

# Nontraditional Cytotoxic Therapies for Relapsed/Refractory Multiple Myeloma

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**Key Words.** Multiple myeloma · Arsenic trioxide · Relapsed disease · Clinical trials

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## LEARNING OBJECTIVES

After taking all of the CME courses in this supplement the reader will be able to:

1. Describe the basic biology of various leukemias, multiple myeloma, and myelodysplastic syndrome (MDS).
2. Discuss new targeted treatment strategies for hematologic malignancies.
3. Understand the rationale for the use of nontraditional cytotoxic agents such as arsenic trioxide in the treatment of hematologic malignancies.
4. Examine the role of arsenic trioxide and other novel agents in early- versus accelerated-stage hematologic disease.
5. Discuss the preclinical and clinical efficacy of arsenic trioxide and various agents in treating acute promyelocytic leukemia, MDS, and multiple myeloma.

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## ABSTRACT

Multiple myeloma remains an incurable disease, with median survival rates of 4-6 years even with aggressive, high-dose chemotherapy, bone marrow transplantation, and intensive supportive care. Additionally, multiple myeloma is primarily a disease of the elderly, many of whom cannot tolerate aggressive chemotherapy. Thus, newer treatments with good safety profiles are needed to improve the quality of responses and, hopefully, to translate into prolonged progression and overall survival. The pathophysiology of multiple myeloma is complex, involving many pathways and interactions among cytokines, adhesion molecules, angiogenesis, and mechanisms of resistance, which, taken together, provide multiple targets for novel therapeutic modalities. Agents currently under investigation

for treating multiple myeloma include thalidomide and its successors, PS-341, and arsenic trioxide. Thalidomide and immunomodulatory drugs both exhibit activity against multiple myeloma by affecting different levels of the immune system. PS-341 is a proteasome inhibitor that halts the cell cycle, resulting in apoptosis; it also inhibits a key transcription factor and may have antiangiogenic activity. Arsenic trioxide activates multicellular mechanisms to induce apoptosis, inhibit angiogenesis, and stimulate immune responses. Preclinical and early clinical data suggest that combination regimens should be pursued, given the different mechanisms of action of these compounds on the immune system and their non-overlapping toxicities at low dosages. *The Oncologist* 2002;7(suppl 1):20-29

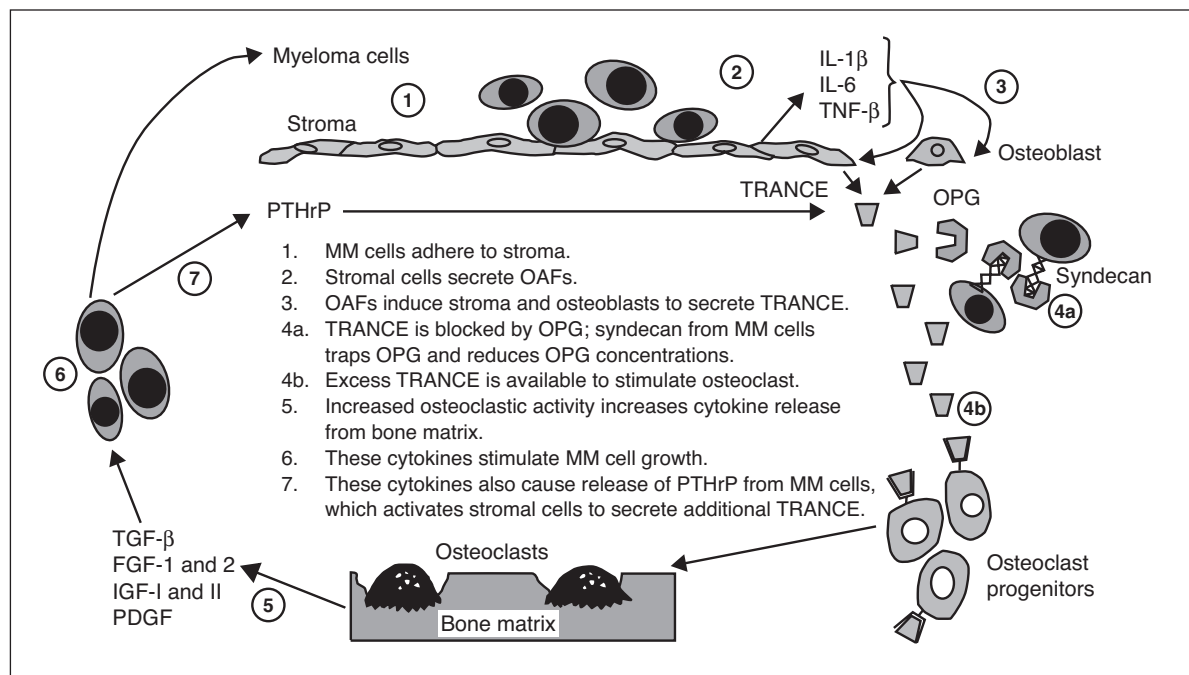
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## INTRODUCTION

Multiple myeloma (MM) accounts for 1%-2% of all cancers and is responsible for 20% of deaths from hematologic

malignancies. Each year, 14,400 cases of MM, leading to approximately 11,200 deaths, are diagnosed in the U.S. [1]. Conventional therapy produces overall response rates of

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**Figure 1.** The vicious cycle of stimulation that supports the growth and proliferation of malignant cells and results in bone destruction in MM. Adapted with permission from Tricot [6]. Abbreviations: OAF = osteoclast-activating factors; OPG = osteoprotegerin; PDGF = platelet-derived growth factor; PTHrP = parathyroid hormone-related protein.

40%-60% and a median survival of 3-4 years [2, 3]. More recent unpublished data from several myeloma centers suggest that the median survival is up to 6-7 years. However, despite these advances, MM remains an incurable disease since almost all patients eventually relapse and develop drug resistance. The ultimate failure of conventional and high-dose chemotherapy in treating these patients, as well as the complex pathogenesis of this disease, provides the rationale for the urgency in investigating novel therapeutic options.

#### POTENTIAL TARGETS IN THE PATHOGENESIS AND PROGRESSION OF MM

Multiple regulatory pathways are involved in the development and progression of MM. The malignant plasma cells in MM are localized in the bone marrow and interact with bone marrow stromal cells through cell adhesion molecules and the secretion of cytokines. The resulting activation of osteoclasts and other stromal cells supports the growth of myeloma cells and contributes to the complications associated with them [4]. These multistep processes and pathways provide many targets for therapeutic intervention.

#### Cytokines

The growth of MM cells in the bone marrow is subject to both autocrine and paracrine regulation. MM cells secrete

a number of cytokines that act on bone marrow stromal cells, which, in turn, secrete factors that contribute to the growth and proliferation of the MM cells (Fig. 1) [5, 6]. The attachment of myeloma cells to bone marrow stroma is mediated by the CXCR4 binding of stromal-cell derived factor-1 $\alpha$ , followed by the upregulation of very late antigen-4 [7]. This process initiates a vicious cycle of events that includes bone resorption and the stimulation of myeloma cell growth [6]. Following the adherence of myeloma cells, stromal cells secrete a number of cytokines, including interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor (TNF- $\beta$ ). These cytokines promote the secretion of TNF-related activation-induced cytokine (TRANCE), also known as osteoprotegerin ligand, a member of the TNF family that stimulates the differentiation and proliferation of osteoclasts [6, 8].

Abnormal IL-1 $\beta$  expression is believed to stimulate the transition from a clinical condition known as monoclonal gammopathy of undetermined significance (MGUS) to frank MM [9]. IL-1 $\beta$  mRNA is not found in healthy individuals, but it is found in 25% of patients with MGUS and in more than 95% of MM patients, all of whom have bone lesions. IL-1 $\beta$  is a key activator of osteoclasts; it increases the expression of adhesion molecules and induces the production of IL-6, which is the central regulatory cytokine in the pathogenesis of MM.

The central role of IL-6 in regulating the proliferation and survival of MM cells is well established [4, 6, 9]. IL-6 is

**Figure 2. IL-6-mediated autocrine and paracrine mechanisms of MM growth.**

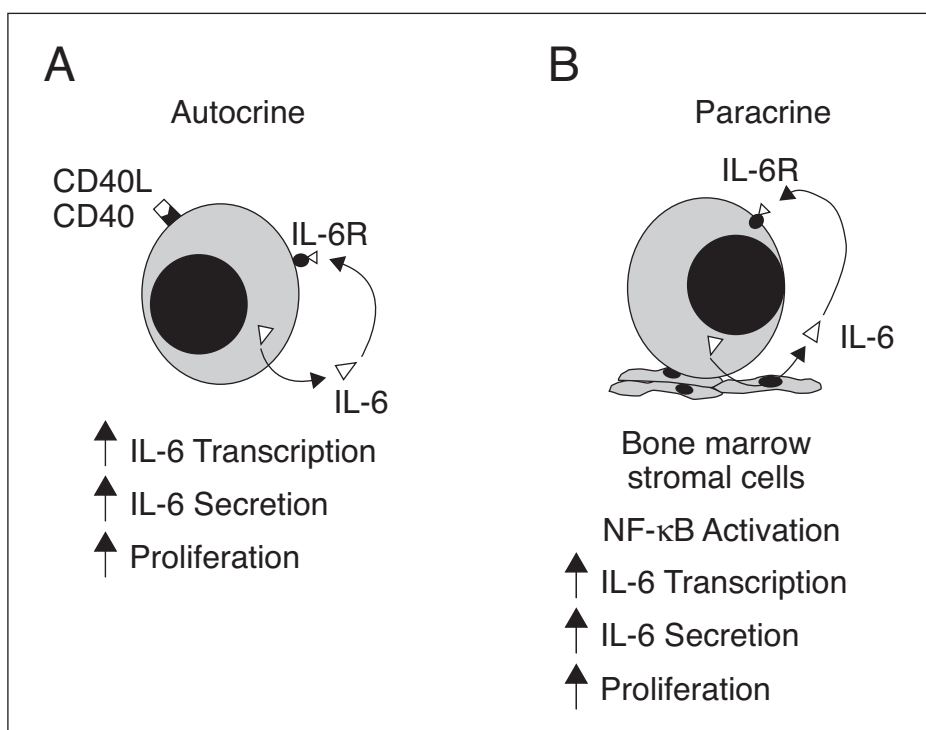
A) Stimulation of the CD40 receptor via CD40 ligand binding on MM cells triggers autocrine IL-6-mediated MM cell growth. B) TGF- $\beta$ 1 production and secretion by both MM cells and bone marrow stromal cells stimulates IL-6 secretion, through NF- $\kappa$ B activation, by both cell types leading to MM cell proliferation. Adapted with permission from Anderson and Lust [9].

a multifunctional cytokine that acts primarily as a paracrine growth factor in the pathogenesis of MM, although it appears to have an autocrine effect as well (Fig. 2) [9]. IL-6 supports the growth and survival of MM cells both by stimulating proliferation and inhibiting apoptosis [4].

The upregulation of IL-6 production by the stromal cells is mediated by the transcription factor NF- $\kappa$ B [10]. In resting cells, NF- $\kappa$ B remains bound to its inhibitor, I $\kappa$ B, in the cytosol [11]. When the cells are stimulated, I $\kappa$ B is degraded and NF- $\kappa$ B moves into the nucleus, where it stimulates the transcription of several genes regulating proliferation. Among these are genes that code for IL-6 and several cell adhesion molecules. NF- $\kappa$ B activation has also been shown to suppress apoptosis induced by TNF- $\alpha$  and the *ras* oncogene.

IL-6 activates Janus kinases (JAKs). These kinases induce the phosphorylation of proteins known as signal transducer and activator of transcription (STAT) proteins, STAT-1 and STAT-3. JAKs also stimulate the Ras growth pathways [4, 6]. Activation of the Ras pathway correlates with the stimulation of myeloma cell growth. Activation of the JAK-STAT pathway may prevent apoptosis of myeloma cells and mediate drug resistance through an increased expression of the cell survival protein Bcl-X<sub>L</sub> [4, 6, 12].

Other cytokines in the bone marrow microenvironment that contribute to the proliferation and survival of MM cells include: A) G-CSF; B) interferon- $\alpha$  (IFN- $\alpha$ ), which influences the myeloma cells by either stimulation or inhibition, depending on the state of the cells; C) IL-10, whose role in regulating proliferation is independent of IL-6, and D) transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), which acts directly on stromal cells to increase IL-6 production and may also aid in inhibiting a normal immune response [4, 5, 13]. GM-CSF, IL-3, stem cell factor, TNF- $\alpha$ , hepatocyte growth factor, and insulin-like growth factors I and II have been shown to stimulate MM cell



growth and differentiation in vitro, often acting synergistically with IL-6. However, in a clinical study in advanced myeloma patients, GM-CSF did not result in disease progression or any adverse events [14].

#### Adhesion Molecules

Most adhesion receptors found on MM cells are also found on normal cells, but adhesion receptors and their ligands play a number of important roles in MM [15]. Both the extravasation of MM cells from blood to bone marrow, a key event in the pathogenesis of MM, and metastasis to distant sites are mediated by adhesion molecules [15]. As previously discussed, the adhesion molecule-mediated binding of MM cells to stromal cells in the bone marrow stimulates the release of cytokines that act as growth factors or that promote the production of growth factors [15]. The adhesion molecules E-selectin and vascular cell adhesion molecule-1 are also important for angiogenesis [16].

#### Angiogenesis

Solid tumors, like normal tissue, rely on blood vessels to supply oxygen and nutrients. They cannot grow beyond 2 mm in diameter without increased blood perfusion, and the density of blood vessels is associated with disease progression. In MM, increased microvessel density and angiogenic activity in the bone marrow are characteristic features and are correlated with the plasma cell labeling index (a measure of proliferative activity) and with progressive disease [17, 18]. The mechanism of angiogenesis in MM is not well defined.

Almost certainly it involves several cytokines, including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). Angiogenesis probably is a by-product, rather than a cause, of these events.

VEGF has been detected in bone marrow aspirates of patients with MM [19]. The myeloma cells expressed VEGF, and the stromal tissue surrounding the myeloma cells expressed both VEGF receptors, Flt-1 and KDR. This suggests an autocrine or paracrine regulation of VEGF secretion that supports myeloma cell proliferation. Levels of bFGF are significantly higher in patients with active MM than in patients with nonactive disease or MGUS. In vitro studies indicate that antibodies against bFGF inhibit angiogenic activity. This has been shown in human endothelial cells when tested with conditioned media from MM cells of patients with active myeloma; it has also been observed in a chick embryo chorioallantoic membrane assay [18]. The association of the bFGF receptor syndecan-1 (CD138) in the growth and progression of MM, and the occurrence of the translocation involving the *FGFR3* gene in MM, provide additional evidence for the role of angiogenesis in the growth and progression of MM [2].

VEGF, a potent stimulator of neovascularization, plays a central role in angiogenesis through the mitogen-activated protein kinase (MAPK) and the JAK/STAT signaling pathways. VEGF is secreted by many types of malignant cells [20]. Evidence suggests that VEGF may also act as a paracrine growth factor for MM cells by stimulating IL-6 production in bone marrow stromal cells and vascular endothelial cells [21]. Recent studies have demonstrated that VEGF induces proliferation and triggers the migration of human MM cells by protein-kinase C (PKC)-independent and PKC-dependent pathways, respectively [22]. These results have formed the basis of research efforts to block MM cell survival by developing agents that inhibit angiogenesis and promote apoptosis.

### Drug-Resistance Mechanisms

MM cells, unlike most other malignant cells, are highly resistant to chemotherapy. The membrane cell survival proteins Bcl-2 and Bcl-X<sub>L</sub> are overexpressed in most MM cells through interaction with the environment. These proteins are associated with the inhibition of apoptosis. Although apoptosis is necessary for normal hematopoietic stem cells, it leads to drug resistance in MM through upregulation of the antiapoptotic members of the Bcl-2 family [4, 23]. In a recent study, Bcl-X<sub>L</sub> overexpression in bone marrow biopsy samples correlated strongly with decreased patient response to melphalan, vincristine, doxorubicin, and dexamethasone. Response rates were 83%-87% in non-Bcl-X<sub>L</sub>-expressing patients but only 20%-31% in Bcl-X<sub>L</sub>-expressing patients [23].

Other mechanisms of chemoresistance include the persistence and subsequent expansion of abnormal CD19<sup>+</sup> clones, the increased expression of survival proteins (e.g., IL-6) and/or antiapoptotic proteins (e.g., NF-κB, Bcl-2, Bcl-X<sub>L</sub>, Mcl-1), and a decreased responsiveness to inhibitory proteins, such as TGF-β [13, 24].

### CURRENT TREATMENT OF RELAPSED/REFRACTORY MM

Therapeutic options remain limited for patients with relapsed or refractory disease. Currently, following tumor reduction with conventional chemotherapy, relapsed or refractory MM patients are treated with high-dose chemotherapy with or without total-body irradiation, usually with autologous stem cell rescue and/or administration of growth factors, if the patient can tolerate these regimens [25]. A proportion of patients can achieve long-term complete remission with this aggressive treatment. *Barlogie et al.* evaluated five high-dose regimens (four including melphalan and one including thiotepa) in patients with primary refractory or relapsed/refractory MM. Overall, 12% of the patients achieved a complete response, 25% of whom were still alive at 10 years [26]. However, this study also demonstrated the extreme toxicity of high-dose regimens, which necessitated intensive supportive care for the first 4-6 months. The mean treatment-related mortality was 17%. The highest mortality occurred among the patients who received the lowest melphalan dose ( $\leq 100$  mg/m<sup>2</sup>), no total-body irradiation, and no cellular support. A recent randomized trial, designed to analyze the outcome of pretreated patients with MM who responded to initial chemotherapy and subsequently received either high-dose therapy/stem cell rescue intensification or a continuation of conventional chemotherapy, showed that the complete response rate was significantly higher in patients who received the intensification. However, the length of progression-free survival and overall survival was similar in both arms, underscoring the need for more innovative therapy [27].

Thus, MM remains an incurable disease despite the use of the most aggressive treatments, which are often poorly tolerated by elderly patients. Therapeutic agents that can potentially target the myriad and complex underlying pathogenic mechanisms are needed, particularly therapies that offer improved safety profiles, which would increase their suitability for use in elderly patients.

### CURRENT NONTRADITIONAL AGENTS WITH BROAD MECHANISMS OF ACTION

#### Thalidomide

Thalidomide, marketed in the late 1950s and early 1960s to prevent morning sickness in pregnant women, was

arguably the greatest drug disaster of all time and resulted in a major overhaul of the drug testing and approval process. Nevertheless, thalidomide was approved by the Food and Drug Administration (FDA) in 1998 for the treatment of erythema nodosum leprosum, an inflammatory condition associated with leprosy. At present, this drug is being investigated as an immunomodulatory agent in a variety of other conditions (e.g., chronic graft-versus-host disease, rheumatoid arthritis) and as an antiangiogenic agent for treating several types of cancer, including glioma; melanoma; renal cell, ovarian, prostate, and breast cancers; Kaposi's sarcoma; and MM [2, 28].

The immunomodulatory action of thalidomide is probably multifaceted and is not fully understood. Thalidomide inefficiently, but selectively, inhibits the production of TNF- $\alpha$  in human monocytes in a dose-dependent fashion, exerting no effect on total protein synthesis or the production of other cytokines [29]. The inhibitory action of thalidomide on TNF- $\alpha$  occurs by enhancing mRNA degradation [30]. Thalidomide has complex effects on T cells, stimulating proliferation and inducing the secretion of IFN- $\gamma$ . Thalidomide also induces cytokine production by type 2 helper T cells, inhibits cytokine production by type 1 helper T cells, and regulates the expression of adhesion molecules, which is probably the principal action behind all of the other effects [2, 28].

The antiangiogenic activity of thalidomide appears to be species specific. It requires activation of the drug after systemic administration [28]. Angiogenesis inhibition is probably effected through the inhibition of bFGF and VEGF activity [2].

#### *Clinical Efficacy of Thalidomide in MM*

Numerous studies have demonstrated the activity of thalidomide in relapsed and refractory MM. Response rates of 25%, with thalidomide used as a single agent, and up to 75%, when used in combination with other agents, have been observed [2, 31-33]. Some of these responses have been durable [2, 32, 34]. *Barlogie et al.* [34] reported an overall 2-year survival rate of 60% and a significant dose-response effect, with superior response and survival rates occurring in patients receiving higher doses of the drug.

The side effects of thalidomide are usually mild to moderate. The most common include sedation, fatigue, constipation, and skin rash. Peripheral neuropathy is also known to occur with this drug and may be permanent following chronic use [31, 32].

Because of its novel activity profile and lack of myelosuppression, thalidomide is being investigated for use with other agents [32]. A review of studies with thalidomide has indicated that combination therapy produces higher rates of

overall response (defined as  $\geq 50\%$  improvement in myeloma protein levels) in relapsed/refractory patients, with a mean of 36% with thalidomide alone, 52% with the addition of dexamethasone, and 62% with the addition of dexamethasone plus chemotherapy [34]. Responses have also been achieved in patients with previously demonstrated resistance to dexamethasone-based regimens and in those who had relapsed after high-dose therapy with autologous stem cell transplantation [35]. At this stage, it is too early to know if overall survival will be affected by the use of thalidomide therapies.

#### **Immunomodulatory Drugs**

Based on the encouraging results with thalidomide, a novel group of thalidomide analogues has been developed that are up to 50,000 times more potent than the parent molecule at inhibiting TNF- $\alpha$  production by bone marrow plasma cells [36]. In preclinical studies, these immunomodulatory drugs (IMiDs) were shown to arrest the growth of G<sub>1</sub> cells and induce apoptosis via a caspase-8-mediated mechanism in MM cell lines and in MM cells taken from patients [37]. These studies also demonstrated that IMiDs downregulate the constitutive MAPK phosphorylation, which is indicative of IL-6-induced growth stimulation [38]. IMiDs have also been shown to target the bone marrow microenvironment by enhancing the modulation of IL-2 and IFN- $\gamma$  levels relative to the levels achieved following treatment with thalidomide [36, 39].

There is some evidence that IMiDs could play a role in overcoming the drug resistance that is characteristic of MM. IMiDs can inhibit the proliferation of MM cells resistant to melphalan, doxorubicin, and mitomycin by 20%-35% and can inhibit proliferation of dexamethasone-resistant cell lines by 50% [38]. IMiDs have been shown to potentiate the anti-MM activity of dexamethasone [37]. These results suggest a potential clinical utility for IMiDs in treating patients who are resistant to currently available MM therapies.

#### **PS-341**

PS-341 is a synthetic boronate proteasome inhibitor with good selectivity [11]. Proteasomes are large complexes of proteolytic enzymes. These enzymes are involved in the intracellular degradation of proteins and the turnover of many regulatory proteins via the ubiquitin-proteasome pathway. The proteolytic enzymes recognize the presence of ubiquitin, which marks the proteins (including regulators of cell proliferation) that have been designated for degradation. Selectively inhibiting proteasome activity "stabilizes" the proteins that regulate the cell cycle, thus disrupting cell proliferation and resulting in apoptosis [11]. The addition of PS-341 to susceptible cells in culture halts the cell cycle at the G<sub>2</sub>-M phase, causing cell death. The activity of some proteasome inhibitors, such as PS-341, is reduced in cells

expressing the multidrug-resistant (*MDR*) gene. PS-341 shows half its normal activity in *MDR*-expressing cells. Other proteasome inhibitors (e.g., lactacystin) are apparently unaffected in these cells. The cytotoxicity of proteasome inhibition appears to be cell-type specific.

Proteasome inhibition can block the activity of the transcription factor NF- $\kappa$ B [11, 40]. The stabilization of I $\kappa$ B by proteasome inhibitors might prevent desensitization of tumor cells to drug- or TNF- $\alpha$ -induced apoptosis. Blocking NF- $\kappa$ B activation also inhibits the TNF- $\alpha$ -induced expression of cell-surface adhesion molecules, which should have several effects: slowing the migration and metastasis of tumor cells, inhibiting the NF- $\kappa$ B-dependent increase in IL-6 production in stromal cells, and inhibiting the paracrine stimulation of MM cell growth. PS-341 has been shown to reduce the number and size of lung metastases in a murine cancer model [11, 41]. Because adhesion molecules are also important in angiogenesis, the inhibition of adhesion molecule expression by proteasome inhibitors should also prove antiangiogenic. This has been demonstrated using the natural proteasome inhibitor lactacystin.

PS-341 is active against many tumor types *in vitro*, including MM, and reduces tumor growth in an established murine model of MM and in other murine cancer models [11, 42]. PS-341 is not subject to drug-induced resistance; it has only slightly reduced affinity for the *MDR* transporter and it is active in chemoresistant cell types overexpressing Bcl-2 [11].

PS-341 added to human MM cell lines and freshly isolated MM cells *in vitro* demonstrates multiple effects. It inhibits proliferation and induces apoptosis, it prevents MAPK growth signaling, and it induces apoptosis despite the induction of p21 and p27 in both p53 wild type and mutant cell lines. PS-341 also overcomes drug resistance and IL-6-mediated resistance to apoptosis. Further, it reduces adherence to bone marrow stromal cells, stabilizes I $\kappa$ B, and inhibits NF- $\kappa$ B-induced IL-6 secretion. Finally, PS-341 enhances the antitumor activity of dexamethasone [42].

*In vitro*, PS-341 also increases the effects of other chemotherapeutic agents, including 5-fluorouracil, paclitaxel, cisplatin, doxorubicin, and irinotecan (CPT-11). For example, CPT-11 increases NF- $\kappa$ B activity, which partially negates its antitumor effect. The addition of a proteasome inhibitor would be expected to block the CPT-11-induced NF- $\kappa$ B activation, thus enhancing activity. PS-341, exhibiting antitumor activity when used alone, produces a synergistic effect *in vitro* when combined with CPT-11. PS-341 might also increase the duration of antitumor activity by inhibiting degradation of the topoisomerase I-camptothecin complex [11].

To date, only limited clinical data are available for the proteasome inhibitors. PS-341 was the first proteasome

inhibitor to reach clinical trials [11]. Phase I trials in diverse hematologic and solid malignancies have shown that PS-341 produces clinical response in patients resistant to standard chemotherapy [43]. Preliminary results of a phase I trial showed that two of three patients with MM demonstrated a clinically significant response [44]. PS-341 was fairly well tolerated, with gastrointestinal toxicity being the major side effect. A multicenter phase II trial of PS-341, alone or in combination with dexamethasone in relapsed or refractory MM patients, is under way. Among the 75 patients enrolled to date, preliminary results suggest that PS-341 has significant antitumor activity in a group of advanced myeloma patients [45].

### Arsenic Trioxide

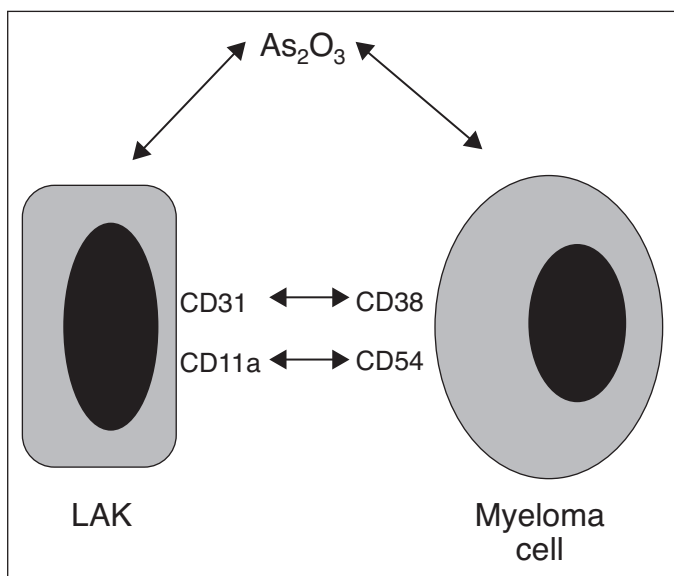
Arsenic has been used as a medical treatment for thousands of years and persists as a folk remedy in Asia [46]. Recent discoveries, particularly dramatic clinical results from China and the U.S., have renewed interest worldwide in the use of arsenicals for treating cancers. In 1997, a study on the use of arsenic trioxide (ATO) in heavily pretreated patients with relapsed acute promyelocytic leukemia (APL) was initiated in the U.S. [47]. Eleven (92%) of 12 patients achieved complete remission with good tolerability of the medication and no evidence of cumulative toxicity [47]. Adverse events were generally manageable and reversible and did not require interruption of therapy.

A subsequent multicenter trial in the U.S., enrolling 40 relapsed APL patients, 19 of whom had experienced two or more relapses, reported similar results. The complete response rate was 85%, the 18-month relapse-free rate was 56%, and the overall survival rate was 66% [48]. Following therapy with ATO, 86% of patients achieved molecular remission, as measured by a negative reverse transcriptase-polymerase chain reaction test for the promyelocytic leukemia-retinoic acid receptor alpha transcript.

### Rationale for ATO in MM

ATO holds therapeutic promise in the treatment of MM. The *in vitro* sensitivity of cultured MM cell lines to 1- to 2- $\mu$ M concentrations of ATO, and the preliminary reports of response in patients with MM, have prompted investigations of its effectiveness in this disease [49-51]. Studies show that ATO may inhibit tumor angiogenesis through direct and indirect mechanisms. *In vitro* experimental data indicate that ATO inhibits VEGF production by a leukemic cell line and induces apoptosis and capillary tubule formation of human umbilical vein endothelial cells [52]. Furthermore, *in vivo* data show that ATO causes vascular shutdown leading to tumor necrosis in a murine fibrosarcoma model [53].

ATO induces growth inhibition and apoptosis in a number of malignant hematopoietic cell lines, including MM and



**Figure 3. Schematic model of a potential mechanism of immunologic cytotoxicity of low-dose ATO in myeloma cells and cell lines.** Arsenic trioxide enhances recognition, adhesion, and lysis of MM cells by LAK cells through the upregulation of specific receptor/ligand systems such as CD38/CD31 and CD11a/CD54. Adapted with permission from Deaglio et al. [57].

freshly isolated human MM cells [49, 50, 54, 55]. These effects appear to be preferential for MM cells, as they are greatly reduced in normal myeloid cells isolated from bone marrow [49]. Exogenous IL-6 does not overcome arsenic-induced growth inhibition or apoptosis [49, 55, 56]. Inhibition of proliferation has been associated with induction of the p21 cyclin-dependent kinase inhibitor protein and apoptosis [50]. Apoptosis occurs through a mechanism that involves collapse of the mitochondrial transmembrane potential, increased caspase-3 activity, and, possibly, the downregulation of Bcl-2 [50, 54, 55].

ATO also induces antitumor activity through immunologic mechanisms [57]. The exposure of human myeloma-like cell lines and freshly isolated MM cells to ATO resulted in increased killing mediated by lymphokine-activated killer (LAK) cells, possibly through the upregulation of the CD38/CD31 and CD11a/CD54 receptor-ligand systems, which increase recognition, adhesion, and lysis (Fig. 3) [57]. Treatment of both effector (LAK) and target (MM) cells with ATO selectively upregulated the expression of adhesion molecules involved in cell-cell interactions (CD38, CD54) and their ligands (CD31, CD11a) on MM and LAK cells, respectively. Blocking these cell-surface molecules with antibody inhibited cytotoxicity, suggesting increased adhesion as a mechanism for the cytotoxic activity. These findings suggest that ATO may be of clinical benefit in boosting the immune response against myeloma cells.

At least two apoptotic signaling pathways exist, one of which is mediated by c-Jun NH<sub>2</sub>-terminal kinases (JNKs) and is unaffected by IL-6 [58]. This pathway is involved in apoptosis induced by irradiation and ATO [58, 59]. The second mechanism, used by dexamethasone, is JNK independent. It is associated with the downregulation of MAPK and P70 and is inhibited by IL-6 [58]. Thus, an agent such as ATO, which downregulates Bcl-2 and induces apoptosis through an IL-6-insensitive apoptotic pathway, might have additive or even synergistic effects with dexamethasone. A National Cancer Institute (NCI)-sponsored multicenter phase II study of ATO in combination with dexamethasone for patients with recurrent or refractory stage II or III MM is under way.

The generation of reactive oxygen species enhances ATO-induced apoptosis, but glutathione reduces this effect [59, 60]. The increased expression or activity of glutathione and glutathione-related compounds is also known to confer resistance to alkylating agents [60]. Therefore, reducing glutathione during treatment with ATO might be expected to increase the drug's effect [60, 61]. Indeed, adding butathione sulfoximine, which is known to deplete glutathione in vivo, greatly increases the cytotoxic effect of ATO on MM and other tumor cell lines [61, 62].

Ascorbic acid is known to decrease glutathione concentrations and is well tolerated in vivo [60, 63]. Ascorbic acid has been shown to decrease glutathione concentrations and to significantly enhance the ATO-mediated killing of drug-resistant MM cell lines and freshly isolated MM cells [60]. Compared with refractory cells, cells from previously untreated patients are much more sensitive to ATO alone, and the addition of ascorbic acid does not enhance apoptosis. ATO and ascorbic acid alone or in combination have little effect on normal bone marrow cells.

The combination of ATO and ascorbic acid has increased the survival of mice implanted with lymphoma cells, which, in vitro, had reacted to combination treatment with enhanced growth inhibition [62]. These data suggest that ATO in combination with ascorbic acid might be clinically useful for treating refractory MM.

#### **Clinical Efficacy of ATO in MM**

ATO received orphan drug designation from the FDA for the treatment of MM. Clinical data indicate that ATO has single-agent activity against MM. In a small phase II trial, nine heavily pretreated, high-risk patients with advanced refractory MM received ATO 0.15 mg/kg as a 2-hour daily infusion (the dose previously used for treating APL patients). The planned duration of treatment was 60 days. Seven of the nine patients had undergone two or more high-dose chemotherapy cycles with autologous stem cell support, and all had received salvage therapy with dexamethasone, cyclophosphamide, etoposide,

and cisplatin chemotherapy, followed by thalidomide. Despite these treatments, their disease ultimately progressed. After the ATO treatment, two patients achieved a >50% reduction in myeloma (M)-protein level, for a response rate of 23%; no patient achieved a complete response. Both of the patients showing the response received the ATO treatment for at least 30 days [56, 64].

A larger multicenter phase II trial is under way to evaluate the role of ATO in advanced MM using higher doses but a less frequent dosing schedule [65]. Patients receive up to six 4-week cycles of ATO 0.25 mg/kg administered by infusion 5 days per week for 2 weeks per cycle. Of the 17 patients enrolled to date, eight had relapsed and nine had refractory disease. Response was evaluable in 10 patients (four relapsed, six refractory). Among the relapsed patients, one had stable disease through four cycles and one had a 25% decrease in M-protein levels. Among the refractory patients, three had decreases in M-protein levels of 32%, 39%, and 40%, respectively.

A phase I/II clinical trial sponsored by the NCI has been initiated to determine dose, safety, and efficacy of ATO combined with ascorbic acid in patients with relapsed or refractory MM [66]. The first three patients received up to three cycles (5 days/week  $\times$  5 weeks = one cycle) at the first dose level (ATO 0.15 mg/kg/day plus ascorbic acid 1,000 mg/day). As no dose-limiting toxicities were encountered in the first three patients, the second dose level (ATO 0.25 mg/kg/day plus ascorbic acid 1,000 mg/day) was tested in three additional patients. All six patients received a median of two cycles of ATO (range, one to five cycles). Grade 1 or 2 fatigue was the only major side effect. Other toxicities included grade 1 or 2 sensory neuropathy ( $n = 4$ ), grade 1 nausea ( $n = 3$ ), grade 1 or 2 skin rash and dry skin ( $n = 4$ ), grade 2 ( $n = 5$ ) and grade 3 ( $n = 1$ ) leukopenia, and grade 1 edema ( $n = 1$ ). No cardiac arrhythmias were seen. Two patients achieved a partial response ( $\geq 25\%$  decrease in disease markers) and four patients achieved stable disease (0%-25% decrease in disease markers). The phase II component of this study is currently under way.

The preliminary results from the clinical studies and new pharmacokinetic data suggest that a 5-day loading

period followed by a biweekly treatment schedule may be adequate for active treatment of MM patients. This schedule should improve tolerability and allow for a longer treatment period, which may be critical for not only increasing the response rate but also improving the quality of response [66].

ATO may be most beneficial when used in combination with other agents that have non-overlapping or complementary mechanisms, such as dexamethasone or ascorbic acid. Dexamethasone produced responses in more than half of patients treated, but resistance developed in more than 90% of patients within 2 years of treatment. Resistance to dexamethasone is associated with high Bcl-2 levels. It is further known that IL-6 protects MM cells from dexamethasone-induced apoptosis [58]. Clinical studies are in development that will address these issues.

## SUMMARY

Disease progression in MM is associated with complex biologic pathways and processes, making it difficult to manage the disease successfully and increasing the probability of relapse. The nature of MM also contributes to the development of resistance to current treatments. Novel treatment strategies are needed to target the underlying pathogenic mechanisms, but they must be safe for a predominantly elderly patient population.

Nontraditional therapeutic agents having novel mechanisms of action are under investigation. These include thalidomide, IMiDs, proteasome inhibitors, and ATO. Preliminary data suggest that these agents, alone or in combination with other therapies, may fill an unmet need in the management of relapsed/refractory MM. Complete results from clinical trials with these agents are eagerly awaited by the medical community.

## ACKNOWLEDGMENT

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