 4 months was achieved in 4 patients. The treatment was well tolerated, and grade 3/4 toxicities were rare. A decrease in median VEGF plasma levels was observed in patients with stable disease compared with patients who had progressive disease.¹³⁵

Bcl-2 Antisense Oligodeoxynucleotide

Defects in apoptotic pathways contribute significantly to resistance of cancer cells to chemotherapeutic drugs.¹³⁶ Bcl-2 is an antiapoptotic protein that has shown to be expressed in both myeloma cell lines and patient samples despite the absence of any molecular rearrangement of its chromosome.^{137,138} Gene transfer-mediated overexpression of Bcl-2 in myeloma cells has been reported to block chemotherapy-induced apoptosis.^{139,140}

Preclinical studies showed that G3139, an 18-mer fully phosphorothioated antisense oligodeoxynucleotide targeted to the first six codons of the Bcl-2 mRNA, was effectively taken up by myeloma cells. It decreased both the mRNA levels and protein levels of bcl-2, and it sensitized myeloma cells to chemotherapeutic agents that included dexamethasone and doxorubicin.^{139,140}

Clinical Studies: In a phase II study of G3139 combined with VAD in 10 heavily pretreated patients, 7 patients responded (4 partial responses and 3 minor responses), including 5 with disease previously refractory to VAD.¹⁴¹ In another study using G3139 in combination with dexamethasone and thalidomide in patients who had a median of three prior treatment regimens, including 6 patients who had progressive disease on thalidomide, response was documented in 12 of 16 evaluable patients (2 complete remissions, 2 near complete remissions, 5 partial remissions, and 3 minimal responses).¹⁴² The toxicities experienced by these patients were not different from those observed with the use of the chemotherapy drugs alone. Interestingly, Bcl-2 protein levels were only mini-

mally reduced after 4 days of G3139 treatment (maximal reduction occurred after 4 days from preclinical studies), and there was no correlation between Bcl-2 protein levels and response.^{141,142}

2-Methoxyestradiol

2-Methoxyestradiol (2ME) is a natural metabolite of estradiol with potent antitumor and antiangiogenic activity. It binds poorly to the estrogen receptor and mediates its antiproliferative effects independently of estrogen-receptor expression or responsiveness.¹⁴³ It overcomes the protective effect of IL-6 and IGF-1 and decreases the secretion of VEGF and IL-6 by BMSCs. It also induces apoptosis in MM cells.^{144,145}

Preclinical and Clinical Studies: 2ME inhibited the growth of and induced apoptosis in drug-resistant MM cells. It also enhanced dexamethasone-induced apoptosis.^{144,145} As a monotherapy in 51 patients with relapsed and plateau phase MM, 2ME was well tolerated with mainly grade 1-2 toxicities and produced a 1-year progression-free survival rate of 24% for the whole cohort, with 51% for those in plateau phase and 10% for relapsed patients. However, no partial responses were achieved.¹⁴⁶

Other Treatments

Some other novel treatments targeting intracellular signaling in myeloma cells and their interaction with BMSCs have produced promising results in preclinical studies. These include the histone deacetylase (HDAC) inhibitors,¹⁴⁷⁻¹⁴⁹ inhibitors of lysophosphatidic acid acyltransferase β (LPAAT),¹⁵⁰ inhibitors of IGF-1 receptor,¹⁵¹ and inhibitors of heat-shock protein 90 (HSP-90).¹⁵²

HDACs affect cell growth at the transcriptional level by regulating the acetylation status of nucleosomal histones. HDAC inhibition induced differentiation, growth arrest, and apoptosis not only in transformed cells but also in MM cells, irrespective of resistance to dexamethasone or conventional chemotherapy. The transcriptional signature following HDAC inhibition in MM cells was characterized by antiproliferative and proapoptotic molecular events and included downregulation of the proteasome pathway.¹⁴⁸ This finding suggests that a therapeutic strategy combining bortezomib and clinically relevant HDAC inhibitors may result in a synergistic increase in cell kill.¹⁵³

The action of LPAAT is to convert lysophosphatidic acid to phosphatidic acid, which is involved in signal transduction. The inhibition of LPAAT resulted in growth arrest of primary MM cells from patients as well as MM cell lines. The most potent of these inhibitors, CT-32176, mediated apoptosis through activation of caspase-8, -3, and -7 and poly (ADP-ribose) polymerase cleavage. Interestingly, these inhibitory effects were not negated by the addition of exogenous IL-6 and IGF-1.¹⁵⁰

IGF-1 is an important growth and survival factor for MM cells in vitro, and MM cells constitutively express IGF-1 receptors. A preclinical study reported that use of neutral-

izing antibodies, antagonistic peptides, or a selective kinase inhibitor, NVP-ADW742, to target this receptor resulted in apoptosis of various tumor cells including MM.¹⁵¹

Lastly, HSP-90 is a molecular chaperone that interacts with target intracellular proteins to facilitate intracellular trafficking, conformational maturation, and 3-dimensional folding required for protein function. Geldanamycin and its analog bind to the critical ATP-binding site of HSP-90, abrogating its chaperoning activity, which has led to the following observations: decreasing IGF-1 receptor and IL-6 receptor expression on MM cells, decreasing growth kinases (eg, Akt, I κ B kinase, and Raf) and antiapoptotic proteins (FLICE inhibitory protein, XIAP, cIAP, and telomerase), and inhibiting activation of NF- κ B and telomerase. The sequelae is the induction of apoptosis of MM cell lines, including those resistant to dexamethasone, anthracyclines, thalidomide, and bortezomib.¹⁵²

Conclusions

Much in our basic understanding of the biology of myeloma has occurred in recent years. With this knowledge, we have expanded our armamentarium of drugs that are available to our patients with this disease. The efficacy of these agents has been tested on various cell lines and in patients and has produced encouraging results.

The development of some of these drugs has been remarkable. For instance, bortezomib was granted orphan-drug status by the FDA in October 2001 and the first completed clinical trial was published in 2002. In mid 2002, bortezomib entered phase II studies in other hematologic cancers, and by early 2003, it entered phase III clinical trials. In fact, the FDA granted fast-track status to bortezomib for the treatment of relapsed or refractory MM in May 2003 based on initial reports of efficacy. It is interesting to note the unusual step of the endorsement given to it in clinical trials despite the lack of understanding its mechanism of action. This goes against our current drug-discovery paradigm.

With this plethora of treatment options, it will be increasingly difficult to decide on which is the best treatment option for our patients. We need to rationally study how to incorporate them into existing therapies, or perhaps a better therapeutic strategy would be to use various combinations of pathway-specific drugs that could lead to additive or synergistic interactions and reduce the possibility of drug resistance.^{153,154} This could be further aided by gene expression profiling that would allow the identification of patients who would benefit from these drugs either through the identification of signatures for specific molecular defects or signatures predictive of response.¹⁵⁵

Although only thalidomide and bortezomib have produced objective responses in MM, most of the other novel agents were effective in stabilizing the disease with minimal toxicity. Therefore, although a cure for MM may not

be currently possible, it is hoped that treatment with these new agents may convert MM into a chronic illness with minimal toxicity while maintaining a good quality of life for our patients.

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