Targeted Therapies in Myelodysplastic Syndromes: ASH 2003 Review

Anna Candoni, Federico Silvestri, Silvia Buonamici, Donglan Li, Poloru Reddy, Naomi Galili, Giuseppina Nucifora, and Azra Raza

The myelodysplastic syndromes (MDS) continue to pose conceptual and practical conundrums because of their heterogeneity and therapeutic challenges. They are not restricted to the presence of clonal cells that are prone to excessive proliferation and premature apoptosis. In MDS the bone marrow microenvironment also is abnormal and exhibits an excess of proinflammatory cytokines, especially tumor necrosis factor (TNF), neoangiogenesis, and poorly defined immune defects. Thalidomide, a drug with anti-TNF, antiangiogenic, and immunomodulatory activities, and other agents with anti-TNF effects, such as pentoxifylline, etanercept, and infliximab, have produced hematologic improvement in 20% to 40% of patients. These agents may provide effective therapy for a subset of lower-risk MDS patients, even if the drugs target the bone marrow microenvironment predominantly. However, in higher-risk MDS patients, especially those with more than 10% blasts, it is important to eliminate abnormal cell clones; drugs used for this purpose have included arsenic trioxide, topotecan, the farnesyl transferase inhibitor tipifarnib, and demethylating agents, such as 5-azacytidine and decitabine. To increase the therapeutic index, a combination strategy may be preferable for higher-risk MDS patients, in whom the seed (clone) and the soil (bone marrow microenvironment) must be targeted simultaneously. The challenge is to recognize the subset that is likely to respond to a given drug so that patients can be preselected for therapy.

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A COLLECTION of heterogeneous clonal hematologic disorders grouped under the term myelodysplastic syndromes (MDS) continues to pose conceptual and practical conundrums. Although refractory anemia and dysplastic morphology are the hallmarks of practically every MDS case, clinical presentation, disease course, and response to therapy can vary broadly, even among patients within defined prognostic categories. In the United States, the incidence of MDS is estimated to be between 10,000 and 20,000 cases annually, and there are approximately 55,000 patients with active disease at any given time in the country. As the population ages, more and more elderly patients are being diagnosed with MDS though no approved therapy exists, justifying the urgency to identify novel therapeutic targets so that new strategies can be developed and therapy can be tailored to individual patients. Stem cell transplantation is a potentially curative option for these patients, but it is restricted to only a few because of their advanced ages.[1] In most MDS patients, the pathologic condition is not restricted to the existence of an abnormal clone of cells that is rapidly proliferating and is prone to premature apoptosis. Rather, the bone marrow microenvironment is also frequently affected by a changed cytokine milieu, neoangiogenesis, and as yet poorly understood immune defects.[2-9] Translation of these insights into novel therapies has been accomplished by the introduction of anticytokine, antiangiogenic, cytoprotective, and immunomodulatory strategies to the more conventional attempts of trying to eliminate the MDS clone of cells. In relatively benign and low-risk forms of MDS, manipulations of the bone marrow microenvironment alone—for example, with the use of thalidomide—have been successful in improving cytopenia in a subset of MDS patients.[10-12] For higher-risk patients and those with more advanced disease, eliminating the MDS clone using chemotherapy or newer agents, such as DNA methyltransferase inhibitors, farnesyl transferase inhibitors, and arsenic trioxide, have produced encouraging results.[13-16] A strategy to combine the two approaches by simultaneously targeting the MDS clone of cells and the bone marrow microenvironment has been tried, and early results suggest that this is a well-tolerated and feasible approach warranting further exploration.[17] Here, we summarize our experience at the Rush MDS Center using thalidomide

From the Section of Myeloid Diseases and MDS Center, Rush University, Chicago; and the Department of Pathology, University of Illinois, Chicago, IL.

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Address correspondence to Azra Raza, MD, Section of Myeloid Diseases and MDS Center, Rush University Medical Center, 2242 W Harrison St, Suite 108, Chicago, IL 60612.

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either alone or in combination with other agents, and we describe some novel strategies.

Identifying Novel Targets Through Biologic Insight

To understand the biologic basis for the variable cytopenias in MDS patients despite cellular bone marrow, we conducted studies of proliferation using the thymidine analogs 5-bromodeoxyuridine and 5-iododeoxyuridine. More cells were found to synthesize DNA in MDS marrow, and they cycled faster than normal cells.[9] A failing marrow could not be the reason for the paucity of cells in the blood. Using a variety of techniques, we measured the rate of apoptosis in the marrow of MDS patients and found that two thirds of the patients had evidence of excessive intramedullary apoptosis and that in one third of the patients as many as 50% of cells could be undergoing programmed cell death in the marrow at any given time.[3] An exploration of the cytokine milieu revealed the presence of a cascade of excess proinflammatory cytokines in most MDS patients. Tumor necrosis factor (TNF) appeared to be the proximal initiator of this cascade.[6,7] Studies of angiogenesis found that there was an increase in microvessel density and clear evidence of neoangiogenesis in the marrow of most MDS patients.[5] Finally, an as yet poorly defined immunologic component was demonstrated in a subset of MDS patients.[18] These biologic insights then led to the identification of the following targeted therapeutic approaches—some possibly novel and others conventional—of attempting to eliminate the MDS clone:

- Anticytokine, especially anti-TNF
- Antiangiogenic therapies
- Cytoprotection
- Immunomodulation

Anti-TNF Therapies

Thalidomide was chosen for clinical trial in MDS because this agent has anti-TNF, anti-angiogenic, and immunomodulatory properties, three effects we desired in the MDS population. In a pilot study of 83 MDS patients treated with thalidomide as a single agent, we documented 16 responses; 10 patients acquired transfusion independence.[10] In this study, most responders were in the refractory anemia (RA) or RA with ringed sideroblasts (RARS) categories, and most hematologic improvements were restricted to the erythroid series. There were some bilineage and trilineage responses, but these numbered fewer than the single lineage erythroid responses. The mechanisms of response were investigated in detail, and it appeared that although thalidomide could produce decreases in the bone marrow TNF-α levels and decreases in microvessel density, neither biologic effect was necessarily translated consistently into a clinical response.[19] Other attempts to directly neutralize the effects of TNF-α included the use of etanercept (Enbrel; Amgen, Thousand Oaks, CA) and infliximab (Remicade; Centocor Pharmaceuticals, Wyeth-Ayerst/Celltech, Malvern, PA). Etanercept is the soluble TNF-α receptor that produced improvements in absolute neutrophil count and platelet count in some MDS patients, but no improvement in the erythroid series could be documented with this approach.[12] On the other hand, the chimeric anti-TNF antibody infliximab has successfully produced a variety of responses in patients with low–Int-1 risk MDS, including multilineage responses.[20] Thalidomide was next combined with etanercept to improve the therapeutic outcome; once again, the responses were restricted to 30% to 40% of MDS patients.[21] It was then decided to test a combination of thalidomide with other agents, including topotecan for patients with Int-2–high-risk MDS,[22] and to try newer MDS agents such as gemtuzumab ozogamicin (Mylotarg; Wyeth-Ayerst/Celltech Group)[23] and the proteasome inhibitor bortezomib (Velcade; Millenium Pharmaceuticals, Waltham, PA).[24] Table 1 summarizes the results and includes clinical characteristics of the patients according to protocol.

Survival of MDS Patients Affected by Anti-TNF Therapies

An important question relates to the long-term effects of TNF suppression on the survival of MDS patients, especially of those who have responded to such therapies: Do these therapies only provide short-term palliation, or is there improvement in the survival of responders? A total of 248 MDS patients have received thalidomide either alone or in combination with other agents at the Rush MDS Center. Analysis of these data shows that the 52 responders appear to have better survival than do the nonresponders ($P = .0002$) (Fig 1).

When protocols that provided for combinations of thalidomide with other agents were compared with that using thalidomide as a single agent, it appeared that the best survival was seen in patients who received the thalidomide and etanercept combination (Fig 2).

However, it must be noted that the populations of patients in the various studies were heterogeneous. For example, 20 of 22 patients in the thalidomide + etanercept study had low–Int-1 risk disease, whereas the arsenic + thalidomide study included six patients with Int-1 disease and 13 patients with high-risk disease, and the thalidomide alone study included all French-American-British (FAB) and International

Table 1. Thalidomide Protocols (248 patients): Synthesis

Protocol No. (combination)	No. of Patients	Sex (M/F)	Age (yr) at Diagnosis*	Years From Diagnosis to Therapy*	FAB (n)	IPSS (n)	Responders (%)
200010 (thalidomide + arsenic trioxide)	28	19/9	65.3 ± 7.5 65.2 (49-81)	1.5 ± 1.6 1 (0.1-7.5)	RA (2) RARS (4) RAEB (12) RAEB-t (5) CMML (2) Unclassified (3)	Low (6) Int-1 (6) Int-2 (3) High (13)	7 (25)
2002 (thalidomide + topotecan)	45	31/14	67 ± 6.4 68 (51-79)	0.7 ± 1.2 0.3 (0.1-6)	RA (1) RARS (0) RAEB (29) RAEB-t (10) CMML (5)	Low (1) Int-1 (18) Int-2 (16) High (10)	12 (27)
2003 (thalidomide + etanercept)	26	15/11	64.2 ± 11.5 66.5 (31-82)	3.2 ± 4.1 1.9 (0.2-18.6)	RA (13) RARS (10) RAEB (3) RAEB-t (0) CMML (0)	Low (10) Int-1 (14) Int-2 (2) High (0)	6 (23)
9821 (thalidomide alone)	83	53/30	66.5 ± 9.1 67 (42-87.4)	1.9 ± 1.9 1.3 (0-9.4)	RA (36) RARS (13) RAEB (26) RAEB-t (4) CMML (4)	Low (14) Int-1 (42) Int-2 (17) High (10)	16 (19)
9914 (thalidomide + PTX + ciprofloxacin + dexamethasone)	66	42/24	67 ± 10 69 (30-86)	2 ± 2 1 (0-14)	RA (35) RARS (14) RAEB (15) RAEB-t (0) CMML (2)	Low (11) Int-1 (43) Int-2 (12) High (0)	11 (17)

*Values are mean ± SD, followed by median (range).

Abbreviations: CMML, chronic myelomonocytic leukemia; FAB, French-American-British; Int, intermediate; IPSS, International Prognostic Scoring System; RA, refractory anemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess blasts; RAEB-t, RAEB in transformation; PTX, pentoxifylline.

Prognostic Scoring System (IPSS) categories. Therefore, the better survival of the thalidomide + etanercept study participants is likely to be a result of better disease rather than an effect of treatment. Obviously, the best approach would be to compare the treated patients with a similar population of untreated persons with similar disease. In the absence of such a control population, what we can say is that, as shown in Fig 1, patients who respond to therapy tend to survive longer than patients who do not.

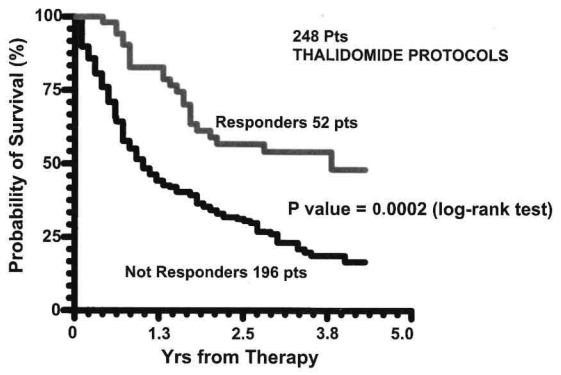

Figure 1. Survival after thalidomide therapy according to response (n = 248).

Targeting the MDS Clone and the Bone Marrow Microenvironment Simultaneously

The bone marrow microenvironment pathology in MDS is different than that in acute myeloid leukemia (AML). Because MDS is more often a chronic disease than AML, there is ample opportunity for the abnormal cell clone to affect, and to be affected by, the microenvironment. If we only focus on eliminating the MDS clone of cells, whatever few cells are left behind after treatment will inevitably enjoy a growth advantage in the abnormal cytokine milieu. Perhaps

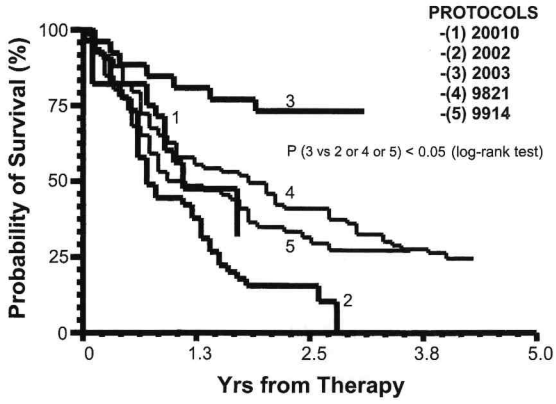

Figure 2. Survival after therapy according to protocol (n = 248). On protocol 2003 (thalidomide + etanercept), 23 of 26 (88%) patients have RA or RARS and 24 of 26 (92%) patients have IPSS low or Int-1. There was no difference in rates of response and survival when we compared thalidomide alone (protocol 9821) with other protocols, but the patients in the different protocols are heterogeneous for IPSS and FAB.

this is one reason why, in MDS patients with excess blasts, even a complete remission induced by drugs is not durable or curative. It was, therefore, proposed that a strategy combining drugs that target the MDS clone and the bone marrow microenvironment might be better than either strategy alone. We combined thalidomide (targets the bone marrow microenvironment) with arsenic trioxide (Trisenox, Cell Therapeutics, Inc., Seattle, WA; targets both the clone and the microenvironment) to treat 28 MDS patients. Arsenic trioxide alters cellular function in a variety of tissues by affecting numerous intracellular signaling pathways, and it has been shown to induce apoptosis, exert anti-angiogenic effects in vitro and in vivo, and produce remissions in a high proportion of patients with acute promyelocytic leukemia (APL).[25-29] Preliminary studies suggested that the pro-apoptotic effects of arsenic trioxide are not restricted to APL cells because patients with MDS and AML were also noted to respond, although not with the same frequency as those with APL.[26]

Arsenic Trioxide and Thalidomide

It is our contention that giving low doses of drugs for longer periods works far better in MDS than trying higher doses for shorter periods. Given the luxury of time in many patients with MDS, as opposed to those with AML, this is often possible. A lower than usual dose of thalidomide was, therefore, proposed for this study. Arsenic trioxide was administered at 0.25 mg/kg intravenously, Monday through Friday for 2 weeks followed by a 2-week rest, for six cycles along with thalidomide at 100 mg by mouth every day. Overall, therapy with the combination of arsenic trioxide and thalidomide was reasonably well tolerated; myelosuppression was the major toxicity observed.[30] No incidence of Q-T prolongation was noted, probably because magnesium and potassium levels were strictly maintained in the high normal range. Despite the combination of two potentially neurotoxic agents, there was no significant increase in the incidence or severity of peripheral neuropathy in the 28 patients treated. Seventeen patients discontinued therapy after a median of three cycles (three discontinued because of disease progression), and only one of these showed a response. On the other hand, six of 10 patients who completed the prescribed six cycles of therapy responded within a median of 3 months, suggesting the importance of longer duration of therapy. The median age on the arsenic trioxide + thalidomide study was 65 years; 19 participants were men. According to FAB classifications, two patients had RA, four RARS, 12 RA with excess blasts (RAEB), five RAEB in transformation (RAEB-t), two chronic myelomonocytic leukemia (CMML), and three unclassified disease. IPSS classifications indicated that six patients were at low risk, six Int-1, three Int-2, and 13 high risk. Adverse effects included myelosuppression, rash, neuropathy, and fluid retention, but most effects were reversible with dose and schedule modifications. Responses were assessed using the International Working Group criteria. Seven patients demonstrated a variety of hematologic responses, including one complete hematologic and cytogenetic response and one with regression in spleen size. Two trilineage responses were seen in patients with the inv(3)(q21q26.2) abnormality, which is otherwise recognized as a marker for resistance to antileukemic therapy and for short survival. This was a surprise, but it also suggested that perhaps some gene(s) on chromosome 3 directly interact with arsenic trioxide to inhibit clonal proliferation (Table 2).

This observation prompted measurement of the level of myeloid malignancy–associated proto-oncogene *EVI1* because of its location at 3q26. As two patients with a trilineage response had an inv(3) abnormality, we hypothesized a possible interaction of arsenic trioxide (with or without thalidomide) with the *EVI1* gene, which is located at that region. *EVI1* was originally described as an integration site for ecotropic retrovirus in myeloblastic leukemias of susceptible mice.[31] Proviral integration in this site activates *EVI1* expression, which encodes for a 145-kd nuclear protein with two zinc-finger repeat domains. *EVI1* is not normally expressed in hematopoietic cells, and ectopic expression blocks differentiation of early myeloid cells in response to granulocyte colony-stimulating factor.[32-35] The human *EVI1* has been mapped to chromosome 3q25-q28, and its expression has been shown to interfere with the transcription of *GATA-1* target genes, impairing respon-

Table 2. Treatment With Arsenic Trioxide and Thalidomide: Responders

FAB	Cytogenetics	Response	Duration (d)
RARS	46,XX	HI-E minor (50% PRBC)	136
RAEB	46,XY,inv(3)(q21q26.2)	Trilineage	200
RAEB	46,XY,del(5)(q15q33)	HI-E major (100% PRBC/Hb)	280
RAEB	46,XY	HI-E major (100% PRBC/Hb)	84
RAEB-t	45,X-Y,inv(3)(q21q26.2)	Trilineage	310
RAEB-t	47,XY,+8	HI-ANC	63
Unclassified	46,XY	HI-E minor (50% PRBC, spleen)	107

Abbreviations: ANC, absolute neutrophil count; FAB, French-American-British; Hb, hemoglobin; HI-E, hematologic improvement erythroid; PRBC, packed red blood cells; RARS, refractory anemia with ringed sideroblasts; RAEB, RA with excess blasts; RAEB-t, RAEB in transformation.

siveness of erythroid precursors to erythropoietin and implying its possible role in MDS pathology.[36] AML and MDS patients with translocations and inversions of chromosome band 3q26 have shown abnormal transcriptional activation of *EVI1* associated with multilineage dysplasia, atypical megakaryocytes, high platelet count, and particularly poor outcomes.[37-44] Most recently, in a group of 319 patients with AML, high *EVI1* expression was found to be an independent, poor prognostic marker for those with the intermediate karyotype, who had significantly shorter overall and event free survival.[45] Using real-time polymerase chain reaction (PCR), we detected high *EVI1* mRNA levels (expressed as the ratio of *EVI1*/ABL) in five of 28 patients on the arsenic trioxide + thalidomide protocol. Among these five patients, two died early during the course of therapy and are therefore not evaluable, whereas three patients who were able to tolerate the treatment were found to have responded. It is important to note that all three patients who had the inv(3) abnormalities had high *EVI1* expression, suggesting a consistent association between the two. Of the other two patients with high *EVI1* levels, one had an abnormal karyotype (46,XY,+1,der(1;7)(q10;p10)[20]) and the other did not, implying multiple signaling pathways through which the *EVI1* gene can be constitutively turned on (Table 3).

Of the two trilineage responders with inv(3), one died of a fungal infection after radiation treatment for carcinoma of the larynx while in partial response, and the other achieved a complete cytogenetic remission, was maintained on thalidomide for 1 year, and then taken off all treatment at the end of 12 months. He has maintained his remission and has been monitored since the end of treatment in July 2003. Interestingly, patient no. 18599 showed a decrease in spleen size from 9 cm below the costal margin before therapy to being barely palpable after 6 months of treatment, suggesting a possible future role for arsenic trioxide (with or without thalidomide) in MDS. This patient stopped taking therapy after the 6 months were completed on the protocol and has since been lost to follow-up. Based on these data, it is possible that clinical responses of MDS patients may be attributed to a previously unsuspected interaction between arsenic trioxide (with or without thalidomide) and *EVI1*. We conclude that patients with high levels of *EVI1* before therapy may be uniquely sensitive to a combination of arsenic trioxide and thalidomide. Whether *EVI1* overexpression is mediated by abnormalities of chromosome 3 or an as yet unknown mechanism, it can be a useful preselection criterion for therapy with arsenic trioxide (with or without thalidomide) if larger studies corroborate these data. The question still unanswered is whether sensitivity to this therapy is the result of the interaction of *EVI1* with arsenic trioxide or thalidomide or both. To address that issue, some in vitro experiments were undertaken to better define the consequences of high *EVI1* expression and the proliferation of cells after exposure to arsenic trioxide in vitro. It was possible

Table 3. *EVI1* mRNA Levels in MDS Patients Before Arsenic Trioxide Treatment

RCI	FAB	Cytogenetics Before Treatment	Ratio of *EVI1*/ABL × 100 Before Treatment
15311	RAEB	46,XY,inv(3)(q21q26.2)[16]/46,XY[4]	1.2740
16607	RAEB	46,XX,inv(3)(q21q26.2)[5]/45,idem,−7[15]	2.3000
16857	RAEB-t	45,X,−Y,inv(3)(q21q26.2)[18]/46,XY[2]	1.2222
17017	RAEB	46,XY,+1,der(1;7)(q10;p10)[20]	1.0392
18599	Unclassified	46,XY[20]	2.8235

Abbreviations: RAEB, refractory anemia with excess blasts; RAEB-t, RAEB in transformation.

to compare the effects of arsenic on cells that express high *EVI1* (K562 cells) with those that do not (HL-60 cells). However, the cell lines differ from each other in multiple biologic characteristics such that it would be impossible to conclude whether *EVI1* was the sole reason for any detected differential sensitivity to arsenic trioxide. Therefore, murine cells were forced to express *Evi-1*, and to be compared with those that did not so that the only difference between the two would be overexpression of a single gene, *Evi-1*, as described below.

In Vitro Studies to Determine the Effects of Arsenic Trioxide on Cells Expressing High *EVI1*

Mouse embryonic interleukin-3 (IL-3)–dependent 32Dcl3 cells were used to determine the effects of constitutive expression of *EVI1* on the sensitivity of cells to arsenic trioxide. For this purpose, the cells were infected with the retrovirus MSCV, which expresses the G418-resistance marker, or with a recombinant retrovirus that also expresses full-length human *EVI1*. The cells were cultured for 7 days without or with arsenic trioxide (0.5 and 1 μmol/L). In addition, murine bone marrow was isolated from the femurs of 4-week-old animals, and lineage-negative hematopoietic cells were enriched by negative selection.[17] After exposure to the retroviral stock, noninfected bone marrow cells were eliminated by culture with the antibiotic G418. Hematopoietic cells were cultured in methylcellulose[17] with or without the addition of arsenic trioxide (1 μmol/L). Colonies were counted under a microscope after 6 days. After colony disaggregation, the cells were counted using with the trypan blue method. During the first 3 days of culture, we did not observe a significant difference in cell number in the presence or absence of arsenic trioxide, but after 7 days of culture with 1 μmol/L arsenic trioxide, control cells were reduced to approximately 70% and *EVI1*-positive cells were reduced to approximately 33% of the cells cultured without arsenic trioxide. These assays were repeated with normal murine bone marrow cells infected with an EVI1 retrovirus or with the empty vector. After 6 days of culture, the colonies were isolated from the medium and disaggregated, and the cells were counted. Results indicated that whereas arsenic trioxide did not significantly affect the number of normal hematopoietic cells, the forced expression of *EVI1* strongly increased the sensitivity of hematopoietic cells to arsenic trioxide with a reduction to less than 20%. These data appear to suggest that *EVI1* increases the sensitivity of cells to arsenic trioxide. What role, if any, the addition of thalidomide plays in this setting remains unclear. A clinical trial is ongoing in which MDS patients in the intermediate and high-risk IPSS categories are receiving arsenic trioxide alone for six cycles, followed by the addition of thalidomide for those who have stable disease or are considered nonresponders. This study should address the role of combination versus single-agent arsenic trioxide therapy.

Therapy Directed at the MDS Clone of Cells

A number of agents, including toptecan, gemtuzumab ozogamicin, 5-azacytidine, decitabine, and the farnesyltransferase inhibitor tipifarnib (Zarnestra; Johnson & Johnson, New Brunswick, NJ), have been tested in MDS that mainly target the abnormal clone of cells.[10-16] We combined the cytotoxic agent topotecan with thalidomide and reported on the improved survival among responders. Perhaps the most exciting strategy has been the use of demethylating agents targeting the enzyme DNA methyltransferase. In a two-arm crossover study using subcutaneous 5-azacytidine,[14] it was found that 47% patients in the treatment arm experienced hematologic improvement compared with 7% in the observation arm ($P = .0001$); 6% of those experiencing improvement achieved complete remission and 10% achieved partial remission. Although no improvement in median survival could be documented between the two arms because of the crossover nature of this study, the time to progression to AML was longer for the treatment arm (22 *v* 12 months; $P = .0034$). Decitabine, an analog of 5-azacytidine, is fivefold more potent at inhibiting DNA methyltransferase and has shown response rates ranging between 30% and 50% in various phase 1 to 3 trials. Both these drugs are being extensively investigated in phase 3 trials and are expected to become accepted in the near future as treatment options for the more advanced forms of MDS.

Conclusion

The past decade has been one of increasing interest and activity devoted to better defining the pathologic basis of cytopenias in MDS and to identifying novel therapeutic targets in this disease. These efforts have met with encouraging biologic insight and have led to the evolution of several new treatment strategies, including anticytokine, antiapoptotic, antiangiogenic, and cytoprotective therapies directed at the bone marrow microenvironment. Although a number of newer therapies have produced encouraging results, the challenge for the investigator is to identify the correct drug for each patient before starting therapy.

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