Original Article

Novel Biospecific Agents for the Treatment of Myelodysplastic Syndromes

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Key Words

Myelodysplastic syndrome, biologic response modifier, thalidomide, CC-5013, farnesyltransferase inhibitor, 5-azacytidine, decitabine

Abstract

Levels of treatment for patients with myelodysplastic syndromes (MDS) fall within 3 broad categories: supportive care, low- and high-intensity therapy. Most approaches remain experimental, and supportive care remains the standard of treatment in MDS. In parallel with the growing knowledge of the multiple pathobiologic abnormalities in MDS, increasing numbers of low-intensity, biospecific agents that target these pathogenetic lesions have entered clinical trial testing. Although the term "biospecific" has been applied to many of these investigational drugs, they often exert pleiotropic effects, many of which remain to be identified. An ongoing challenge will be to more fully characterize the mechanisms of action of these drugs and to characterize biologic correlates of response. With these data in hand, it will be increasingly feasible to treat patients with combinations of biospecific drugs with non-overlapping actions and toxicities, a therapeutic approach that is likely required to effectively overcome the barriers posed by the biologic heterogeneity of MDS. This review focuses on recent therapeutic approaches using such biologic response modifiers to treat MDS. (*JNCCN* 2003;1:473–480)

Several issues require careful consideration when tailoring therapy for individuals with MDS. Disease-related factors such as cytopenia-related complications and the potential for evolution to leukemia often intersect with patient-related factors including the advanced age and frequent co-morbidities of the MDS population. The FAB (French-American-British) and International Prognostic Scoring System (IPSS) classification systems have been useful in stratifying patients into prognostic risk groups, which in turn aid the selection of either low- or high-intensity treatment approaches. In the MDS practice guidelines set forth by the NCCN, IPSS risk category, which has been shown to improve outcome analysis, is the initial factor used to assess therapeutic options. Patient age and performance status are 2 key additional determinants in the NCCN treatment algorithms for MDS. Biologic rather than chronologic features often play an important role in age-related risk, and the arbitrary threshold of 60 years partly reflects the age cut-off used for certain high-intensity treatments such as acute myelogenous leukemia (AML)-type chemotherapy or stem cell transplantation. The rational development of more biologically tailored anti-tumor agents, with increased specificity and decreased toxicity, should provide an opportunity to extend treatments to MDS patients who would have otherwise received supportive care or are not suitable candidates for intensive therapy. In the case of hematologic malignancies, sampling of the tissue of interest (bone marrow or peripheral blood) allows longitudinal study of the biologic targets of agents with diverse modes of action. Table 1 lists novel therapies for MDS, currently in clinical trials, some of which are the subject of this review.

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Thalidomide

Thalidomide's activity in multiple myeloma and MDS is explained by multiple biologic actions, including modulation of the immune system, anti-angiogenic effects, and improvement of the bone marrow microenvironment.[1,2] Thalidomide's effects on the bone marrow milieu may be mediated by several mechanisms, including enhancement of adherent stromal cell growth, and neutralization of proinflammatory and inhibitory hematopoietic cytokines.[3–5]

Table 1 Bio-Specific MDS Therapies in Clinical Trials

Compound	Mode(s) of Action	Trial Phase	Examples of Potential Biomarkers of Effect
Thalidomide (Thalomid®)	Anti-angiogenesis, anti-TNF-α, immunomodulation BM microenvironment effects	III	Bone marrow (BM) microvessel density (MVD), ΔVEGF-receptor autophosphorylation, downstream signaling, hemopoietic colony formation
CC-5013 (Revimid®)	Same as above	II	Same as above
R115777 (tipifarnib, Zarnestra™)	Farnesyltransferase inhibitor	II	*RAS* mutation status, Δ phosphorylation of downstream effectors (MAP kinase, STAT3, AKT)
Arsenic trioxide (Trisenox®)	Induction of apoptosis, cell cycle arrest, anti-angiogenesis, differentiation	II	Apoptosis assays (e.g. TUNEL, Annexin V)
Tumor necrosis factor-α fusion protein (Etanercept, Enbrel®)	Anti-TNF-α	II	Marrow / plasma TNF-α levels
5-azacytidine/decitabine	Hypomethylation/differentiation	II/III	p15 methylation status
Agents in Early Clinical Trials			
Darbepoetin-α (Aranesp)	Long-acting erythropoietin	I/II	BFU-E colony formation, apoptosis assays
ICL-670	Oral iron chelator	I/II	Liver iron content by biopsy or SQUID; serum ferritin
Anti-VEGF Antibody (Bevacizumab, Avastin™)	Anti-angiogenesis	I/II	BM MVD, apoptosis assays, hemopoietic colony formation

Abbreviations: Δ, change; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; BFU-E, burst forming unit-erythroid; SQUID, Superconducting quantum interference device.

A pilot study of thalidomide in MDS was conducted using a starting dose of 100 mg daily, allowing dose escalation up to 400 mg daily as tolerated.[6] Among the 83 patients enrolled (FAB subtypes refractory anemia [RA; 36 patients], RA with ringed sideroblasts [RARS; 13 patients], RA with excess of blasts [RAEB; 24], RAEB in transformation [RAEB-T; 6], and chronic myelomonocytic leukemia [CMML; 4]), only 51 patients completed at least 12 weeks of therapy. Discontinuation of thalidomide was attributed to drug-related side effects in 17% of individuals. Sixteen patients (19%) experienced hematologic improvement, including 10 patients who became red blood cell transfusion-independent. Dose escalation to 400 mg daily was achieved in 34 of 51 patients by one month, but only 8 patients could tolerate this higher dose for the remainder of the 12-week course because of substantial side effects, particularly in these elderly patients.

Moreno-Aspitia et al.[7] studied thalidomide in 73 MDS patients stratified into favorable (IPSS $\leq$ 1.0) and unfavorable (IPSS $\geq$ 1.5) groups. Red blood cell (RBC) or platelet transfusion dependence was present at baseline in 69% and 40% of the favorable and unfavorable groups, respectively. Thalidomide was started at 200 mg daily and increased as tolerated by 50 mg weekly to a targeted maximum dose of 1000 mg daily. A maximum dose of 300 mg daily was reached in at least one-half of patients in each group (range 200–1000 mg daily). By International Working Group (IWG) criteria, only one favorable group patient (3%) and 6 unfavorable group patients (21%) exhibited hematologic improvement or a partial response that were maintained for at least 2 months. Therapy was terminated early in most patients due to adverse reactions.

Musto et al.[8] evaluated thalidomide doses of up to 300 mg daily in 25 transfusion-dependent MDS patients (17 RA/RARS, 8 RAEB). Five patients (20%) became transfusion independent between 4 and 9 weeks. Because of lack of efficacy, 10 patients discontinued treatment after 2 months, and the remaining 10 patients discontinued thalidomide because of adverse effects.

In a more positive study of 34 MDS patients (22 RA/RARS, 4 RAEB, 5 RAEB-T, 3 CMML), a remarkably high median thalidomide dose of 400 mg daily was tolerated.[9] Four patients discontinued study within 5 weeks because of adverse events and one patient died of heart failure after 4 weeks. Hematologic

improvement was shown in 19 patients (56%) and occurred after a median of 2 months of therapy. Four patients (one each of RARS, RAEB, CMML, and RAEB-T) exhibited major responses (RBC or platelet transfusion independence), 6 showed minor responses, and partial remissions were achieved in 9 responders distributed among lower- and higher-risk IPSS groups.

Thalidomide has been combined with other agents including arsenic trioxide (Trisenox, Cell Therapeutics, Inc., Seattle, WA),[10] etanercept,[11] and darbepoetin-α.[12] The development of thromboembolic events (2 deep venous thromboses, 1 fatal pulmonary embolism) in 3 of the first 7 patients enrolled in the combination trial of thalidomide and darbepoietin-α prompted early discontinuation of the study.[12] In the trial of thalidomide plus Trisenox, 2 patients with trilineage responses had an inv(3)(q21q26.2) karyotype abnormality, a region in which the *Evi* gene is located.[10] Three of 4 patients with high pretreatment *Evi* mRNA levels (detected by real-time quantitative PCR) responded, suggesting that one or both of these agents may be particularly useful for this subgroup of patients.

In summary, thalidomide elicits erythroid responses in a proportion of patients, but its relatively unfavorable safety profile, particularly in more elderly individuals, limits its utility as a single agent in MDS.

CC-5013 (Revimid)

CC-5013 belongs to a class of thalidomide analogues referred to as immunomodulatory drugs (IMiDs). In phase I studies of CC-5013 in refractory and relapsed multiple myeloma, myelosuppression was the dose-limiting toxicity.[13] However, compared with thalidomide, CC-5013 exhibited a more favorable safety profile including less sedation, neurologic side effects, and constipation.

In preclinical studies, CC-5013 exhibited greater potency than thalidomide in several respects. These include stimulation of T-cell proliferation, production of interleukin-2 (IL-2) and interferon-gamma (IFN-γ), and suppression of tumor necrosis factor-α (TNF-α) secretion.[14,15] The clinical activity of CC-5013 in phase I and II studies of refractory multiple myeloma reflects several mechanisms of action, which were shown with either cell lines or patient cells: 1) decreased binding of myeloma cells to bone marrow stromal cells; 2) inhibition of cytokines (e.g., IL-6, vascular endothelial growth factor [VEGF], and TNF-α mediating growth of myeloma cells; 3) blockade of angiogenesis; 4) induction of G1 growth arrest and apoptosis; and 5) augmentation of host anti-myeloma natural killer cell immunity.[13,16–18]

VEGF elicits trophic effects in malignant myeloid progenitors through paracrine and autocrine pathways.[19] Molecular modeling was used to show high-affinity binding of CC-5013 to the fibroblast growth factor receptor-1 (FGFR-1) and VEGF receptor 1 and 2 through docking to the phosphorylated adenosine 5′-triphosphate (ATP) binding sites.[20] CC-5013 inhibited VEGF-induced clonogenic responses in VEGFR-1 and VEGFR-2 expressing KG1 AML cells. CC-5013 also abolished cytokine-induced AKT phosphorylation and suppressed VEGF-induced adhesion of KG1 cells to bone marrow microvessel endothelial cell layers.[20]

Preliminary results of a phase I/II study of CC-5013 for MDS patients with transfusion-dependent or symptomatic anemia were presented at the American Society of Hematology meeting in December 2002.[21] Although CC-5013 was commenced at a dose of 25 mg daily, myelosuppression led to a reduction in the dose to 10 mg daily in the initial 9 evaluable patients, and subsequent patients were treated with 10 mg daily. Six of 9 evaluable patients (66%) showed hematologic benefit, including one patient with a bilineage response (6 of 7 patients [86%] with IPSS low/intermediate-1 risk MDS). Hematologic responses included 4 patients who showed RBC transfusion-independence, one patient with a 50% decrease in RBC transfusion frequency, one patient with a 1.5 g/dL increase in hemoglobin, and one major platelet response. One partial and 2 complete cytogenetic responses were seen among 5 patients evaluable for karyotypic analysis. CC-5013 was generally well tolerated with toxicity mostly related to grade 3+ myelosuppression and grade 1-2 pruritus at the 25 mg daily dose. The high erythroid-remitting activity and favorable safety profile of CC-5013 make this ImiD a promising drug for MDS. Expanded phase II multicenter trials of CC-5013 are being initiated to further characterize erythroid responses in patients with lower-risk MDS.

Farnesyltransferase Inhibitors (FTIs)

The pathogenesis of solid and hematologic malignancies is linked to mutationally-activated oncogenic

Ras or other abnormalities of Ras signaling. Up to 30% of MDS patients show Ras mutations (usually *N-RAS*), although this frequency may be higher in the CMML subtype.[22] Through a process termed farnesylation, a 15-carbon lipid moiety is added to the carboxy-terminus of Ras, a post-translational step critical to its functional localization at the cytoplasmic plasma membrane.[23] Besides Ras, many farnesylated proteins are involved in cancer-driven pathways that are potential targets of farnesyltransferase blockade. FTI R115777 (Zarnestra, tipifarnib) is a potent inhibitor of farnesyltransferase in vitro and in vivo,[24] with activity in patients with refractory/relapsed AML.[25] In a phase I/II study of 21 MDS patients (median age 66 years, <2 prior therapies), Zarnestra was studied at a dose of 300 mg twice daily for 21 days every 28 days and dose escalated by 100 mg daily until the maximum tolerated dose was achieved.[26] A 25% response rate was observed among 20 evaluable patients, consisting of one complete response, one partial response, and three with hematologic improvement. Responses were observed among all dose levels (300–900 mg daily) and did not correlate with *RAS* mutation status. No relationship was found between clinical response/toxicity and biologic effects (e.g., changes in phosphorylation) on signaling effectors such as MAP kinase, STAT3, and the downstream anti-apoptotic cellular kinase AKT. At the 900 mg total daily dose cohort, consisting predominantly of elderly (>70 years old) patients, dose limiting toxicities were encountered. Overall, cumulative myelosuppression was the most commonly observed toxicity, with gastrointestinal side-effects and skin rash noted in several patients. The maximum tolerated dose was determined to be 400 mg twice daily. An international, open-label expanded phase II trial is currently evaluating Zarnestra in high-risk MDS patients starting at a dose of 300 mg twice daily given on the same dosing schedule.

Arsenic Trioxide

Arsenic trioxide, commercially available in the U.S. as Trisenox®, is FDA-approved for the treatment of refractory or relapsed acute promyelocytic leukemia (APL). Pre-clinical investigations of the mechanisms of action of arsenic trioxide in APL are relevant to other hematologic malignancies, particularly MDS. In APL cells, arsenic trioxide induces differentiation in part by targeting and promoting the degradation of the leukemogenic fusion protein PML-RARα. In non-APL cells, arsenic trioxide causes differentiation through histone hyperacetylation and interference with nuclear co-repressors and their transcription factor partners.[27–31]

Arsenic trioxide has been shown to induce apoptosis of APL cells by activation of caspases, down-regulating bcl-2 expression, favoring a pro-apoptotic state.[29,31] Another means by which arsenic also may induce apoptosis is through binding of microtubular proteins and interference with the mitotic apparatus.[32]

In addition to its differentiating and pro-apoptotic effects, arsenic trioxide can inhibit proliferation and angiogenesis by modulation of cytokine activity and disruption of cell cycle progression. The antiproliferative effects of arsenic have been linked to both prolongation of the cell cycle and arrest of hematopoietic cells in the G1 and G2/M phases.[33] The ability of arsenic trioxide to inhibit proliferation and angiogenesis, and promote non-terminal differentiation and apoptosis make it an appealing agent for both low- and high-risk MDS.

Preliminary results of an ongoing European multicenter phase 1/2 study of Trisenox® in MDS patients were recently reported.[34] Trisenox® was administered as a loading dose of 0.30 mg/kg/day for 5 days, followed by a maintenance dose of 0.25 mg/kg/day twice weekly. Eighteen of the 31 patients were transfusion-dependent, and abnormal cytogenetics were present in 16 of 28 patients with baseline karyotype analysis. Five patients were discontinued from study early. With Trisenox® therapy, 7 (5 high risk [HR], 2 low-risk [LR]) of the remaining 26 patients (27%) exhibited unilineage or bilineage hematologic responses. These consisted of 3 major and 1 minor erythroid, 1 major and 1 minor neutrophil, and 2 major and 1 minor platelet responses. Three of the 7 responding patients became transfusion independent and 1 had decreased transfusion requirements. Two HR patients had significant reductions in bone marrow blast counts after 2 to 4 months of therapy. Thirteen (7 HR, 6 LR) (50%) had stable disease for 1.7 to 6.8 months (median 4.6 months). Three patients transformed to AML, 2 had disease progression, and 1 died from disease-related complications. No QT prolongation was observed and the safety profile was generally favorable.

In a U.S. phase II multicenter trial, Trisenox was administered on a different schedule of 0.25 mg/kg, 5×/weekly followed by 2 weeks off. Data from 32 (16 LR and 16 HR) of the 44 patients enrolled have been

analyzed. A 34% hematologic response rate was observed among 5 HR and 6 LR patients, similar to the 27% tally of the European trial. One major and 3 minor erythroid responses, 3 major and 1 minor neutrophil responses, and 2 major platelet responses were observed. In selected patients, durable responses were noted, including >10 month remissions in 2 patients, and a remission lasting 17 months after completion of arsenic trioxide therapy. Responses were documented 31 to 141 days (median 85 days) after initiation of treatment. The most common serious non-hematologic adverse events included febrile neutropenia, diarrhea, and pneumonia.

These encouraging preliminary data from the European and U.S. trials show that arsenic trioxide exerts trilineage activity in both low- and high-risk MDS patients. Ongoing accrual will provide more data regarding cytogenetic and pathologic responses, and the impact of arsenic trioxide on the survival of patients.

Tumor Necrosis Factor Receptor-Fusion Protein

The hemopoietic dysregulation occurring in MDS is related to both abnormalities within the patients' hemopoietic stem cells and in their marrow stromal support mechanisms.[36,37] The stem cell abnormalities in MDS include altered levels of apoptosis (programmed cell death), immune dysregulation, telomere shortening and enhanced oxidative stress susceptibility. The marrow stromal derangements in this disorder that enhance apoptosis include increased paracrine production of inhibitory cytokines (e.g., TNF-α, transforming growth factor-β, fas ligand) and suboptimal production of stimulatory cytokines (granulocyte-colony stimulating factor [G-CSF], granulocyte macrophage-CSF [GM-CSF], IL-3, erythropoietin), which together diminish the survival and differentiation of these human hematopoietic stem cells (HSCs).[38–46] Such paracrine anomalies enhance intracellular activation of apoptosis-generating proteases (caspases), which underlie much of the pathogenesis of this disorder.[47]

Experimental therapeutic approaches are being aimed at blocking many of these lesions. Studies in rheumatoid arthritis patients showed clinical benefit and improvement in their associated anemias when treated with TNFR-FP.[48] In vitro investigations with MDS marrow showed that TNFR-FP resulted in significantly increased hemopoietic colony formation.[49]

To attempt to diminish the inhibitory effects of TNF-α and its associated cytopenias in MDS, 14 patients were treated in a Phase I/II trial with TNFR-FP.[50] The patients were treated with TNFR-FP 25 mg subcutaneously twice weekly for 8 weeks, followed by either continued therapy at the same dose for responders or at 3 doses/week for another 8 weeks for non-responders. All patients had previously received red blood cell or platelet transfusions (or both), and one or more treatment modalities. Overall treatment was well tolerated, although 4 patients developed antibiotic-responsive infections (2 requiring hospitalization and in whom TNFR-FP was discontinued prematurely). Among the remaining 12 patients, there were 5 responding patients: 4 had erythroid responses with improvements in hemoglobin by 1 to 1.5 g/dL or a 50% decrease in transfusion requirement; 2 of these patients also showed 54% and 73% increases in platelet counts. Two patients (1 additional to the erythroid responders) showed 63 and 120% increments in neutrophils. Except for an increase in marrow blasts from 15 to 25% in one patient, marrow blasts remained stable after treatment, and marrow cytogenetics did not change. Pre-treatment marrow plasma TNF-α levels were elevated relative to controls in all patients, but did not correlate with in vivo responses.

In another Phase I/II study using TNFR-FP, 3 of 15 MDS patients had minor hematologic responses.[51] Similar to thalidomide, these studies may reflect the impact of modifying TNF levels in MDS, one of the possible mechanisms of action of TNFR-FP.

Results of these trials provide evidence for biologic activity and some clinical efficacy of agents potentially blocking inhibitory cytokines such as TNF in patients with MDS. However, given the limited responses, these data suggest that factors additional to TNF-α inhibitory activity contribute to the development of these patients' cytopenias. Further studies are warranted using anti-TNF/anti-inhibitory cytokine approaches in combination with hemopoietic stimulatory drugs plus other agents capable of abrogating the effects of additional inhibitory mechanisms in MDS.

5-Azacytidine

As a form of low-intensity chemotherapy for MDS, encouraging data have been reported with the

hypomethylating agent 5-azacytidine (AzaC). In a randomized Phase III trial from Cancer and Leukemia Group B (CALGB), AzaC decreased the risk of leukemic transformation and, in a portion of the patients, improved their survival.[52] This trial evaluated 171 MDS patients, comparing AzaC (75 mg/m^2/d subcutaneously for 7 days every 28 days, predominantly as an outpatient) with supportive care. Patients in the supportive care arm whose disease worsened were permitted to cross-over to AzaC. Patients were stratified according to their FAB category (RA 19%, RAEB 42%, RAEB-T 21%) and symptomatic cytopenias. Responses occurred in 60% of patients on the AzaC arm (7% complete response, 16% partial response, 37% improved) compared with 5% (improved) receiving supportive care. Median time to leukemic transformation or death was 21 months for AzaC versus 13 months for supportive care. Transformation to AML occurred in 15% of patients on the AzaC arm and in 38% receiving supportive care. Eliminating the confounding effect of early cross-over to AzaC, a landmark analysis after 6 months showed a median survival of an additional 18 months for AzaC compared to 11 months for supportive care. Quality-of-life assessment found significant major advantages in physical function, symptoms, and psychological state for patients initially randomized to AzaC.[53] Thus, AzaC treatment resulted in significantly higher response rates, improved quality of life, reduced risk of leukemic transformation, and improved survival compared with supportive care. This agent appears to provide a new treatment option that is superior to supportive care for patients with MDS.

Decitabine

The related hypomethylating agent decitabine (5-aza-2′-deoxycytidine), with doses that required hospitalization of most patients, also demonstrated encouraging results for the therapy of patients with high-risk MDS, with some patients demonstrating cytogenetic conversion.[54–56] In a phase II study of 66 high-risk MDS patients, a dose of 45 mg/m^2/d was administered for 3 days every 6 weeks.[54] Patients were classified by the IPSS as intermediate-1 (24%), intermediate-2 (38%), and high-risk (38%). Myelosuppression was common, suggesting cytotoxic effects of the drug, and 5 patients (7%) died during treatment, primarily due to infection. The overall response rate was 49%, with a 64% response rate in patients with a high-risk IPSS score. The median response duration was 31 weeks, and the median survival from the start of therapy was 15 months (14 months for the high-risk IPSS patients). In 31% of patients, normalization of clonal cytogenetic abnormalities (often high-risk) was seen.

Comparison of the studies with either of these hypomethylation agents demonstrated a substantial degree of similarity in results (Table 2). Similar proportions of "good responses," AML transformation, and duration of survival were seen. Cytogenetic and molecular studies were more commonly performed and initially reported in the decitabine trials.[55,56] A higher proportion of cytogenetic responses was seen with decitabine. In the AzaC study, 36% patients developed an abnormal clone (vs. 20% in the supportive care arm).[57] In the decitabine studies, 65% patients had basal marrow cell hypermethylation that was reversed in most responders[56] Further trials with these agents are ongoing to attempt to confirm and extend these findings.

Conclusions

The data reviewed herein describe initial and current studies using biospecific therapies in an attempt to block presumed pathogenetic mechanisms in MDS. As indicated by the relatively limited disease responses to most agents, the multiple mechanisms causing the patients' cytopenias will likely require effective combinations of agents to elicit more substantial clinical

Table 2 Hypomethylation Therapy in Myelodysplastic Syndromes

Clinical Parameters	5-Azacytidine[52] Phase III, *n* = 96*	Decitabine[54] Phase II, *n* = 66
Prognostic subgroups	FAB: RAEB/RAEBT–63%	IPSS: Int-2/High–76%
Total 'good' responses	60%	49%
Median Survival	18 months	15 months
Median Response duration	18 months	8 months
AML transformation	15%	26%
Major cytogenetic response	8%[57]	31%
p15 hypomethylation	—	75%[56]

*Treatment arm patients only.

benefit. The clinical research challenges for the immediate future will be to determine the best combinations of these agents or to unearth newer, more basic biologic targets amenable to such therapy. Toward this end, clinical trials remain the mainstay of treatment for most MDS patients.

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