Novel Therapeutic Agents for the Treatment of Myelodysplastic Syndromes

Bruce D. Cheson, James A. Zwiebel, Janet Dancey, and Anthony Murgo

Few chemotherapy agents have demonstrated activity in patients with myelodysplastic syndromes (MDS) and supportive management remains the standard of care. An increasing number of new drugs in development are being directed at specific molecular or biological targets of these diseases. Topotecan, a topoisomerase I inhibitor, has shown single-agent activity and is now being combined with other agents, including cytarabine. The aminothiol amifostine induces responses in about 30% of patients; however, its role is still being clarified. Agents that inhibit histone deacetylase and target DNA hypermethylation, thus permitting derepression of normal genes, include 5-azacytidine, decitabine, phenylbutyrate, and depsipeptide. Arsenic trioxide has demonstrated impressive activity in acute promyelocytic leukemia and preclinical data suggest the potential for activity in MDS. UCN-01 is a novel agent that inhibits protein kinase C and other protein kinases important for progression through the G_1 and G_2 phases of the cell cycle. Dolastatin-10 has extremely potent in vitro activity against a variety of tumor cell lines. Since its dose-limiting toxicities include myelosuppression, it is being studied in acute myelogenous leukemia (AML) and MDS. *Ras* may play a role in MDS, and activation of this gene and its signaling pathways may require farnesylation. Several farnesyl transferase inhibitors are now available for study in patients with MDS. An increasing body of data suggests a possible role for angiogenesis in MDS, and several antiangiogenesis agents are in clinical trials, including thalidomide, SU5416, and anti–vascular endothelial growth factor (VEGF) antibodies. Development of new drugs and regimens will be facilitated by recently developed standardized response criteria. Future clinical trials should focus on rational combinations of these agents and others with the goal of curing patients with MDS.

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THE MYELODYSPLASTIC syndromes (MDS) are a heterogeneous group of hematopoietic disorders characterized by pancytopenia, generally in the setting of a hypercellular bone marrow. MDS have historically been referred to as oligoblastic leukemia, refractory anemia, smoldering acute leukemia, or preleukemia. In 1982, the French-American-British (FAB) group presented a classification, modified in 1985, which currently is the most widely used.[1,2] The FAB group separated MDS into five categories: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB), and RAEB in transformation (RAEB-T). The distinction between RAEB-T and acute myelogenous leukemia (AML) is based on histopathology, not clinical features. As a result, patients with MDS may exhibit a clinical picture consistent with AML with rapidly increasing numbers of blasts, but without the requisite number to fulfill the criteria for the diagnosis of AML.[3] Recently, a World Health Organization (WHO) steering committee proposed changes to the MDS subtypes with the major modifications including reclassifying chronic myelomonocytic leukemia (CMML) as a myeloproliferative disorder and decreasing the threshold for diagnosing AML from 30% blasts to 20%.[4] This system may eventually replace the FAB.

The likelihood of transformation to AML varies by FAB subtype[5-8]: approximately 10% to 20% for RA or RARS, 20% to 30% for CMML, 40% to 50% for RAEB, and 60% to 75% for RAEB-T. Nevertheless, the MDS are uniformly fatal, even without progression to AML, because of infection and bleeding.[9,10]

Over the years, a number of scoring and prognostic systems have been published to facilitate comparisons among reports of various treatments for MDS. Recently, the International Prognostic Scoring System (IPSS) has been widely adopted.[11] Factors taken into consideration included bone marrow blasts, cytogenetics, and cytopenias. Groups were identified with relative risks for transformation to AML and overall survival. Patients in the good cytogenetics group were those with a normal karyotype; poor risk included patients with complex abnormalities or with an involved chromosome 7; intermediate-risk patients consisted of all others (Table 1).

There are no curative therapies other than stem cell transplantation, which is an option for only a subset of patients. Therefore, numerous therapies

From the Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD.

Address reprint requests to Bruce D. Cheson, MD, National Cancer Institute, Executive Plaza North–Room 741, Bethesda, MD 20892.

Table 1. International Prognostic Scoring System for the Myelodysplastic Syndromes

Prognostic Variable	Score				
	0	0.5	1.0	1.5	2.0
Bone marrow blasts (%)	<5	5-10		11-20	21-30
Karyotype	Good	Intermediate	Poor		
Cytopenias (no. of lineages)	0/1	2/3			

NOTE. The total of the values for each prognostic variable is used to place patients in 1 of 4 risk groups: low, intermediate-1, intermediate-2, and high. These risk groups have significantly different outcomes.

have been and are being investigated to improve the outlook for these patients. Drugs selected for study in MDS have typically been those with significant activity in AML. Thus, cytarabine has been most widely evaluated, dating back more than 30 years when Ellison et al[12] first reported complete remissions with doses of cytarabine as low as 10 mg/m^2/d. Response rates were clearly dose-dependent, which encouraged the development of higher dose regimens. Subsequently, anecdotal reports and small series were published in which cytarabine at 10% to 20% of the standard dose administered either subcutaneously or by continuous intravenous infusion appeared to be effective in the treatment of AML and MDS.[13-22] Additional studies and a randomized phase III trial failed to support a major role for this therapy.[23-25]

Anthracyclines and related compounds have had been studied as single agents only to a limited extent.[26,27] In a study in which hydroxyurea and etoposide were compared in patients with CMML, response rates and survival were not impressive with either agent, but favored the former.[28] Other drugs that have been evaluated include 6-thioguanine and homoharringtonine, but both showed limited activity.[29,30]

NEW AGENTS

Several agents with unique mechanisms of activity are currently or will soon be evaluated in clinical trials for patients with MDS.

Topoisomerase I Inhibitors

Topotecan is a topoisomerase I inhibitor whose activity in acute leukemia led to its testing in MDS. The initial report included 47 patients with RAEB, RAEB-T, or CMML.[31] They were a poor-risk group, as demonstrated by the fact that the median age was 66 years, 70% exhibited cytogenetic abnormalities, and more than half were thrombocytopenic before topotecan therapy. Topotecan was delivered at a dose of 2 mg/m^2 as a continuous 24-hour infusion for 5 days. Treatment resulted in 28% complete remissions and an additional 13% of patients who experienced significant hematologic improvement. All eight patients with cytogenetic abnormalities before treatment and who achieved a complete remission became cytogenetically normal once in complete remission. The median remission duration was 7.5 months with 38% of patients still alive 1 year following treatment. Whether chronic oral topotecan is effective is undergoing evaluation.

The same investigators have shown that combination of topotecan and cytarabine is extremely active in patients with MDS. Beran et al[32] reported on 86 patients with MDS and CMML, most of whom (66%) were previously untreated, but who were considered high risk based on age or cytogenetic abnormalities. Topotecan was administered at a dose of 1.25 mg/m^2 by continuous infusion daily for 5 days, and cytarabine at 1 g/m^2 by a 2-hour infusion daily for 5 days. A complete remission was attained in 56% of patients, with 7% treatment-related deaths and a median survival of 60 weeks.[32] Preliminary results have been published of aggressive combination of topotecan, fludarabine, cytarabine, and granulocyte colony-stimulating factor (G-CSF); there were 50% complete remissions and 40% partial remissions, and the regimen appeared to be well tolerated.[33]

Amifostine

Amifostine (Ethyol; Alza Pharmaceuticals, Palo Alto, CA) is a phosphorylated aminothiol that protects bone marrow progenitors and other normal tissues from the toxicities associated with chemotherapy or radiation therapy. It was developed by the Walter Reed Army Medical Institute

(thus, the military code name WR-2721) during the Cold War as part of a classified research project to identify an agent that would protect military personnel from radiation in the event of nuclear war. Amifostine was found to afford greater protection against radiation than more than 4,000 other compounds screened. Nevertheless, the Army terminated development of this compound in 1988 because of its poor oral bioavailability and the prohibitive nausea, vomiting, diarrhea, and abdominal cramps with the oral formulation.

Further research was encouraged by the observation that amifostine stimulates hematopoiesis in both animal models and in vitro studies, and that it enhances the formation of hematopoietic progenitors from MDS bone marrow. In the initial phase I/II study,[34] the drug was administered at doses of 100, 200, or 400 mg/m^2 three times per week or 740 mg/m^2 weekly for 3 weeks. These investigators treated 18 patients at a median age of 73 years. FAB types included RA (seven patients), RARS (n = 5), RAEB (n = 4), and RAEB-T (n = 2). Seventeen patients were anemic, 15 of whom were transfusion-dependent; 12 had an absolute neutrophil count less than 1,000/μL and 14 were thrombocytopenic. Hematologic improvement was observed in 83% with the three-times-a-week schedules, including either an increase in neutrophils or a reduction in red blood cell transfusion requirements. More than 40% of patients had a rise in their platelet counts. However, there was acceleration to AML in several patients with RAEB-T. Although 61% of patients had clonal cytogenetic abnormalities before therapy, the abnormalities persisted even in patients with a hematologic response. No data regarding duration of response were provided, although responses were reported to persist during continuation therapy.

List et al[35] reported the results of a subsequent multicenter trial of amifostine in 117 patients, 104 of whom were evaluable at the time of presentation. A neutrophil response occurred in 10 (33%) of 30 patients, and was considered major in nine and minor in the other. A red blood cell response was evaluable in 66 patients, and a major response occurred in seven, with three experiencing a minor response. A major improvement in platelet count was seen in seven of 27 patients, with a minor response in three others, and 21% of patients had an increase in the reticulocyte count. A decrease in myeloblasts and sideroblasts occurred in 28% and 31%, respectively. The overall response rate was 30%, which is significantly lower than in the previous trial. Adverse events that were moderate or severe included fatigue (14%, 18%), nausea (19%, 36%), and vomiting (14%, 27%). In a smaller series,[36] a single or multilineage response was noted in five of 12 patients (58%). The absolute neutrophil count increased in 25% (by 102 to 1,560/μL), platelets in 50% (by 24,000 to 49,000/μL), reticulocytes in 25% (1.9% to 20%), and hemoglobin in 16% (5.3 to 5.6 g/dL).

In other reports, results with this agent were disappointing.[37,38] Hofmann et al[38] described 32 patients with RA/RARS (n = 26) and RAEB/RAEB-T (n = 15) treated at a dose of 200 mg/m^2 three times per week followed by a 2-week interval, for four courses. Limited benefit was observed even in patients with low- or intermediate-risk disease by the IPSS.

The role of amifostine in MDS is still being clarified. Nevertheless, combinations of amifostine with other agents such as 5-azacytidine are being evaluated.

Agents That Target Transcription

Recent developments in understanding the molecular basis for transcriptional repression and activation have presented new possibilities for cancer therapy. Two mechanisms of gene silencing, promoter hypermethylation and histone deacetylation, appear to be interrelated. The utility of targeting DNA hypermethylation and histone deacetylation is being explored clinically. Agents shown to inhibit histone deacetylase in vitro include sodium phenylbutyrate, depsipeptide, hybrid polar compounds,[39] and MS-27-275.[40] Hypomethylating agents include 5-azacytidine and 5-aza-2-deoxycytidine. The exploration of these agents in the clinic, either alone or in combination with retinoids, demethylation agents, and chemotherapeutic agents, is a novel and promising area of cancer therapeutics.

Hypomethylating agents. 5-Azacytidine and 5-aza-2-′deoxycytidine are pyrimidine analogs that have been extensively evaluated in patients with MDS. These compounds are metabolized intracellularly to triphosphates and subsequently incorporated into newly synthesized DNA, where they directly inhibit DNA synthesis and inhibit the activity of DNA methyltransferase, the enzyme required for 5′-cytosine methylation of cytosine-

guanosine (CpG) dinucleotides.[41-45] As a result, cytosine methylation is blocked in newly replicated DNA, but not in the DNA of resting or nondividing cells. Inhibition of methylation by 5-azacytidine and decitabine is associated with transcription of genes previously silenced by methylation of promoter region CpG-rich islands, and with cellular phenotypic changes; these effects can occur at concentrations that are too low to inhibit DNA synthesis directly or to cause substantial cytotoxicity.[41,43-45] The potential application of 5-azacytidine and decitabine as inhibitors of DNA methylation and inducers of cell differentiation of normal and neoplastic hematopoietic progenitor cells is an area of active investigation.[43,45-48]

5-Azacytidine initially demonstrated activity in AML,[49-53] but with considerable toxicity at doses required for response. Since the drug also induces in vitro cellular differentiation in association with hypomethylation of DNA, it was of interest for study in MDS. Chitambar et al[54] used a relatively low dose (10 to 35 mg/m^2/d for 14 days) to treat 13 patients, three of whom achieved a partial response. Cancer and Leukemia Group B (CALGB) investigators[55] conducted a phase II trial of 5-azacytidine at 75 mg/m^2/d by continuous infusion for 7 days every 28 days in 48 patients with MDS and noted 11% complete remissions and 25% partial remissions. Major toxicities included nausea and vomiting; one patient died of neutropenic sepsis. Subcutaneous administration resulted in slightly lower response rates—7% complete remissions, 17% partial remissions, and 14% with trilineage improvement, but less than a partial response.[56] These findings are similar to those achieved with low-dose cytarabine.

The CALGB recently reported the preliminary results of a phase III randomized trial of 5-azacytidine versus observation in 191 patients with MDS.[57] The patients were stratified by FAB subtype (19% RA, 4% RARS, 42% RAEB, 21% RAEB-T, 6% CMML); patients with RA or RARS had, in addition, symptomatic cytopenias. 5-Azacytidine was administered subcutaneously at a dose of 75 mg/m^2/d for 7 days every 4 weeks for four cycles. Patients on the observation arm could receive 5-azacytidine upon progression. Hematologic responses were significantly higher in patients randomized to receive 5-azacytidine compared with observation ($P < .0001$): 63% (6% complete response, 10% partial response, and 47% improvement) versus 7% (all improvement, no complete or partial responses). The median time to leukemic transformation or death was 22 months for patients on the treatment arm, compared with 12 months for the patients randomized to observation ($P = .0034$). The 12- and 24-month overall survival rate was higher in patients randomized to receive azacytidine (70% and 41% versus 62% and 25%, respectively), as was the median survival time (18 versus 14 months), but the differences were not yet significant. Treatment with 5-azacytidine was associated with subjective improvement in quality of life as measured by fatigue, dyspnea, physical functioning, positive affect, and psychologic distress.[58] Whether 5-azacytidine improves overall survival or reduces transformation to leukemia will require additional follow-up evaluation.

5-Aza-2′deoxycytidine (decitabine) is another hypomethylating agent with potent in vitro activity. In earlier studies, decitabine administered as an intermittent intravenous infusion achieved brief responses in a small series of patients with MDS; however, the majority experienced life-threatening neutropenia and/or thrombocytopenia.[59] Wijermans et al[60] reviewed the experience with this agent in MDS and found a 54% response rate of 29 elderly patients, although there were 17% toxic deaths. This drug is under development for MDS both in Europe and the United States.[46,60]

Histone deacetylation and DNA hypermethylation. Retinoids, other hormone receptors, and the Myc/Mad/Max network of growth regulators exert their effects on gene expression by interacting with nuclear corepressor complexes that are present on the DNA of promoter regions.[61,62] Gene silencing occurs with the recruitment of histone deacetylases and the formation of a nuclear corepressor-histone deacetylase complex (NCHDC). Histone deacetylase catalyzes the removal of acetyl groups from histone proteins, inducing a conformation change that results in an environment unfavorable to gene transcription. A NCHDC has been found to play an important role in acute promyelocytic leukemia (APL), where the NCHDC is recruited by both the PML-RARa and PLZF-RARa fusion proteins, which form as a consequence of chromosomal translocations t(15;17) and t(11;17), respectively.[63-67] A NCHDC is also recruited by ETO, a component of the fusion product resulting from the t(8;21) chromosomal translocation in AML.[68,69] Moreover, inhibitors of histone deacetylase have

been found to overcome transcriptional repression and to potentiate retinoid-induced differentiation of APL and AML cells.[63-66,69] A clinical test of this observation was performed in a patient with APL who had become refractory to both chemotherapy and all-*trans* retinoic acid (ATRA). Administration of both ATRA and a histone deacetylase inhibitor, sodium phenylbutyrate (see below), resulted in a complete remission. The clinical response was associated with acetylation of histone proteins in the leukemic cells.[70]

While methylation of CpG islands in gene promoter regions has long been known to be associated with gene silencing, it was not known how such DNA hypermethylation exerts its effect on gene transcription. Recent studies have shed light on both the role of DNA hypermethylation in the inactivation of tumor suppressor genes, as well as the mechanism of transcriptional repression. Examples of genes associated with CpG hypermethylation include, among others, *RB* in retinoblastoma, *VHL* (the von Hippel-Lindau gene) in renal carcinoma, *p16INK4A* and *p15INK4A* (cyclin-dependent kinase inhibitors) in solid tumors and in hematologic malignancies, and *hMLH1* (a DNA mismatch repair gene) in colon cancer.[71] The mechanism of gene silencing by DNA hypermethylation now appears to involve the recruitment of a NCHDC by the methyl-CpG-binding protein, MeCP2.[72,73] In fact, the combined administration of a demethylating agent and a histone deacetylase inhibitor has been shown to synergize in reactivating genes that were silenced in cancer cells.[74] This finding not only links the processes of DNA hypermethylation and histone deacetylation, but also presents therapeutic targets for agents that are relatively nontoxic, or used at nontoxic doses.

Phenylbutyrate

Phenylbutyrate (PB) is a low–molecular-weight phenyl-fatty acid that been used clinically to treat hyperammonemia in children with inborn errors of urea synthesis.[75] It also been shown to enhance fetal hemoglobin production in some patients with hemoglobinopathies.[76] A number of mechanisms have been proposed for the antitumor effect of PB, including (1) elimination of glutamine necessary for nucleic acid and protein synthesis in rapidly growing normal and tumor cells[77,78]; (2) inhibition of the mevalonate pathway of cholesterol synthesis leading to interference of post-translational processing of proteins, modification of lipid metabolism, inhibition of protein isoprenylation, and regulation of gene expression through DNA hypomethylation[79,80]; (3) activation of a peroxisome proliferator-activated receptor by PB, a transcriptional factor regulating lipid metabolism and cell growth[81]; and (4) regulation of gene expression through histone hyperacetylation via inhibition of nuclear histone deacetylases.[82-84]

PB has been shown to induce differentiation, tumor cytostasis, and reversion of malignant phenotype in several in vitro models.[80,85-89] PB, as a histone deacetylase inhibitor, may have synergistic activity with ATRA in the treatment of APL.[65,67,90] The PML-RAR fusion protein was shown to recruit a transcriptional corepressor complex that includes a histone deacetylase. ATRA alone could partially dissociate the complex, allowing increased transcription, but butyrate (or other inhibitors of histone deacetylases) in combination with ATRA was able to completely abrogate the inhibition of transcription. In light of these observations, an APL patient who experienced multiple relapses after ATRA treatment was treated with PB in combination with ATRA under compassionate release, and achieved a complete remission.[70]

Depsipeptide

Depsipeptide (NSC 630176) is a bicyclic peptide originally isolated from *Chromobacterium violaceum*, strain 968, by Fujisawa Pharmaceutical Co (Osaka, Japan). In the original observations, depsipeptide selectively decreased the mRNA expression of the c-*myc* oncogene and inhibited the growth of the Ha-*ras*–transformed NIH3T3 clonal cell line, Ras-1, but had no effect on Ha-*ras* mRNA expression.[91] It did not affect DNA synthesis, but caused cell cycle arrest at G_0/G_1. Recently, it has been shown to be a histone deacetylase inhibitor.[92]

Byrd et al demonstrated that incubation of chronic lymphocytic leukemia cells with depsipeptide resulted in an alteration in apoptosis-associated proteins: an increase in *Bax* with no change in *Bcl-2*, and a decrease in *p27* expression.[93]

In collaboration with Fujisawa Pharmaceutical Co, the National Cancer Institute (NCI) is currently sponsoring two phase I trials of depsipeptide administered as a 4-hour intravenous infusion. In one trial, a once-weekly infusion schedule (days 1,

8, and 15 every 28 days) is used, while the other trial evaluates a twice-weekly (days 1 and 5 every 21 days) schedule.

MS-27-275

MS-27-275 is a benzamide derivative that was synthesized by Mitsui Pharmaceuticals (Tokyo, Japan) in a search for novel antitumor agents.[40] The compound was found to have histone deacetylase activity in vitro at micromolar concentrations. In addition, when administered orally, MS-27-275 inhibited the growth of a number of tumor xenografts. The NCI, in collaboration with Mitsui Pharmaceuticals, plans to sponsor phase I trials of this agent in the near future.

Hybrid Polar Compounds

Hexamethylene bisacetamide (HMBA) was the first of the class of hybrid polar compounds to be evaluated as an antitumor agent in MDS and AML. The limited clinical activity that was observed was attributed to the inability to achieve the plasma concentrations that were required to induce differentiation in cells in vitro and to dose-limiting thrombocytopenia.[94] Subsequently, Richon et al[39] described compounds structurally related to HMBA, but which exhibited 3-log greater potency in inducing terminal differentiation and apoptosis in transformed cell lines. In addition, these compounds possess histone deacetylase inhibitory activity at micromolar concentrations. Recently, one such compound, M-carboxycinnamic acid bishydroxamide (CBHA), was found to induce apoptosis in human neuroblastoma, and the effect was associated with CD95/CD95 ligand expression by the tumor cells.[95] These agents should be entering into early clinical trials in the near future.

Arsenic Trioxide

Arsenic was used as a medicinal 2,400 years ago in the time of the ancient Greeks and Romans. Paul Ehrlich used organic arsenicals for the treatment of syphilis. Arsenicals are still included as ingredients in folk remedies of some cultures, particularly in China and other parts of Asia. Arsenic was widely used to treat syphilis before the advent of penicillin, and the organic arsenical melarsoprol is a recognized treatment for the meningoencephalitic stage of African trypanosomiasis.[96] Fowler's solution (1% arsenic trioxide in potassium bicarbonate), formulated in the 18th century to treat a variety of infectious and neoplastic disorders, was reported by US physicians in the 1930s to be useful in the treatment of chronic myelogenous leukemia (CML), and more recently by hematologists in China to treat various forms of leukemia, including CML.[97]

Recent interest in the development of arsenic trioxide as an anticancer agent emanates from reports by Chinese investigators[98,99] of its efficacy in the treatment of APL. These favorable results in APL were confirmed in the United States by investigators at Memorial Sloan-Kettering Cancer Center (MSKCC).[100]

Preclinical studies have shown that human APL cells are very sensitive to the growth-inhibitory and cytotoxic effects of arsenic trioxide.[101,102] Sensitivity to arsenic trioxide in vitro has also been demonstrated against a variety of other tumor types, including those derived from myeloid leukemias other than PML,[101] myeloma,[103] lymphoid leukemia and lymphoma,[104-107] prostate cancer,[108] and a various other solid tumors.[109]

The mechanism of antitumor activity of arsenic trioxide is not understood, but it appears to depend to some degree on dose and tumor type. The principal target in APL cells is the promyelocytic leukemia gene-retinoic acid receptor-alpha fusion transcript (PML/RAR-α).[102,110] Arsenic trioxide causes the degradation of the PML/RAR-α and wild-type PML proteins, thus inhibiting their affect on growth and differentiation.[102,111,112] Unlike ATRA, arsenic trioxide does not downregulate wild-type RAR-α.[102] In NB4 cells, a human APL cell line with t(15;17) and the PML/RAR-α fusion protein, the effects of arsenic trioxide treatment are dose-dependent[102]: induction of partial (nonterminal) differentiation at relatively low concentrations (0.1 to 0.5 μmol/L) with predominantly apoptosis at higher concentrations (0.5 to 2 μmol/L). However, induction of apoptosis by arsenic trioxide involves mechanisms other than modulation of PML or PML/RAR-α.[101] For example, growth inhibition and apoptosis induced by arsenic trioxide has been associated with downregulation of *bcl-2* expression in APL and other myeloid cell lines.[101,102] Arsenic trioxide is known to react with sulfhydryl (SH) groups and thus alter many different enzyme systems, including those that affect protein tyrosine phosphorylation.[113,114] The antitumor properties of arsenic trioxide have been attrib-

uted, at least in part, to effects on mitochondria.[105,115,116] Studies with isolated systems suggest that arsenic trioxide induces apoptosis by directly influencing the mitochondrial permeability transition pore, which can be inhibited by *Bcl-2*, an endogenous antagonist of permeability transition pore function.[116] Mitochondrial transmembrane potential collapse has been demonstrated in malignant lymphocytic cells undergoing arsenic trioxide–induced apoptosis.[105] Cells become more sensitive to arsenic trioxide when combined with inhibitors of glutathione (GSH) synthesis, including ascorbic acid.[105,117] Additional data suggest that induction of mitotic arrest and apoptosis by arsenic trioxide is related to the binding of this trivalent arsenical to two cysteine residues in tubulin, thus blocking the guanosine triphosphate (GTP) binding site and leading to disruption of microtubule formation during mitosis.[118]

Most clinical experience with arsenic trioxide comes from trials conducted in China and the United States in patients with refractory or relapsed APL.[98-100,119,120] In a study by Shen et al,[99] arsenic trioxide was administered intravenously at a fixed dose of 10 mg/d, either alone or in combination with other chemotherapy. Nine of 10 patients treated with arsenic alone and all five of the remaining patients treated with a combination regimen achieved complete hematologic remissions. Niu et al[120] recently reported the results of arsenic trioxide treatment in 58 patients with APL. Clinical complete remission was obtained in eight of 11 patients (72.7%) with newly diagnosed APL and 40 of 47 patients (85.1%) with relapsed disease. In a pilot study conducted at MSKCC in the United States,[100] arsenic trioxide was administered intravenously over 2 to 4 hours at doses ranging from 0.06 to 0.2 mg/kg/d; the initial course of treatment was continued until the bone marrow was morphologically clear of leukemia. The median duration of induction therapy was 33 days (range, 12 to 39 days), with one to five courses administered to responding patients. A complete hematologic remission was achieved in 11 of the 12 patients. The median duration of remission was 5 months (range, 1 to >9 months). In eight patients, the bone marrow tested negative by reverse-transcription polymerase chain reaction (RT-PCR) for PML/RAR-α after two courses of therapy. The three patients whose bone marrow remained RT-PCR–positive relapsed during the second course of treatment. None of the patients whose bone marrows were RT-PCR–negative relapsed at the time of the report. The results of this single-institution study are being confirmed in a multicenter trial.[121] A dose-finding phase I study of arsenic trioxide is being conducted by investigators at MSKCC to determine the recommended phase II dose in hematologic malignancies other than APL.[121]

Major toxicities attributed to arsenic trioxide involve a variety of organ systems. Skin changes are common, including dryness, hyperkeratosis, pruritus, rash, erythema, and hyperpigmentation. Gastrointestinal toxicity includes nausea, vomiting, abdominal pain, anorexia, and stomatitis. Renal and hepatic toxicity and hyperglycemia are also observed. Patients can experience a variety of constitutional symptoms and muscular skeletal complaints, such as lassitude, fatigue, weight gain, arthralgia, bone pain, myalgia, toothache, and headache. Cardiovascular and neurologic complications may be a particular concern in patients who receive more than a few courses of therapy.[119] Neurotoxicity includes peripheral motor or sensory neuropathy and seizures. Some patients experience lightheadedness, dizziness, and hypotension during the 1- or 2-hour infusion, but this is usually alleviated by extending the infusion to 4 hours. Other cardiac effects that have been reported include second-degree heart block, prolongation of the QT interval, and torsades des pointes; patients exposed to amphotericin B, or those with hypokalemia or hypomagnesemia for any reason, may be at higher risk for these cardiac abnormalities.[100]

Of interest is that patients with APL treated with arsenic trioxide can develop fluid retention and a syndrome similar to the "retinoic acid syndrome,"[122,123] with fever, fluid retention, weight gain, dyspnea, pneumonitis, and leukocytosis. Signs or symptoms of the syndrome are usually manifested within 1 to 3 weeks of initiating therapy.[122] The syndrome can be effectively treated with high doses of dexamethasone without the need to interrupt arsenic administration.

Based on the favorable clinical results in APL and promising preclinical data suggesting a broader range of activity, the NCI will further develop arsenic trioxide as an anticancer agent in patients with variety of hematologic malignancies, including MDS.

Protein Kinase C Inhibitors

UCN-01 (NSC 638850). UCN-01 or (3α,9β,10α,11α,13β)-(+)-2,3,10,11,12,13-hexahydro-3-hydroxy-10- methoxy-9-methyl-11-(methylamino)-9,13-epoxy-1H,9H-diindolo[1,2,3- *gh*:3′,2′1′-*im*]-pyrrolo[3,4- *j*] [1,7]benzodiazonin-1-one, is the 7-hydroxy analog of staurosporine. It is a fermentation product isolated from the culture broth of a *Streptomyces* species. UCN-01 was originally identified as a selective inhibitor of calcium and phospholipid-dependent protein kinase C (PKC).[124-127] Subsequently, it has been found to inhibit a number of serine/threonine kinases in a concentration dependent manner that results in arrest of cells in G_1 and abrogation of the G_2/M checkpoint. It was selected for clinical development because of its potent in vitro and in vivo antiproliferative activity, as well as for its novel mechanism of action.

UCN-01 directly inhibits of a number of other protein kinases important for orderly progression through the G_1 and G_2 phases of the cell cycle. UCN-01–induced G_1 arrest is associated with the accumulation of the dephosphorylated pRb, reduction in expression of cyclin A, and the induction of the CDK-inhibiting proteins *p21* and *p27*.[128] The G_2/M transition is regulated by Cdc2, which is activated by the phosphorylation of threonine residue 161[129] and inactivated by phosphorylations of threonine-14 and tyrosine-15.[130,131] UCN-01 abrogates G_2 checkpoint by inhibiting Chk-1 kinase. The downstream effect of inhibiting Chk-1 is loss of inhibitory phosphorylation of Cdc-2. Persistent activation of Cdc-2 results in progression through G_2 to M.[132]

UCN-01 demonstrated cytotoxic effects in vitro and against a variety of murine and human malignant cell lines in vivo. Initial screening studies in the NCI human tumor cell line screen suggested preferential inhibition of renal, CNS, lung, and leukemia cell lines. In vitro, UCN-01 at low micromolar concentrations can induce apoptosis K562 and HL60 leukemia cells lacking functional *p53* and resistant to apoptosis induced by DNA-damaging agents.[133] Experiments assessing treatment schedule suggested extended durations of exposure to UCN-01 resulted in a more prolonged antitumor response.[134] UCN-01 has also been shown to enhance antitumor effects of several chemotherapeutic cancer agents, including cytarabine and fludarabine, as well as 5-fluorouracil, gemcitabine, the camptothecins, and cisplatin, in vitro and in vivo.[133,135-138] UCN-01 can circumvent resistance of *bcl-2*–overexpressing leukemic cells to cytarabine-induced apoptosis.[139] These results suggested that UCN-01–induced abrogation of G_2 checkpoint prevents repair of DNA damage induced by standard therapies.

Phase I studies evaluating schedules of 72 hours and 3-hour intravenous infusions were initiated in the United States and in Japan, respectively. Although drug accumulation was not noted in the animal studies, pharmacokinetic data from the first patients administered UCN-01 revealed a very long half-life of several weeks, extremely small volume of distribution at steady-state, and a very low systemic clearance, in contrast to the large distribution volume and rapid systemic clearance in animal models.[140-142] Subsequent studies showed that UCN-01 binds tightly to human α-acid glycoprotein (hAGP); this tight binding alters the clearance of UCN-01, producing prolonged exposures after a single dose.[143] To prevent drug accumulation, doses after the first cycle were reduced by half in both phase I studies.

To date, only the NCI study has been completed. The highest dose reached was 53 mg/m^2/24 h × 72 hours (×36 hours for cycles ≥2) every 4 weeks.[144] Dose-limiting toxicities were transient and usually asymptomatic hypoxia, self-limited hyperglycemia, lactic acidosis with hyperglycemia, nausea/vomiting, and transient elevation of liver transaminases. Other frequently observed toxicities were transient, asymptomatic hypotension, headache, fatigue, anemia, fever, myalgia, and anorexia. With a two-compartment model fitted to the observed plasma concentrations, the median value for apparent volume of the central compartment (Vc) was 2.6 L (range, 0.1 to 9.1 L), clearance was 0.01406 L/h (range, 0.001 to 0.04 L/h), and the terminal half-life was 574 hours (range, 199 to 4,099 hours). Because of extensive plasma protein binding, salivary drug concentrations were measured as a surrogate for free plasma concentrations. The median salivary "free" UCN-01 concentration at the maximum tolerated dose was 45 nmol/L (range, 29 to 182 nmol/L), which is associated with G_2 checkpoint abrogation.

Given the prolonged half-life, phase I studies of UCN-01 on 1- and 3-hour infusions every 3 to 4 weeks have been initiated. Preclinical studies sug-

gest that the potential benefit is greatest when UCN-01 is combined with DNA damaging and S-phase–specific agents. Given the evidence of activity as a single agent and in combination with antimetabolites and nucleoside analogs, studies assessing the activity of UCN-01 in combination with cytarabine in RAEB/RAEB-T and AML and fludarabine in lymphoproliferative disorders are planned.

Dolastatin-10. Dolastatin-10 is a naturally occurring peptide isolated from the marine mollusk *Dolabella auricularia*. It is a linear pentapeptide (molecular weight 785) with four of its five subunits derived from modified amino acids.[145] This antimitotic agent has potent cytotoxic activity and causes cells to accumulate in metaphase arrest. Dolastatin-10 binds tubulin and inhibits microtubule assembly and tubulin-dependent GTP binding, and noncompetitively interferes with vincristine and vinblastine binding to tubulin.[146,147] Examination of configurational isomers and peptide segments of dolastatin-10 indicated that the middle amino acid residue, dolaisoleucine, was most critical for interaction with tubulin and cytotoxic effect.[148] Studies with radiolabeled dolastatin-10 established tight binding of the peptide to tubulin and substantial accumulation of dolastatin-10, in comparison to vinca alkaloids. The level of intracellular accumulation correlated with cytotoxic potency.[149] The drug is modulated by the multidrug resistance (*MDR*) gene product.[149]

Dolastatin-10 is one of the most potent in vitro cytotoxic anticancer compounds, with a 50% inhibitory concentration (IC_{50}) for P388 murine leukemia cells of 5×10^{-11} mol/L. Complete synthesis of the natural product permitted extensive preclinical assessment. Total growth inhibition of sensitive cell lines in the NCI cell line screen was noted at 0.1 to 1.0 nmol/L of drug. The compound's antitumor activity has been established against human leukemia and lymphoma cell lines and against solid tumors, including melanoma, prostate cancer, and small cell lung cancer cells, in a variety of in vitro assays.[150-155] Maximal cytotoxic effect was time-dependent up to 8 hours of exposure. The growth inhibitory activity of dolastatin-10 for a lymphoma cell line could be potentiated by pretreatment with bryostatin 1, a PKC inhibitor.[156] Dolastatin-10 associated growth inhibition also occurs at drug levels that are inadequate for microtubule disruption; like other tubulin-binding compounds, cytotoxic effects of the compound may be related to modulation of apoptosis-associated proteins. Tumor cells exposed to dolastatin-10 undergo apoptosis and the compound induces phosphorylation of the antiapoptotic *bcl-2* protein.[157,158] Expression of *bcl-2* and other antiapoptotic proteins (eg, *Bcl-XL, Bag-1* and *XIAP*) decreased in 30% to 60% of cells from AML patients being treated with dolastatin-10, while comparable increases in proapoptotic family members *Bcl-XS* and *Bax* were measured.[158]

In vivo studies of dolastatin-10 administered by several routes (intraperitoneally, intravenously, and orally) in the treatment of early- and late-stage human xenograft models established that dolastatin-10 produced growth delays and complete regressions of subcutaneously implanted LOX melanomas and NCI-H522 non-small cell lung cancers cell lines and the intraperitoneally administered promyelocytic leukemia cell line HL-60. Single-dose bolus scheduling was most effective when compared with multiple-dosing administration.[159] Because of extensive protein binding and rapid metabolism of the compound, a critical threshold level of free drug was necessary for maximal antitumor efficacy. The maximal antitumor effect was achieved when plasma levels between 1 and 10 ng/mL could be maintained for 5 to 8 hours. Pharmacokinetic studies in CD2F1 mice indicated that the drug had a half-life of approximately 5.6 hours.[160] Preclinical toxicology studies established that myelosuppression was the dose-limiting toxicity following single-dose administration in CD2F1 mice, Fischer-341 rats, and beagle dogs. Mice were the least sensitive species. The cytotoxic effects of dolastatin-10 were examined in cultures of hematopoietic progenitor cells and these studies confirmed that mouse cells were almost 1,000-fold less sensitive than canine and human marrow progenitor cells.[161]

Dolastatin-10 phase I studies of patients with solid tumors have been conducted at M.D. Anderson Cancer Center (MDACC) and the Mayo Clinic to assess an intravenous bolus administration every-3-week schedule. Maximal tolerated doses of 300 and 400 $\mu g/m^2$, respectively, were identified and appeared to correlate with the extent of prior cytotoxic therapy. Reversible myelosuppression was the dose-limiting toxicity. A median time to granulocyte nadir of 19 days was noted, with recovery usually within 3 to 8 days.

Extramedullary toxicities, including phlebitis, fatigue, diarrhea, and neurosensory changes, were infrequent and relatively mild. No objective responses were noted.[162,163] Three compartment pharmacokinetic modeling established a γ half-life of 16 to 19 hours and plasma clearance rates that were relatively slow (range, 0.82 to 12.63 L/h/m) and characterized by high intrapatient and interpatient variability. Pharmacodynamic assessment identified a correlation between dolastatin-10 area under the curve (AUC) and decline in neutrophil count.[164] The MDACC phase I trial also evaluated the potential for dolastatin-10 dose escalation with cytokine support, with the objective of maximizing the duration that plasma levels exceeded 1 ng/mL. Patients managed with prophylactic G-CSF received dose escalations starting at 300 $\mu g/m^2$ and were able to receive up to 660 $\mu g/m^2$ of dolastatin. Higher doses were associated with development of dose-limiting thrombocytopenia and granulocytopenia, but there were no significant nonhematologic toxicities observed during the trial. Objective responses were not observed in any of the 19 patients treated. Pharmacokinetic analysis from this trial confirmed that plasma levels of dolastatin-10 above the targeted 1 ng/mL could be maintained for greater than 30 hours in these patients.[165]

Since myelosuppression remained the dose-limiting toxicity, a phase I trial of dolastatin-10 was initiated at MDACC to establish a dose of dolastatin-10 for treatment of patients with acute leukemia. More than 16 patients with acute myeloid leukemia and two patients with acute lymphoblastic leukemia have been treated with doses ranging from 400 $\mu g/m^2$ to 1,200 $\mu g/m^2$. Patients were refractory to prior therapy or had relapsed after a median first complete response duration of 33 weeks. One patient achieved a partial remission with decrease in bone marrow blasts to 9% and platelet recovery; 60% of patients with circulating blasts (>20%) had a significant decrease in blast counts with an increase in neutrophils, and 17 of 18 patients developed at least a transient decrease in white blood cell counts. The response to dolastatin-10 appeared to correlate with *MDR* expression. Nonhematologic toxicities were minimal even at doses threefold higher than administered to solid tumor patients; drug-related toxicities reported included grade 1 hypokalemia and grade 2 nausea.[166] Continued dose escalation of dolastatin 10 is planned for this trial. The promising indications of activity may lead to further assessment of dolastatin-10 on a more frequent dosing schedule.

Faranesyl Transferase Inhibitors

The family of *Ras* genes encode 21-kd proteins that function as molecular switches that regulate diverse signaling pathways involved in cell growth, differentiation, and apoptosis. *Ras* is synthesized as an inactive cytosolic precursor molecule that achieves functional localization by integrating into the plasma membrane. The first step in the processing involves the enzyme farnesyl transferase. Therefore, farnesyl transferase inhibitors are of interest in MDS because they inhibit an enzyme required for *RAS* activation of a number of signaling pathways.[167] Several agents in this class are entering clinical development, including R115777 (Janssen Pharmaceuticals, Titusville, NJ), BMS-214662 (Bristol-Meyers-Squibb, Wallingford, CT), and LB-42908 (LG Chem, Taejon, Korea).[168,169] However, these agents may act at sites other than farnesyl transferase, such as those involving geranylgeranly protein transferase.[167]

R115777

R115777 (NSC 702818) is a potent nonpeptidomimetic inhibitor of farnesyl protein transferase (FPTase).[170] The antitumor activity of R115777 is possibly related to blocking the post-translational farnesylation of *Ras* proteins (H-, N-, and K-) that are frequently mutated in a variety of cancers. This farnesylation is a critical step in the process of membrane anchorage of these proteins and is an essential requirement for the activation of the Ras signaling pathway.[171]

Preclinical studies demonstrated that R115777 inhibits the growth of H-*ras*–, K-*ras*–, and N-*ras*–transformed cell lines and tumor xenografts. In vitro, R115777 inhibited the proliferation of NIH 3T3 cells transfected with the T24 H-*ras* oncogene and two human colon tumor cell lines bearing K-*ras* mutations. In vivo, R115777 significantly reduced the growth of the T24 H-*ras*–transfected 3T3 cells and the LoVo human colon and the CAPAN-2 human pancreatic tumor xenografts after oral administration.[170]

One phase I trial at the NCI using 5-day twice-daily dosing every 2 weeks revealed no dose-limiting toxicity up to the highest dose of 1,300 mg twice daily.[172] Nausea, vomiting, and

fatigue were the most common adverse events, but no ophthalmologic or hematologic toxicities were noted. Two other regimens, 21-day twice daily every 4 weeks and continuous administration for 4 weeks, are being investigated in three other ongoing phase I studies. In another ongoing phase I study of chronic twice-daily dosing, one instance of dose-limiting febrile neutropenia and thrombocytopenia was observed at the 500-mg twice-daily dose level; other adverse events included one instance of grade 3 skin hypersensitivity and one of grade 2 leukopenia.[173]

In collaboration with Janssen Pharmaceuticals, the Cancer Therapy Evaluation Program (CTEP) is planning a development program of R115777, including phase II studies in colorectal, pancreas and prostate adenocarcinoma, non-small cell lung cancer, glioblastoma, melanoma, and MDS. Phase I studies will include combination trials with topotecan, as well as trials in patients with hematologic malignancies with a leukemic phase, in which tumor cells can be easily sampled for drug effects.

Angiogenesis Inhibitors

An increasing body of data suggests that there may be a role for angiogenesis in multiple myeloma, non-Hodgkin's lymphomas, acute leukemias, and MDS.[174-181] Angiogenesis and tumor-associated neovascularization have been implicated in the pathogenesis of a number of human tumors by leading to tumor growth, invasion, and metastasis. These complex processes involve multiple steps and pathways that depend on a balance between positive and negative regulatory factors, as well as interactions among the tumor, its vasculature, and the surrounding extracellular tissue matrix. Tumors appear to remain dormant for an indefinite period of time, with their growth being controlled by apoptosis. Eventually they acquire an angiogenic phenotype, at which time progression occurs with increased tumor growth and metastasis.

Several lines of evidence suggest a potential role for antiangiogenesis agents in MDS. MDS may result from a defect in apoptosis. In other leukemia systems, angiogenesis factors such as basic fibroblast growth factor (bFGF) upregulate *bcl-2*, delaying programmed cell death.[180] Several new antiangiogenesis agents are available for clinical trials, including thalidomide and SU5416.[182-184] Deliliers et al[185] evaluated bone marrow angiogenesis in 81 patients with various subtypes of MDS, and the results were compared with 10 normal controls. They used immunostaining with anti-CD31, anti-CD34, and anti–vascular endothelial growth factor (VEGF), and examined microvessel density (MVD). MVD was significantly higher in patients than controls, but lower than in patients with AML or myeloproliferative disorders. There was a correlation between MVD and cytologic subtypes, with higher levels detected in patients with RAEB-T and CMML. Based on these data, Raza et al[184] studied thalidomide in patients with RA. They reported on 33 patients with a median age of 69 years; 20 had RA or RARS, 12 RAEB, and two CMML. Of these 20 evaluable patients, eight had either a greater than 50% reduction in red blood cell requirements or a rise in hemoglobin of $\geq$2 g/dL; four showed greater than 30,000/μL increase in platelets, and one had a neutrophil response greater than 500/μL.

Angiogenesis inhibitors have a number of potentially attractive features. They have a unique mechanism of action and are relatively without toxicity, which makes them of interest for combination regimens. Angiogenesis agents also differ from traditional chemotherapy, since they may be more cytostatic than cytotoxic.

Immunosuppressive Therapy

Several lines of evidence have suggested an immunologic contribution to the pathogenesis of MDS. As a result, drugs such as azathioprine, cyclosporine, and antithymocyte globulin (ATG) have been studied and anecdotal responses reported.[186-190] Molldrem et al[187] described 25 transfusion-dependent patients with MDS and less than 20% blasts who were successfully treated with a single course of ATG at a dose of 40 mg/kg for 4 days. Barrett et al[187,191] updated this experience with 60 MDS patients, showing that they were able to render one third independent of red blood cell transfusions, with 87% of responders free of progression at 2.5 years. Further study of this approach is warranted.

SUMMARY AND CONCLUSIONS

We have clearly moved beyond the empiric study of new chemotherapy drugs and biologic agents for treating patients with MDS. This strategy is no longer appropriate given our increasing knowledge of the molecular and immunologic

abnormalities associated with this diverse group of disorders. We have a large number of new agents that are either currently or will soon be in clinical trials that target biologic or molecular pathways. It is important to rapidly accrue patients to these studies. Unfortunately, patients often receive new investigational drugs after failing extensive prior therapy, when they are least likely to respond. Patients with MDS who are not suitable transplant candidates can be considered for treatment with a new agent as part of their initial therapy, until an effective treatment for this disease becomes available.

To identify new effective agents, standardized response criteria are needed to ensure comparable patient groups among studies and collection of complete prognostic factor information; the availability of uniform response criteria would facilitate the interpretation of data and the ability to compare results among various studies. Therefore, standardization would help us to identify new agents with promising activity. In addition, criteria provide a framework upon which to test scientific correlative studies of new biologic and immunologic insights into MDS. Recently, an international group of researchers with expertise in MDS developed a series of recommended guidelines to achieve these goals.[192]

Also critical for evaluating and clarifying outcomes of treatments and for designing trials is to effectively and prospectively stratify patient selection. Studies in MDS should provide risk-based criteria for patient entry and evaluation (ie, FAB group, or IPSS category[11]). Such stratification could include age, performance status, and prognostic risk category, such as the IPSS.

It is unlikely any of the single agents will make a major impact on patient outcome. However, failure of a single agent to induce significant benefit may not be the fault of the drug, but rather the manner in which it was tested: clinical trials evaluating a suboptimal dose or schedule may lead to an erroneous impression that a compound is inactive. Negative results in preliminary trials may lead to the premature discarding of potentially valuable agents. For example, antiangiogenic drugs are likely to be cytostatic and, therefore, may not induce traditional responses in phase II studies. Future strategies must identify the appropriate therapeutic targets and to design rational drug combinations to attack those targets with the goal of eventually curing patients with MDS.

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Editors John W. Yarbro, MD, PhD • Michael J. Mastrangelo, MD

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John C. Byrd, MD, Michael A. Caligiuri, MD,
and Michael R. Grever, MD, *Guest Editors*

Contributors

Kenneth C. Anderson • Karen K. Ballen • Pamela S. Becker
Ivan Borello • John C. Byrd • Michael A. Caligiuri • Bruce D. Cheson
Janet Dancey • Stefan Faderl • Ian W. Flinn • N. Gökbuget
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