New Approaches to the Treatment of Myelodysplasia

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ABSTRACT

The therapeutic dilemma that confronts the management of patients with myelodysplastic syndromes (MDS) is illustrated by the absence of a Food and Drug Administration-approved agent with an indication for this disease. Clinical heterogeneity and inadequate understanding of the disease pathobiology have limited progress in the development of novel therapeutics. Preclinical investigations indicate that reciprocal interaction between the malignant clone and the microenvironment serve to create a hostile milieu that reinforces ineffective blood cell production. Ineffective hemopoiesis, the hallmark of MDS, arises from impaired progenitor responsiveness to normal trophic signals and excess local generation of inhibitory cytokines, which promote accelerated apoptotic loss of progenitors and their progeny. Evidence to support this model derives from cytokine neutralization studies and the direct relationship between plasma tumor necrosis factor-α concentration and DNA oxidation and glutathione depletion in malignant CD34+ progenitors. Recent investigations indicate that angiogenic molecules generated by malignant myelomonocytic precursors represent integral diffusable signals that reinforce leukemia progenitor self-renewal while promoting the generation of proapoptotic cytokines and medullary angiogenic response. The potential for leukemia evolution is compounded by epigenetic events including methylation silencing of the p15 proto-oncogene or activating ras point mutations. Delineation of such biologic features that are central to the pathobiology of MDS provides a reliable framework for the development of novel therapeutics.

Antiangiogenic agents in clinical testing include vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors, thalidomide and related analogues, and the recombinant VEGF neutralizing antibody, bevacizumab. Agents whose actions may restore differentiation programs, such as the DNA methyltransferase inhibitors or histone deacetylase inhibitors, offer the prospect to promote effective hematopoiesis while impacting the potential for leukemia evolution. RAS farnesyl transferase inhibitors have shown encouraging preliminary results in acute myeloid leukemia and are currently under investigation in advanced MDS and chronic myelomonocytic leukemia.

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leukemia. Arsenic trioxide (ATO) interacts with a spectrum of biologic targets that may be uniquely suited to MDS. ATO is a potent inducer of apoptosis in thiol-depleted malignant progenitors and neovascular endothelium, while promoting differentiation through histone acetylation and inactivation of transcriptional corepressors. The identification of relevant biologic targets in MDS has raised expectations for the development of disease-specific therapies for MDS in the years that follow. *The Oncologist* 2002;7(suppl 1):39-49

**INTRODUCTION**

The myelodysplastic syndromes (MDS) have emerged as one of the most common hematologic malignancies affecting adults. The estimated crude incidence of MDS ranges from 3.5-12.6/100,000 per year in the U.S. However, the relative risk increases with age, reaching 15-50/100,000 per year in persons older than 70 years [1]. Although there is no national registry of patients with MDS, an estimated 15,000 to 20,000 new cases are diagnosed annually, primarily affecting individuals between 58 and 74 years of age [1]. These figures probably underestimate the true incidence of MDS, since patients with low-grade disease have a paucity of symptoms and display subtle bone marrow changes [1]. As the population of the industrialized world ages, and as physician awareness and diagnostic recognition of MDS increase, the incidence of MDS is expected to rise dramatically over the next few decades [2].

The major manifestations of MDS reflect the signature biologic feature, i.e., ineffective hematopoiesis, which arises from accelerated apoptotic death of affected multipotent hematopoietic progenitors and their progeny. Patients with MDS typically present with an unexplained anemia often accompanied by reticulocytopenia [3]. In a recent analysis, anemia was the presenting manifestation in 85% (99/117) of MDS patients [4].

No uniformly effective treatment exists for patients with MDS. Allogeneic stem cell transplantation and intensive induction chemotherapy have had limited success, particularly in the elderly, who are poor candidates for such aggressive procedures. Therefore, there is a tremendous need for novel approaches in the management of MDS. Advances in our knowledge of the pathobiology of MDS and insight into the molecular biology of the disease have led to innovative approaches to this hematologically diverse group of bone marrow malignancies. Among these treatments are DNA methylation inhibitors, aminothiols, clonal suppression with new classes of agents such as the topoisomerase I inhibitors and farnesyltransferase inhibitors (FTIs), angiogenesis inhibitors, and arsenic trioxide (ATO). This article provides a succinct overview of the pathobiology and classification of MDS and presents experimental and clinical experience to date with these new therapeutics.

**PATHOPHYSIOLOGY OF MYELODYSPLASIA**

**The Seed (Clone) and the Soil (Microenvironment)**

MDS is characterized by refractory multilineage cytopenias that result in dependence on transfusion, excessive risk of infection or hemorrhage, and heightened potential for transformation to acute myeloid leukemia (AML). Ineffective hematopoiesis results from a complex interaction between hematopoietic progenitors and the microenvironment, resulting in premature apoptotic death of progenitors and their maturing progeny. Myelodysplastic bone marrow progenitors display deficient growth of multipotent and primitive erythroid progenitors, retarded maturation capacity, and impaired responsiveness to growth factors despite normal receptor display and ligand-binding capacity [5-9]. Chronic myelomonocytic leukemia (CMML) represents the sole exception, demonstrating myeloid progenitor hypersensitivity to GM-CSF associated with activated Ras point mutations. Erythroid progenitors display both impaired phosphorylation of the signal transduction factor STAT-5 and induction of the erythroid-specific factor GATA-1, in response to erythropoietin stimulation [10]. Although normal erythroid precursors constitutively express fas receptor (CD95), receptor density is low, thereby fostering a high level of resistance to fas ligand-induced apoptosis [11-13]. In contrast, in myelodysplastic CD34+ cells, both fas receptor and ligand display are increased, permitting autocrine and paracrine activation of the cell death program [14-17]. The intensity of CD95 expression on MDS CD34+ cells inversely correlates with blast percentage, whereas fas ligand density remains preserved, indicating that with disease progression, myeloblast populations emerge that are more resistant to fas ligand-induced cell death [16, 18].

CD95 upregulation in hematopoietic progenitors is mediated in part by the actions of tumor necrosis factor-α (TNF-α) and other inflammatory cytokines [12]. Overproduction of inflammatory cytokines, such as TNF-α and interleukin-1β (IL-1β), is demonstrable in both the bone marrow microenvironment and the plasma of patients with MDS [11, 12, 19-22]. These cytokines promote the apoptotic death of long-term initiating cells as well as of primitive and committed progenitors [12]. Indeed, in vitro neutralization of TNF-α promotes the outgrowth of MDS bone marrow progenitors [23]. There is a direct correlation...
between the concentration of TNF-α in the bone marrow plasma and both nucleotide pyrimidine oxidation and depletion of cellular glutathione in CD34+ bone marrow mononuclear cells, supporting an effector role for these cytokines in the impaired hematopoiesis of MDS [22].

**VEGF and Medullary Angiogenesis in MDS**

Delineation of biologic signals responsible for the induction of proapoptotic cytokines in MDS represents a focus of active investigation. Bone marrow microvessel density (MVD) is increased in trephine biopsies, and the density directly correlates with myeloblast percentage [24]. A critical factor contributing to neoplastic angiogenesis is vascular endothelial growth factor (VEGF), a potent homodimeric peptide with biologic actions that extend to the regulation of hematopoietic stem cell development, extracellular matrix remodeling, and the generation of inflammatory cytokines [25-32]. Transcriptional regulation of VEGF is influenced by numerous upstream signals, including Ras activation, which converge upon the oxygen-sensitive, hypoxia inducible factor, HIF1α. We have shown that VEGF is overexpressed and secreted by myelomonocytic precursors in MDS and AML, which also display the high-affinity receptor, VEGFR-1 (Flt-1), whereas erythroid precursors are receptor naïve [33, 34]. This pattern of VEGF and receptor coexpression is demonstrable in central myeloblast clusters (i.e., abnormal localized immature precursors) in MDS biopsy specimens, monocyctic precursors of CMML, and AML myeloblasts.

Neutralization of VEGF inhibits leukemia colony formation (CFU-L) in advanced MDS and CMML, whereas recombinant human (rHu)-VEGF stimulates CFU-L formation in clinical specimens [33, 34]. Indeed, using receptor-competent AML cell lines as an in vitro model, we have shown that rHu-VEGF exerts direct trophic effects leading to increases in colony size and number and leukemia self-renewal [34].

Although VEGF may impart a trophic signal for myeloid elements, excessive local elaboration of VEGF may accelerate apoptotic death of receptor-naïve erythroid progenitors. In vitro observations that antibody neutralization of VEGF suppressed the generation of TNF-α and IL-1β and promoted the formation of multipotent and erythroid progenitors from MDS bone marrow mononuclear cells support this notion [33, 34]. Similarly, Broxmeyer et al. [35] showed that rHu-VEGF promotes in vitro expansion of myeloid progenitors while inhibiting the formation of erythroid bursts and multipotent progenitors. Prolonged in vivo administration of rHu-VEGF results in intense medullary hyperplasia of immature myeloid elements and arrest of erythroid maturation in animal models [36].

**Cytogenetic Abnormalities**

Clonal cytogenetic abnormalities are demonstrable in 40%-50% of patients with primary MDS and in up to 80% of patients with secondary or treatment-induced MDS [2, 3]. The specific chromosome affected and the number of chromosomal abnormalities (i.e., complexity) offer powerful prognostic information. Evolution to AML, however, may be influenced by epigenetic events. For example, methylation silencing of the proto-oncogene p15INK4b is detected in >70% of patients experiencing AML evolution [37, 38].

**Apoptosis Regulation**

Accelerated apoptosis (programmed cell death) and abnormal apoptosis regulation in hematopoietic progenitors and their progeny represent important elements of the pathobiology of MDS. In low-risk MDS, characterized by refractory cytopenias and low leukemic burden, the apoptotic index and the proliferative fraction are markedly increased, resulting in ineffective hematopoiesis and bone marrow hypercellularity [1, 18]. With progression to more advanced stages of the disease, the apoptotic index decreases, accompanied by further impairment in maturation potential with a corresponding elevation in blast percentage. A number of biologic events have been implicated in the seeming reversal in survival signal regulation; these include upregulation of the Bcl-2 and/or Bcl-XL antiapoptotic proteins, methylation silencing of the p15INK4b proto-oncogene, and downregulation of the Fas receptor. Mutations of p53 and other tumor suppressor genes are infrequent, but may contribute to leukemia clonal expansion.

**Classification and Prognosis of Myelodysplasia**

For a classification scheme to be useful, it must be easily applied, reproducible, and clinically relevant. Since 1976, MDS has been categorized by the French, American, and British (FAB) morphologic scheme into five subgroups based upon the number of ringed sideroblasts, degree of monocytosis, and the percentage of myeloblasts (Table 1) [39, 40]. While this classification has prognostic utility, owing primarily to the discrimination in blast percentage, some aspects remain controversial. These include the validity of defining CMML as a myelodysplastic disorder despite its myeloproliferative features, the wide range in survival among patients with refractory anemia with excess blasts (RAEB), and the failure to recognize a form of MDS characterized by dysplasia confined to a single cell lineage. The World Health Organization (WHO) proposed changes to the classification of neoplastic diseases of the hematopoietic system [41, 42]. The WHO reclassified proliferative CMML (i.e., leukocyte count >13,000/µl) as a disorder with mixed myelodysplastic and myeloproliferative features, and divided RAEB into two
categories using a myeloblast threshold of 10%: RAEB-I, with 5%-10% blasts, and RAEB-II, with 11%-20% blasts (Table 2) [41, 42]. The category of RAEB in transformation (RAEB-t), with 21%-30% myeloblasts, was eliminated in favor of a lower blast threshold for AML. Although this classification is not universally endorsed, it offers a more refined prognostic discrimination, as demonstrated in a recent retrospective analysis of 1,600 patients [43, 44].

A number of independent analyses have attempted to improve upon the prognostic value of the FAB classification with the creation of weighted prognostic scoring systems. These systems incorporate recognized prognostic features, such as blast percentage, biopsy features, number and severity of cytopenias, age, lactate dehydrogenase level, and cytogenetic pattern. In 1997, Greenberg et al. proposed an International Prognostic Scoring System (IPSS) for MDS [45]. Using blast percentages, karyotype, and number of cytopenias, they generated a scoring system (Table 3) that reliably estimates survival and risk of AML transformation for de novo MDS of all FAB types except CMML (Table 4). Four risk groups were identified, providing a simple and useful tool to discern prognoses that should aid in the selection of appropriate interventions for a given individual.

### Standard Care of the Patient With Myelodysplasia

Treatment options for patients with MDS are influenced by age and individual clinical and prognostic factors. Standard care of patients with MDS consists of supportive measures such as transfusions to correct anemia, administration of hematopoietic growth factors and cytokines, stem cell transplantation, and chemotherapy [46-49].

The only curative therapy demonstrated to date for patients with MDS is high-dose chemotherapy with stem cell transplantation. Younger patients with low-grade MDS (e.g., IPSS Intermediate-I), favorable cytogenetics, and an appropriately matched sibling or unrelated donor can be treated with allogeneic transplantation with expectations for cure ranging from 40%-60% [46, 47]. However, allogeneic transplantation is associated with high morbidity and mortality (30%-50%), which increases with age. An alternative investigational therapy is high-dose

### Table 1. FAB classification of myelodysplasia

<table>
<thead>
<tr>
<th>Classification</th>
<th>Mean Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade MDS*</td>
<td></td>
</tr>
<tr>
<td>Refractory anemia (RA)</td>
<td>35 months</td>
</tr>
<tr>
<td>Refractory anemia with ring sideroblasts (RARS)</td>
<td>35 months</td>
</tr>
<tr>
<td>High-grade MDS</td>
<td></td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia (CMML)</td>
<td>12 months</td>
</tr>
<tr>
<td>Refractory anemia with excess of blasts (RAEB)</td>
<td>18 months</td>
</tr>
<tr>
<td>Refractory anemia with excess of blasts in transformation (RAEB-t)</td>
<td>6 months</td>
</tr>
</tbody>
</table>

*Based on survival and <5% blasts

1≥15% ring sideroblasts

25%-20% blasts

321%-30% blasts

From Gallagher et al. [85]

### Table 2. World Health Organization classification of myelodysplasia

- Refractory anemia (RA)
- Refractory anemia with ringed sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia
- Refractory anemia with excess blasts I
- Refractory anemia with excess blasts II
- Myelodysplastic syndrome, unclassifiable
- Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality (5q- syndrome)

1CMML with leukocyte >13,000/µl was reclassified as a disorder with both myelodysplastic and myeloproliferative features. RAEB-t (MDS with 21%-30% blasts) is now reclassified as AML.

25%-10% blasts

311%-20% blasts

From Bruning et al. [42]

### Table 3. International prognostic classification of myelodysplastic syndromes

<table>
<thead>
<tr>
<th>Prognostic variable</th>
<th>Score 0</th>
<th>Score 0.5</th>
<th>Score 1.0</th>
<th>Score 1.5</th>
<th>Score 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow blasts (%)</td>
<td>&lt;5</td>
<td>5-10</td>
<td>—</td>
<td>11-20</td>
<td>21-30</td>
</tr>
<tr>
<td>Karyotype*</td>
<td>good</td>
<td>intermediate</td>
<td>poor</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cytoopenias</td>
<td>0/1</td>
<td>2/3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Good = normal, -Y, del(5q), del(20q); poor = complex (≥3 abnormalities) or chromosome 7 abnormalities; and intermediate = other karyotypic abnormalities

From Greenberg et al. [45]
chemotherapy followed by autologous stem cell transplantation [46]. Although this procedure decreases the period of posttransplantation cytopenias and eliminates the risk of graft-versus-host disease, which complicates allogeneic stem cell transplantation, the risk of relapse from graft contamination by clonal progenitor cells is pronounced. Chemotherapy as applied in AML, with cytarabine and an anthracycline, alone or in combination with other chemotherapeutic agents or hematopoietic growth factors, has been employed as a less toxic, moderately effective treatment for patients with MDS [50, 51]. Although generally not curative, this approach can suppress leukemia burden and improve cytopenias, and it may induce complete remissions in some patients [46, 50]. Patients with the greatest potential for sustained remission are young individuals with normal or more favorable cytogenetic patterns.

**NOVEL THERAPEUTIC INTERVENTIONS**

Since few treatments, other than stem cell transplantation, offer the prospect to alter the natural history of MDS, a number of novel interventions have been and remain under investigation. Promising agents include DNA methylation inhibitors, aminothiols, and agents intended to suppress the MDS clone such as topoisomerase I inhibitors and FTIs, angiogenesis inhibitors, and ATO.

### DNA Methylation Inhibitors

DNA methylation allows mammalian cells to modify gene expression epigenetically. Methylation occurs when DNA methyltransferase covalently links a methyl group to the 5-position of cytosine residues [52]. Promoter hypermethylation is the most common type of epigenetic DNA modification in human malignancies that silences gene transcription. In normal physiology, DNA methylation also serves to recruit methyl-binding proteins, promote X chromosome inactivation, facilitate imprinting, and provide nuclear protection against viral DNA insertion [53]. An increase in DNA methyltransferase activity appears universal in neoplastic cells, contributing to inactivation of tumor suppressor genes by transcriptional repression [52].

DNA methyltransferase inhibitors, such as 5-azacytidine, promote hypomethylation of DNA, leading to expression of previously silenced genes. The Cancer and Leukemia Group B reported the results of a randomized controlled trial of 5-azacytidine versus observation in 191 patients with MDS [54]. The majority of patients were elderly (median age 68 years), male (69%), and transfusion dependent (71%). Patients of all FAB subtypes with significant hematologic deficits were randomly assigned to a 16-week course of treatment with 5-azacytidine 75 mg/m²/day s.c. for 7 days in 28-day cycles × 4 (n = 99) or observation (n = 92). Patients in the observation arm were permitted to cross over to the 5-azacytidine arm after 16 weeks or if disease progression occurred.

5-azacytidine therapy produced responses in 63% of patients (47% hematologic improvement, 10% partial remission, and 6% complete response), while only 7% of patients receiving supportive measures experienced improvement (p < 0.0001). 5-azacytidine prolonged the median time to progression to AML by 10 months (12 months versus 22 months; p = 0.0034) and decreased the probability of leukemia transformation (31% versus 11%) (p = 0.003). Quality of life was significantly improved with 5-azacytidine treatment [54, 55], including symptoms such as fatigue (p = 0.001), dyspnea (p = 0.0014), physical functioning (p = 0.0002), positive affect (p = 0.0077), and psychological distress (p = 0.015) [55].

The effects of 5-azacytidine on both hematologic response and AML evolution indicate that it may have an impact on relevant methylation targets in MDS, thus emerging as the first of a new class of agents with tangible therapeutic benefit in these patients. At present, 5-azacytidine is available only for compassionate release by the National Cancer Institute, but it is being considered for orphan drug approval by the Food and Drug Administration (FDA).

### Aminothiols

Organic thiols can neutralize both the free radicals generated in tissues exposed to cytotoxic drugs as well as the reactive metabolites of those drugs [56]. Amifostine is a
phosphorylated aminothiol that was developed as a radioprotective agent at the Walter Reed Army Medical Institute [57]. Following systemic administration, the prodrug is dephosphorylated by tissue alkaline phosphatase [56]. Because the activity of alkaline phosphatase is much greater in normal tissue than in tumor tissue, active free thiol accumulates to a greater degree in nontumor tissue. These properties have allowed amifostine to be administered as a selective radioprotective and a cytoprotective agent in solid tumors [58].

In preclinical models and in vitro studies, amifostine protects primitive hematopoietic progenitors from chemotherapy-induced toxicity and stimulates hematopoiesis by promoting the formation of hematopoietic progenitors [57, 58]. In a canine model, a single dose of i.v. amifostine produced reticulocytosis and thrombocytosis, accompanied by an increase in white blood cell count and hemoglobin [57]. In vitro, amifostine stimulates the formation of primitive hematopoietic progenitors up to sevenfold [57]. The greatest activity is apparent in multipotential and erythroid progenitors, as revealed by in vitro assays for colony-forming unit-granulocyte, erythrocyte, megakaryocyte, macrophage, and BFU-E. The stimulatory effects of amifostine remain erythropoietin dependent [57].

In a phase I/II study in patients with MDS, i.v. amifostine was administered to four cohorts of patients at doses of 100, 200, or 400 mg/m² three times per week, or 740 mg/m² once weekly, for three consecutive weeks, followed by an observation period of 2 weeks [59]. Single-lineage or multilineage responses were observed in 83% of patients (15/18) treated with the three-times-per-week dose schedule. Of these patients, 78% (14/18) demonstrated a ≥50% increase in neutrophil count, and 43% (6/14) of patients with thrombocytopenia experienced a similar increment in platelet counts. One third (5/15) of the transfusion-dependent patients had decreased transfusion needs [59]. Although amifostine treatment improved blood counts in the treated population, abnormal karyotypes persisted. The number of blasts increased in three patients who enrolled with excess blasts, and evolution to AML occurred in two treated patients and persisted after withdrawal of amifostine. Patients treated with amifostine doses >200 mg/m², three times weekly, experienced dose-limiting toxicities of grade 2 nausea, vomiting, and fatigue [59]. Results of phase II trials using more stringent response criteria suggest that response rates are somewhat lower when amifostine is used as a single agent.

Recent studies investigating the potential for added benefit when used in combinations appear promising [60-63]. Amifostine has been applied in combination with other antiapoptotic strategies using pentoxifylline, ciprofloxacin, and dexamethasone for the treatment of patients with MDS [63]. Pentoxifylline is a xanthine derivative that interferes with lipid-signaling pathways used by proapoptotic cytokines (e.g., IL-1β, TNF-α, transforming growth factor-β [TGF-β]) to reduce their activity. Ciprofloxacin was added as a pharmacologic inhibitor of the hepatic metabolism of pentoxifylline, whereas dexamethasone was added to downregulate mRNA translation of TNF-α. Amifostine was administered intravenously at doses of 200, 300, and 400 mg/m², three times weekly, to cohorts of 10 patients each [63]. Twenty-nine patients completed at least 12 weeks of treatment. Patients were elderly (median age 67 years) and were classified as having refractory anemia (n = 20), refractory anemia with ringed sideroblasts (n = 3), RAEB (n = 5), or CMML (n = 1). A correlation between amifostine dose and response rate was not apparent. However, an improvement in cytopenias was observed in 76% (22/29) of the patients. In addition, 86% (19/22) of the patients showed improvements in neutrophil counts, 32% (7/22) had improvement in thrombocytopenia, and 50% (11/22) had a >50% decrease in transfusion requirements [63]. These results suggest that the combination of cytoprotection and hematopoietic stimulation with amifostine to decrease apoptosis is a fertile avenue for continued investigation in the treatment of MDS.

Clonal Suppression

**Topoisomerase Inhibitors**

Topotecan is an agent that stabilizes the topoisomerase I (Topo I)-DNA complex, thereby inhibiting cell growth and triggering apoptosis [64]. Topotecan has demonstrated activity in patients with acute leukemia [58]. Since patients with high-grade MDS have excess blasts that may approach the numbers seen in patients with AML, single-agent therapy with topotecan has been investigated in patients with RAEB, RAEB-t, and CMML [64]. Topotecan 2 mg/m² was administered by continuous infusion over 24 hours daily for 5 days every 4-6 weeks for two courses. This was followed by administration of a maximum tolerated dose of 1-2 mg/m² by continuous infusion over 24 hours daily for 5 days once every 4-8 weeks, for a maximum of 12 courses. Complete responses occurred in 31% (19/60) of patients. These responses were often accompanied by disappearance of clonal cytogenetic abnormalities.

Encouraging response rates with single-agent topotecan have fostered combination trials with other antineoplastic agents active in AML. Topotecan 1.25 mg/m²/day administered by continuous i.v. infusion daily for 5 days was combined with cytarabine 1.0 g/m²/day by infusion over 2 hours for 5 days. This treatment produced complete responses in 51% of evaluable patients (30/59) [65]. The benefit of this therapy was
significantly greater in patients with RAEB or RAEB-t than in those with CMML ($p \leq 0.05$). The benefit was equally pronounced in patients with poor prognostic features, such as adverse karyotypes and secondary MDS. Combination therapy with topotecan, fludarabine, cytarabine, and G-CSF has also produced responses in patients with high-grade MDS [66]. Although the activity of the topotecan combination appears encouraging, a recent retrospective comparison with anthracycline/cytarabine combinations in patients with advanced MDS or AML shows that the topotecan combination yields inferior response rates and remission durations [67].

**FTIs**

Another class of agents that may offer selective application in MDS is the FTI class. By targeting the Ras mitogen-activated protein kinase pathway, FTIs retard neoplastic cell proliferation [68, 69]. Evidence shows that the antineoplastic properties of FTIs additionally result from their ability to inhibit the farnesylation of signal transduction proteins other than Ras. FTIs target proteins that contain the CAAX amino acid consensus sequence motif requisite for plasma membrane insertion and activation, including Rho B and Lamins A and B [70].

Several FTIs are currently at various stages of preclinical and clinical development. Phase I and II trials are under way, testing the antineoplastic activity of several FTIs, both alone and in combination with other agents [71]. The most intensive clinical investigations involve the FTIs SCH66336 (Schering-Plough Corporation; Kenilworth, NJ) and R115777 (Janssen Pharmaceutical Products, LP; Titusville, NJ).

A dose-escalation phase I trial is being conducted with R115777 in 19 MDS patients representing all FAB subtypes who had failed $\geq 2$ prior therapies. Preliminary results have shown either hematologic improvement or partial remission in 6 of 18 (33%) evaluable patients, only two of whom harbored mutations in the ras genes [72]. Dose-limiting toxicities were observed at 900 mg administered twice daily, and included myelosuppression, fatigue, and gastrointestinal toxicity. Interim results from phase II trials evaluating the efficacy of R115777 in MDS demonstrated complete remissions in 2 of 16 evaluable patients (13%) (RAEB, $n = 1$; RAEB-t, $n = 1$) following 8 weeks of therapy at 600 mg twice daily. These results suggest that FTIs have substantial antineoplastic activity in MDS and CMML and merit further investigation [73].

**Angiogenesis Inhibitors**

As in solid tumors, angiogenic factors may contribute to the pathobiology of hematologic malignancies [34]. The effect of angiogenic factors on tumor neovascularization can be assessed by estimating MVD. This can be done using immunohistochemical staining for blood vessel antigens such as CD31 and factor VIII antigen or by the expression profile of angiogenic factors such as VEGF [34, 74]. Analysis of bone marrow MVD in 81 patients with MDS demonstrated significantly higher MVD in these patients than in controls ($p < 0.001$) [74]. MVD was higher in patients with RAEB-t and CMML, and the density correlated with blast percentage, but MVD remained significantly lower in patients with MDS than in those with AML. In normal bone marrow, immunohistochemical staining for VEGF is demonstrable in macrophages, megakaryocytes, and erythroid islands at low intensity [34]. In patients with MDS, intense expression is demonstrable in myelomonocytic precursors, particularly in areas of abnormal localization of immature precursors. Both VEGF and its receptors (VEGFR-1 and VEGFR-2) are detected in MDS myelomonocytic precursors. This suggests that neoangiogenesis in MDS is accompanied by autocrine cytokine stimulation of the leukemic clone. A pathogenic role for VEGF in high-grade MDS is supported by the finding that antibody neutralization of VEGF inhibits cell proliferation in specimens from patients with CMML and RAEB-t, whereas rHu-VEGF promotes CFU-L [34].

**SU5416 and SU6668**

Following ligation of VEGF receptors, receptor dimerization activates tyrosine kinases on the receptor’s cytoplasmic tail [75]. SU5416 and SU6668 are potent, small-molecule inhibitors of receptor tyrosine kinases whose spectrum of activity extends to receptors for stem cell factor (c-kit), platelet-derived growth factor, and other so-called type III receptors [75, 76]. Administration of SU5416 to an elderly woman in second relapse of AML restored remission after 12 weeks of therapy. With the exception of persistent moderate thrombocytopenia, peripheral blood findings were also restored to normal [75]. This response continued for more than 4 months. The remission was accompanied by restoration of the elevated MVD into the normal range over a 2-month period [75]. Phase II studies with SU5416 are nearing completion in a broad range of bone marrow malignancies.

**Thalidomide and Thalidomide Analogues**

Thalidomide represents the antiangiogenic agent with the broadest investigation in hematopoietic malignancies to date. Thalidomide displays both antiangiogenic and anti-TNF-α properties [77]. In a phase II trial of thalidomide in MDS, in which 100 mg of thalidomide was given at bedtime escalating to a maximum dose of 400 mg, 15 of 51 evaluable patients (29%) who completed 12 weeks of treatment achieved red blood cell transfusion independence or a >50% decrease in red blood cell transfusion requirements.
Responses in other lineages were less frequent [77]. The potential clinical benefit of thalidomide on erythropoiesis in MDS is currently under investigation in a national randomized, placebo-controlled trial.

Novel orally bioavailable thalidomide analogues have demonstrated 10- to 1,000-fold greater activity than the parent compound in TNF-α inhibition assays and a more potent antiangiogenic effect [78, 79]. These analogues are divided into two classes: selective cytokine inhibitory drugs, which inhibit TNF-α but do not enhance T-cell activation, and immunomodulatory drugs (IMiDs), which stimulate T-cell proliferation as well as IL-2 and interferon-γ production. Preclinical studies indicate that treatment of multiple myeloma cells with IMiDs inhibits DNA synthesis and promotes growth arrest [80]. Given the greater potency of the thalidomide analogue, the IMiDs may provide a potent and less toxic alternative to thalidomide in MDS [77].

**Arsenic Trioxide**

Arsenicals have a long history as chemotherapeutic agents for treatment of patients with hematologic diseases. In the 1900s, arsenic in the formulation of Fowler’s solution was demonstrated to have efficacy in patients with chronic myelogenous leukemia (CML) [58]. Until supplanted by radiation and modern chemotherapy, Fowler’s solution remained a first-line drug for CML. Recently, a resurgence in the use of arsenicals followed FDA approval of ATO for use in the treatment of patients with relapsed and refractory acute promyelocytic leukemia (APL). This novel agent has diverse mechanisms of action that vary by tumor type. These actions include degradation of the APL PML/RARα protein product and wild-type PML transcripts, downregulation of Bcl-2, alterations in mitochondrial membrane permeability, caspase activation, enzyme inactivation by binding to protein sulfhydryl groups, and disruption of microtubules of the mitotic spindle [58]. These activities of ATO appear to contribute to the high rate of complete responses, including molecular remissions, that are observed in patients with APL after treatment with this drug [81].

In vitro studies have shown that exposure of MDS bone marrow mononuclear cells to micromolar concentrations of ATO promotes apoptosis in short-term cultures. An apoptotic response was demonstrated in 7 of 11 patients (64%) with advanced MDS (RAEB-t, CMML, and AML-MDS), whereas pretreatment with GM-CSF increased the apoptotic index [82]. Administration of ATO to a 56-year-old woman with RAEB-t restored neutrophil counts to normal and eliminated the need for red blood cell and platelet transfusions [83]. Bone marrow examination performed at completion of the ATO treatment showed improved maturation of myeloid, erythroid, and megakaryocytic elements and a return to a normal blast percentage (<5%). Eight months after therapy was initiated, the patient remained in remission and transfusion independent. The only complication of therapy was a peripheral neuropathy that improved over time [83].

ATO has now received orphan drug designation from the FDA for the treatment of MDS. At this time, a phase II multicenter trial is ongoing to evaluate its full potential in this patient population [3]. The trial is an open-label, two-stage study in adults representing all FAB subtypes. Patients are treated in 4-week cycles consisting of ATO 0.25 mg/kg/day for 4 days per week in weeks 1 and 2 of each cycle and a treatment hiatus in weeks 3 and 4. The International Working Group Response Criteria proposed for trials in MDS will be applied to characterize responses [84]. Response analyses will be segregated into two cohorts according to risk score as calculated by the IPSS criteria. For lower-risk patients, the primary efficacy measure is the proportion of patients with hematologic improvement, whereas in higher-risk patients (IPSS Intermediate-2, High), the primary end point is the proportion of patients with partial or complete response. Among the 11 evaluable patients enrolled to date, a partial response was achieved in one high-risk patient, and stable disease has been achieved in four high-risk and seven low-risk patients. The drug has been well tolerated, and most patients, including the elderly, are continuing in the study, having received between one and six cycles of therapy. These preliminary results provide evidence for the clinical activity of ATO in patients with advanced transfusion-dependent MDS that merits further investigation.

**CONCLUSIONS**

Over the last several decades, the number of patients diagnosed with MDS has steadily increased. With the exception of allogeneic transplantation, none of the current therapeutic interventions are curative. Rather, treatment has remained primarily supportive, involving transfusions, hematologic growth factors, and antibiotics. More effective treatment options are urgently needed to prevent or delay the progression to AML and to prolong survival and improve patients’ quality of life. New and investigational agents that may help meet these goals include DNA methyltransferase inhibitors, aminothiols, topoisomerase I inhibitors (alone and in combination with other agents), FTIs, angiogenesis inhibitors, and ATO. The prospect that one or more of these agents will benefit patients with MDS is greater now than at any time in the past.

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