REVIEW

Revisiting Use of Growth Factors in Myelodysplastic Syndromes

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Abstract

Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal hematologic neoplasms characterized by morphologic dysplasia, aberrant hematopoiesis and peripheral blood refractory cytopenias. MDS is recognized to be associated with an increased risk of symptomatic anemia, infectious complications and bleeding diathesis, as well as a risk of progression to acute myeloid leukemia, particularly in patients with a high IPSS score. The advent of use of hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) and recombinant erythropoietin (EPO) has improved symptoms in MDS patients in addition to some data that suggest there might be an improvement in survival. G-CSF is an effective therapeutic option in MDS patients, and it should be considered for the management of refractory symptomatic cytopenias. G-CSF and EPO in combination can improve outcomes in appropriate MDS patients such as those with lower-risk MDS and refractory anemia with ring sideroblasts (RARS) . This article reviews use of growth factors for lower-risk MDS patients, and examines the data for G-CSF, EPO and thrombopietic growth factors (TPO) that are available or being developed as therapeutic modalities for this challenging disease.

Keywords: Myelodysplastic syndromes - anemia - G-CSF - erythropoiesis stimulating agents

Asian Pacific J Cancer Prev, **13**, 1081-1091

Introduction

The myelodysplastic syndromes (MDS) represent a diverse group of clonal hematologic neoplasms in which abnormal multipotent progenitor cells are involved. MDS is characterized by morphologic bone marrow dysplasia, aberrant hematopoiesis and peripheral blood refractory cytopenias. They are accompanied by increased risk of symptomatic anemia, infectious complications and bleeding diathesis, as well as propensity to evolve to treatment-resistant acute myeloid leukemia in 30% of patients, particularly in those with high grade MDS. It is well-recognized that immunologic dysregulation plays an important role in the pathogenesis of MDS, leading to ineffective hematopoiesis with progressive cytopenias. The common presenting symptoms include fatigue, dyspnea, bleeding and infection (Albitar et al., 2002; Niner, 2008; Tefferi et al., 2009). In the United States the epidemiological data of MDS has been collected only in the past decade, and reportable to the Surveillance, Epidemiology, and End Results (SEER) program sponsored by the National Cancer Institute since 2001. MDS is mostly a disease of older adults (median age 69 years), males are affected slightly more than females (55% vs. 45%), and whites more than blacks. Average annual age-adjusted incidence rate of MDS for 2001 was 3.3 and for 2003 was 3.6 per 100,000 which is almost equal to 10,000 new cases a year. The latest annual number of cases of MDS in US is approximately 15,000 cases that has roots in better reporting system. The annual incidence among individuals older than age 70 exceeds between 22 and 45 per 100,000 persons. Overall, MDS affects approximately 1 in 500 persons over 60 years of age, making it the most common hematologic malignancy in this age group (Sekerers, 2011; Bennett et al., 1982; Vardiman et al., 2009).The best survival for MDS patients is in the category of refractory anemia (RA) with a median of 28 months, and the worst median survival seen in refractory anemia with excess blast (RAEB) with median of 11 months (Ma et al., 2007). The common causes of death in a cohort of 216 MDS patients included bone marrow failure (infection/hemorrhage) 88%, and AML transformation 28% (Greenberg et al., 1997). 90% of cases are primary (de novo) whereas 10% are secondary to chemotherapeutic agents, radiation, and chemical exposures such as benzene and its derivatives. It is very common for MDS patients to require blood transfusions, about 22% of low risk and 68% of high risk patients are red cell transfusion dependent. In one study, about 65% had received blood transfusion, and 52% had received transfusion in the past three months (Sekerers et al., 2008; Sekers, 2010).

Allogenic hematopoietic stem cell transplantation is the only available potentially curative treatment for MDS

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patients, but due to advanced age, donor availability, and presence of multiple comorbidities not every patient would be a good candidate for stem cell marrow transplantation. As discussed below, thrombopietic stimulating agents (TSAs), erythropoiesis stimulating agents (ESAs), colony stimulating factors (CSFs), antithymocyte globulin (ATG), lenalidomide, and hypomethylating agents are some of the non-transplantation options for MDS patients, and treatment should be individualized on the basis of MDS grade (Komrokji et al., 2011). Prognosis is still poor despite all of the available novel drugs and supportive measures (Garcia-Manero, 2011).

Diagnosis and Classification

The process of diagnosis and classification of MDS is usually started with a comprehensive history and physical examination, complete blood count with manual differential, reticulocyte count, measurement of serum ferritin, vitamin B12, folate, erythropoietin levels, and iron status level to rule out blood loss, inflammatory disorders, and vitamin and mineral deficiencies. The most common laboratory finding is anemia (80%), and most commonly is macrocytic, but can be microcytic, or normocytic. Some patients may develop thrombocytopenia in the course of disease, and present with different types of bleeding.

Clinicians should be aware of the possibility of recurrent infections and should anticipate that during the course of MDS, life-threatening septicemia secondary to neutropenia may occur. Bone marrow biopsy and aspiration are needed for all patients to evaluate bone marrow architecture and cellularity, and in the presence of dysplasia with a hypercellular or hypocellular bone marrow, this would be highly suggestive of the diagnosis. Bone marrow cytogenetic findings are present in half of the patients, and it is considered to be an important laboratory finding of a significant prognostic and therapeutic implications. The dysplasia may involve one (refractory anemia, refractory thrombocytopenia) or more cell lineages that affect clinical presentation and prognosis (Barzi et al., 2010; Greenberg 2010; Garcia-Manero et al., 2011). There are some conditions which can lead to the development of secondary dysplasia which includes megaloblastic anemias, toxic exposures to arsenic and alcohol, dysplasia due to recent exposure to cytotoxic and growth factor therapy, recent intercurrent illness, human immunodeficiency virus, other viral infections, and copper deficiency (Sekers, 2011).

It can be a challenging process to be able to differentiate MDS from chronic anemia with a secondary dysplasia. When a patient is found to have anemia, an extensive work up for treatable and common causes of anemia including gastrointestinal, cardiovascular, and renal system is needed to rule out non-hematologic etiologies. In case of intravascular hemolysis, PNH should be ruled out.

There are two distinct classification systems of French-American-British (FAB) (Rollison et al., 2008) and World Health Organization (WHO) (Hoffman et al., 1996), and several prognostic scoring systems, the most widely accepted is the International Prognostic Scoring System (IPSS) (Sanz et al., 1989). There is no "staging system" for MDS, and each system has its own limitations. The IPSS predicts overall survival, and divides patients to two major categories: a two relatively low risk groups (which constitute approximately 75% of MDS patients) and 2 higher risk groups (constituting 25% of cases) at the time of diagnosis. In lower risk MDS, involved cell lines have shorter survival (early apoptosis) by mechanisms that are not fully understood, whereas pre-proliferative factors are responsible in high risk MDS. Median survival of patient depends on the score value.

In addition to its prognostic and survival significance, the IPSS determines the risk of transformation to AML. IPSS does not determine lower risk patients with poor prognosis, which is the most important disadvantage of it. Recently, Garcia-Manero et al proposed a new scoring system for lower risk patients which divides them to three categories 1 (median survival of 80.3 months), 2 (median survival of 26.6 months), and 3 (median survival of 14.2 months) (Garcia-Manero et al., 2008). The prognostic system is based upon the presence of the following factors which by their presence would indicate a likely poorer prognosis: older age, male gender, poor performance status, comorbidities, low absolute neutrophil count, low platelet counts, RBC transfusion requirements, high serum ferritin, high LDH, bone marrow fibrosis, low CD11b, high HLA-DR, counts of CD34, CD13, CD45, clonal granulocytes, multiple chromosomal abnormalities, short telomerase and long telomerase activity (Mittleman et al., 2010).

Overview of Management

Several factors including age, IPSS score, life expectancy, cytogenetic changes, and performance status should be considered when choosing a therapeutic modality. In view of the recent advances in our understanding of the biology of MDS, treatment for MDS has drastically changed in the past few years (Sanchez, 2011). MDS patients are often elderly individuals with multiple comorbidities; clinicians should consider three clinical parameters before starting any treatment modalities: 1. age, 2. performance status and 3. the international prognostic scoring system (IPSS)-defined risk category (Myelodyspastic syndromes v.2 2010). Anemia, neutropenia and thrombocytopenia, particularly refractory cytopenias are major causes of substantial morbidity and mortality in MDS patients. Therefore, therapeutic goals in MDS patients should include control of symptoms due to cytopenias.

This goal is often accomplished by supportive care measures such as blood transfusions. The major goals of therapy at all ages are to reduce morbidity and mortality, improve quality of life, and minimize treatment-related toxicities. As mentioned above, anemia is the most common cytopenia, and MDS patients need frequent transfusions during the course of their disease. Lifelong transfusions can lead to secondary iron overload which shortens survival. However, due to low survival rate the risk and benefit of iron chelation therapy is not clear, and should be individualized (Barzi et al., 2010; Bryan et al., 2010; Tefferi 2010).

Supportive Care

Erythropoiesis stimulating agents (ESA)

Symptomatic anemia and associated fatigue are usually considered to be one of the major problems in MDS, which can cause significant morbidity and the majority of the patients will need red blood cell transfusion during the course of their disease. Erythropoietin (EPO) is the primary stimulus of normal erythropoiesis (Erslev, 1991). Although ESAs have not been formally approved by FDA, they are frequently the first step in management of anemia, and American Society of Clinical Oncology recommends this for low risk MDS (Steinbrook 2007; Rizzo et al., 2010).

Several studies show that recombinant EPO can correct anemia in MDS patients (Geissler et al., 1997; Moyo et al., 2008; Park et al., 2010). Not all patients need, or benefit from ESAs. Patients who could benefit from EPO replacement depends on the patient's serum erythropoietin level, bone marrow cellularity, and the degree of marrow fibrosis. Asymptomatic anemia does not need any treatment. About 74% of low risk MDS patients who have low transfusion needs and low erythropoietin levels respond to EPO, while this chance drops significantly to 7% in high risk MDS patients who have high transfusion needs and high erythropoietin levels (Sekerers et al., 2007; Santini et al., 2010). The only exception is del 5q MDS subgroup, a low-risk group with a poor response to EPO but a remarkable cytogenetic and hematopoietic response to lenalidomide (Itzykson et al., 2009).

Neutropenia is another common cytopenia in MDS patients, and infection is a serious complication in this patient population. The majority of MDS patients with neutropenia respond to G-CSF (Kobayashi et al., 1989; Yoshida et al., 1991; Kaczmarski et al., 1993; Negrin et al., 1996).

Granulocyte colony-stimulating factor (G-CSF)

A natural product of various body tissues, Granulocyte colony-stimulating factor, also called CSF 3, is the principle factor that is necessary for proliferation, differentiation of myeloid precursor cells into neutrophils, and survival of neutrophils. CSF 3 intensifies multiple neutrophil functions, such as motility and migration of hematopoietic progenitor cells from bone marrow into the circulation, and is required for stress granulopoiesis in response to some types of infection. G-CSF is encoded on chromosome 17q21-q22. G-CSF levels increase by IL-1β, TNFα, bacterial polysaccharides, and myelosuppression. G-CSF systemic levels are regulated by IL-17 via an unknown mechanism (Christopher et al., 2007; Panopoulos et al., 2008; Greenbaum et al., 2011). Neutropenia may increase IL-23 production, which in turn enhances IL-17 secretion, and promotes G-CSF induced granulopoiesis.

In normal conditions, G-CSF is produced at a very low level, and is minimally detectable in plasma. G-CSF is rapidly eliminated by the kidneys. Any defect in G-CSF or its signaling pathways affects the production of both macrophage and granulocyte populations (see below). G-CSF has only one receptor (G-CSFR) which is a member of the hematopoietic (class 1) cytokine receptor superfamily. Interestingly, erythropoietin receptor deficient mice die in utero due to lack of erythropoiesis, but G-CSFR deficient mice can survive regardless of low numbers of mature granulocytes indicating that granulopoiesis has both G-CSF dependent and G-CSF independent pathways. Nevertheless, it is well established that G-CSFR signaling pathway is the main promoter of basal granulopoiesis in normal conditions. G-CSFR deficient mice have normocellular bone marrow with normal number of committed myeloid progenitor cells. G-CSF has a pivotal role in basal granulopoiesis by enhancing the proliferation and differentiation of committed myeloid progenitor cells; however, in G-CSFR deficient mice the number of granulocyte-macrophage colony forming units is slightly decreased. It is a very well-known fact that lack of G-CSFR is accompanied by severe neutropenia; arguably G-CSF is the treatment of choice in severe congenital neutropenia. G-CSF probably has its effect on intermediate myeloblasts and promyelocytes. However, treating AML patients with G-CSF for differentiation induction of blast cells, and increasing their apoptosis had variable results (Richards et al., 2003; Beekman et al., 2010; Gurion et al., 2011).

Both hematopoietic and non-hematopoietic tissues (cardiomyocytes, neuronal precursors, endothelial cells) express G-CSFR, and binding of G-CSF to its receptor leads to activation of different and complicated intracellular signaling pathways. In this case, ras/MAPK (mitogen-activated protein kinase) and phosphatidylinositol 3-kinase (PI3 kinase) are common signaling pathways of EPO and G-CSF, but G-CSF is also engages Janus Kinase 2 (Jak2)/signal transducer and activator of transcription 3 (STAT3) pathways. What was found to be consistently present in all of different studies is that STAT3 plays a key role in the differentiation and survival of both macrophages and neutrophils, STAT5 significantly contributes to myeloid cell proliferation and malignancy (Millot et al., 2001; Ward, 2007; Liongue et al., 2009). Research shows that Jak/Stat, MAPK, and apoptosis pathways are involved in MDS. Interestingly, MAPK is an apoptotic negative regulator of erythroblasts, and commonly is downregulated in cases of refractory anemia with ring sideroblast (RARS). As mentioned above, early apoptosis is the main pathophysiologic mechanism involved in the pathogenesis of the lower risk categories of MDS, and G-CSF has a strong apoptosis inhibitor (anti-apoptotic) effect (Nikamae-Akahori et al., 2006; Nikpour et al., 2010; Molineux 2011).

G-CSF is frequently used in the clinical management of MDS patients to improve their anemia and neutropenia; and conceivably it is a beneficial therapeutic intervention, which has a prominent place in the supportive care plan of those patients. Nonetheless, the benefit of adding G-CSF to EPO has not been confirmed yet. It has been suggested that rHuG-CSF should be considered when there is no response to EPO after 6-8 weeks of therapy (Steensma et al., 2006). It has not been shown yet that rhG-CSF alone can improve survival in MDS patients (Kobayashi et al., 1989; Negrin et al., 1989; Ohyashuki et al., 1989; Negrin et al., 1990; Yoshida et al., 1991; Kaczmarski 1993). In

association with a hypomethylating agent or lenalidomide or in case of severe infection, G-CSF can be used for the management of neutropenia after chemotherapy. There is in vitro evidence that G-CSF is able to repair functional abnormalities of neutrophils in MDS patients (You et al., 1987). Most MDS patients treated with G-CSF show incremental improvements in their peripheral blood neutrophil counts, which is indicative of some bone marrow reserve in those patients. There is no evidence from randomized studies showing an advantage of maintenance G-CSF in MDS patients. A preliminary report of 5 MDS patients treated with intravenous G-CSF (50-1600 μg/m2) showed improvement of neutropenia (Kobayashi et al., 1989). Eighteen patients were enrolled in a phase I/II clinical study of subcutaneous injection of G-CSF (0.1-0.3 μg/kg/day), and 16 of them showed an increase in their neutrophil counts from 5 to 40 fold (Ohyashuki et al., 1989). 11 patients from the cohort participated in a long-term maintenance program with subcutaneous injection of G-CSF, and 10 patients achieved improved neutrophil counts for up to 16 months. Subsequently, this study showed patients with neutrophil counts maintained at > 1.5 x 109/L had fewer bacterial infections than those with lower neutrophil counts (Negrin et al., 1990).Severely neutropenic patients may benefit from prophylactic therapy with low-dose G-CSF (Negrin et al., 1996).

A preliminary phase III multi-institutional randomized trial report of 102 high-risk patients with MDS (RAEB or RAEB-t) showed no increased risk of AML-evolution in the treatment arm of 50 patients (Greenberg et al., 1993). There is in vitro evidence that G-CSF is associated with a growth advantage of an existing subclone of cells with monosomy 7 over diploid cells (Sloand et al., 2006). The initial impression of G-CSF administration in MDS patients was very promising and G-CSF used in a small number of high grade MDS cases (Vadhan-Raj et al., 1987; Brito-Babapulle et al., 1989). These studies used peripheral blood counts, bone marrow changes, and immediate side effects as therapeutic measures without focusing on survival rate, improvement of quality of life, and cost effectiveness, the area that controversy still exists (see below). In a small case control study of 14 patients, there was less dysplastic bone marrow after treating MDS-AML patients with GM-CSF/TAD. In this study, responders to GM-CSF/TAD had less toxic complications without much difference in survival rate (Bernell et al., 1994). In a randomized prospective study on 93 patients with RAEB-t and MDS-AML, the addition of GM-CSF to standard induction chemotherapy schedule not only did not improve the complete remission and survival rate, but also was associated with more side effects. This study suggested that GM-CSF should be used cautiously in elderly due to increased risk of cardiovascular events (Smith et al., 2006). In another prospective randomized double-blind placebo-controlled trial of 31 patients with high risk MDS, administration of GM-CSF with standard-dose chemotherapy regimen was not superior to cytoreductive therapy alone, did not improve response rate, or duration of neutropenia (Verbeek et al., 1997).

To address efficacy of G-CSF administration in high risk MDS, 105 high risk MDS patients or MDS transformed to AML (secondary) entered in a randomized clinical study. In this study 52 patients received chemotherapy and 53 underwent chemotherapy and G-CSF administration. G-CSF improved clinical condition by significantly shortening post-chemotherapy neutropenic phase and increasing of remission rate, but did not prolong survival (Bernasconi et al., 1998). The 2006 updates of American Society Of Clinical Oncology (ASCO) recommends intermittent colony stimulating factors administration in MDS patients with severe neutropenia and recurrent infection. In regard to comparing clinical activity of G-CSF and GM-CSF, the latter has higher rate of fever, however, there is no study which suggests there is any difference in hematopoietic efficacy between the 2 cytokines (Hast et al., 2003).

Combination of granulocyte colony-stimulating factor and erythropoietin

Serum EPO levels usually show an inverse relationship with the degree of anemia in MDS patients, with the highest concentrations being found in patients with erythroid hypoplasia (Jacobs et al., 1989). The hallmark of MDS is increased apoptosis, and there is in vitro evidence that EPO functions as a survival factor with anti-apoptotic properties (Silva et al., 1996). EPO induces anti-apoptotic protein Bcl-XL (Gregory et al., 1999); it also activates the anti-apoptotic PI3-kinase (Uddin et al., 2000). Similarly G-CSF has also anti-apoptotic effects both in vitro and in vivo (Colotta et al., 1992; Watson et al., 1999; Hassan et al., 1999; Molineau 2011).There is in vitro evidence that G-CSF has anti-apoptotic function through inhibiting Fas-triggered caspase activity in bone marrow cells isolated from RARS patients. It also promotes erythroid colony growth and differentiation of stem cells from RARS patients (Schmidt-Mende et al., 2001; Molineau 2011).There is evidence that G-CSF changes the survival abilities of the mobilized CD34+ cells and it was found that peripheral blood stem cell mobilization with G-CSF results in a significant reduction in the number of apoptotic CD34+ cells in comparison with a more apoptotic CD34+ cells collected form an unstimulated mobilization (Philpott et al., 1997). Additionally it has been shown by in vitro experiments that G-CSF blocks spontaneous cytochrome c release and mitochondria-dependent apoptosis in hematopoietic progenitor cells of RARS patients and improves erythropoiesis in MDS (Tehranchi et al., 2003; Sung 2007; Molineau 2011).

As mentioned earlier, recombinant EPO has been used extensively to treat anemia in MDS patients (Bessho et al., 1990; Hellström-Lindberg et al., 1990; Rose et al., 1995). The efficacy of EPO alone is relatively low, and overall erythroid response rates from 7.5% to 36% have been reported (Hellström-Lindberg 1995; Geissler et al., 1997). There is in vitro evidence of a synergistic effect of G-CSF and EPO combination on erythropoiesis (Greenberg et al., 1992).A clinical response to G-CSF and EPO combination has also been demonstrated; morphologically, in bone marrow biopsies of MDS patients which does show a reduced number of apoptotic precursors compared with the pre-treatment samples (Hellström-Lindberg et al., 1997).

Hematopoietic growth factors such as EPO and G-CSF not only block apoptosis of erythroid precursors but also promote growth of cytogenetically normal progenitors in MDS patients (Tehranchi et al., 2005).

Clinical responses have been investigated with EPO and G-CSF combination in MDS patients (Table1). Two phase II clinical trials of G-CSF and EPO combination in MDS patients (mainly RA, RARS, and RAEB) demonstrated erythroid response rates of 42% (10 of 24 patients) and 38% (8 of 21 patients), respectively (Hellstron-Lindberg et al., 1993; Negrin et al., 1993). These studies showed strong erythroid responses in terms of improved hemoglobin levels and reduced RBC transfusion requirements, and the response rates were considerably better than with EPO alone. Subsequently, another clinical trial with 55 MDS patients showed a 48% erythroid response rate (21 of 44 evaluable patients), and 81% of responders maintained their response during an 8-week maintenance period. This study also revealed an interesting finding that approximately 50% of the MDS patients with a response to G-CSF and EPO combination lost their response with G-CSF withdrawal, and some of those patients regained a response when G-CSF was restarted (Negrin et al., 1996). A small phase II clinical study of G-CSF and EPO combination in MDS patient has also been reported from Japan, which did not show promising results; 10 patients received the combination therapy for 10 weeks, and only one patient had a delayed erythroid response, although 80% (8 of 10 patients) had a neutrophil response (Imamura et al., 1994).An American-Scandinavian study of 98 MDS patients treated with G-CSF and EPO combination showed a similar response rate of 36% to treatment. This study revealed that patients with serum EPO concentrations < 500 U/l had a favorable response to G-CSF and EPO combination if the RBC transfusion need was < 2 units per month (Hellström-Lindberg et al., 1997). A subsequent phase II randomized clinical trial of 56 MDS patients (RA, RARS, RAEB) treated with G-CSF and EPO combination showed an overall erythroid response of 38%. The response rates for patients with RA, RARS, and RAEB were 20%, 46%, and 37%, respectively. In this study patients were randomized to two treatment groups: arm A primed with G-CSF for 4 weeks followed by the G-CSF and EPO combination for 12 weeks, and arm B started with EPO for 8 weeks followed by the combination for 10 weeks. The response rates were identical in the two treatment groups. This study clearly confirmed the *in vivo* synergy between G-CSF and EPO, and this synergistic effect was more pronounced in RARS patients (Hellström-Lindberg et al., 1998). A Spanish non-randomized clinical trial of 32 MDS patients (RA and RARS) treated with G-CSF and EPO combination showed an erythroid response rate of 50% and a multivariate analysis confirmed the predictive value of the American-Scandinavian scoring system (Remacha et al., 1999). A German non-randomized clinical trial of 33 MDS patients (RA, RARS and RAEB) treated with G-CSF and EPO combination demonstrated an erythroid response rate of 61% after 12 weeks, which rose to 80% after 36 weeks (Matovani et al., 2000). The predictive value of low serum EPO concentrations (< 150 U/L) was observed in a Greek phase II clinical study in 281 MDS patients (RA, RARS, and RAEB) and an overall erythroid response rate of 45.1% was rEPOrted (Terpos et al., 2002). The Scandinavian MDS group has published the results of a prospective study of 53 MDS patients treated with G-CSF and EPO combination, which showed an overall erythroid response rate of 42%, and it validated the American-Scandinavian predictive model and scoring system. It demonstrated response rates of 61% in the good predictive group and 14% in the intermediate group. This study also showed that responding patients had a significantly better quality of life (Hellström-Lindberg et al., 2003). However, a French randomized controlled clinical trial did not show any significant difference in quality of life between the treatment arm and supportive care arm; the study demonstrated an erythroid response rate of 42% (Casadevall et al., 2004). The Scandinavian group published the results of 129 MDS patients treated with G-CSF and EPO combination that were followed for up to 42 months, and it showed an erythroid response rate of 39% and median response duration of 23 months. They did not find any difference in survival between treated and untreated patients (Jadersten et al., 2005). An Italian randomized prospective study compared EPO with G-CSF and EPO combination in 30 low-risk MDS patients; it showed an erythroid response in 6 of 15 (40%) patients in the EPO arm and in 11 of 15 (73.3%) patients in the G-CSF and EPO combination arm. In 4 of 9 (44.4%) patients who did not have a response to EPO, adding G-CSF induced an erythroid response at 16 weeks (Balleari et al., 2005). A pooled analysis of retrospective data from 162 already-published articles consisting of 2592 MDS patients with RA and RARS suggested that growth factors may improve survival in MDS (Golshayan et al., 2007). A recent French-Belgian retrospective study examined erythroid response rate and overall survival in 403 MDS patients treated with EPO with or without G-CSF; and 62% and 50% response rates were seen in the EPO alone arm and G-CSF and EPO combination arm, respectively. This study reported a better overall survival in the EPO alone arm but, results were not adjusted for all currently employed prognostic factors and RBC transfusion requirement in the multivariate analysis, and

Table 1. Clinical Studies of G-CSF and EPO in MDS

Reference	No of patients	Response Rate
Negrin et al, 1993, (83)	24	42%
Hellstrom-Lindberg et al, 1993,(84)	21	38%
Imamura et al, 1994,(86)	10	Nil
Negrin et al, 1996,(85)	55	48%
Hellstrom-Lindberg et al, 1997,(87)	98	36%
Hellstrom-Lindberg et al, 1998,(88)	56	38%
Remacha et al, 1999,(89)	32	50%
Mantovani et al, 2000,(90)	33	61%
Terpos et al, 2002,(91)	281	45%
Hellstrom-Lindberg et al, 2003,(92)	53	42%
Casadevall et al, 2004,(93)	60	42%
Jadersten et al, 2005,(94)	129	39%
Park et al, 2008,(97)	403	50%
Jadersten et al, 2008,(98)	121	39%
Gotlib et al, 2009,(99)	24	47%
Greenberg et al, 2009,(100)	12	31

selection bias is another potential confounding factor (Park et al., 2008). A Scandinavian retrospective study compared 121 MDS patients treated with G-CSF and EPO combination with 237 untreated patients, and an erythroid response rate of 39% and median response duration of 23 months were reported. This study demonstrated an encouraging survival benefit in a multivariate analysis, but its results are potentially confounded by patient selection bias. No increased rate of AML was observed (Jadersten et al., 2008). An American phase II intra-patient dose-escalation clinical trial of EPO with or without G-CSF in 24 MDS patients showed that addition of G-CSF resulted in an erythroid response of 47% in patients who did not have a good response to EPO. A weekly weight-based EPO regimen was used in the study (Gotlib et al., 2009). A recently published phase III prospective randomized clinical trial of 73 MDS patients treated with EPO with or without G-CSF plus supportive care versus supportive care alone demonstrated an erythroid response of 31% in 12 of 39 patients who received G-CSF and EPO combination (Greenberg et al., 2009). This study did not reveal any difference in overall survival between the EPO and supportive care arms with a median follow-up of 5.8 years. Adding G-CSF to EPO does not show any negative impact on patient's survival or increased incidence of progression to AML. However, a survival benefit was observed in the erythroid responders versus non-responders. EPO has been considered as part of the best supportive care, but only a certain subgroup of MDS patients benefit from this treatment and improving hematologic parameters not necessarily means that this modality improves survival (Nachtkamp et al., 2009; Stone 2009). A meta-analysis of 15 clinical studies in MDS patients treated with EPO with or without G-CSF or M-CSF suggested that higher doses of EPO (60 to 80,000 U/week) may be more effective than standard dose of EPO (30 to 40,000 U/week) (Mundle et al., 2009). The guideline group of UK-based medical experts in the clinical management of MDS suggests that EPO with or without G-CSF is effective in highly selected MDS patients. The MDS subgroups who benefit from EPO are patients with refractory anemia and refractory anemia with excess blast not suitable for chemotherapy/allogenic bone marrow transplantation, patients with transfusion need less than 2 units per month, and patients with EPO level less than 200 U/L. In case of no response to EPO, G-CSF can be added to the schedule or EPO dose doubled for more 6 weeks. The goal of G-CSF therapy is to keep WBC in the range of 6 to 10 x 109/l. Combination of EPO and G-CSF therapy is recommended for RARS, symptomatic anemia, patients with basal EPO level less than 500U/L, and transfusion needs less than 2 units per month. The G-CSF schedule can be 3 times a week, and reduce to the lowest possible dose (Bowen et al., 2003; Akhtari 2011). There is no clinical benefit of prophylactic G-CSF therapy despite of good tolerance by the MDS patients.

Predictors of erythroid response to G-CSF and EPO combination

A multivariate analysis of an American-Scandinavian clinical study demonstrated that two pre-treatment clinical variables, baseline serum EPO value and initial low RBC transfusion need had prognostic values to predict response to G-CSF and EPO combination. By using pre-treatment serum EPO values as a ternary variable (<100, 100-500, or > 500 U/l), and RBC transfusion requirement as a binary variable (<2 or ≥2 units per month), a predictive model and scoring system for erythroid response was developed. Patients were separated into three predicted erythroid response-rate groups of high (74%), intermediate (23%) and poor (7%) (Table2). This study revealed that patients with serum EPO concentrations < 500 U/l had a favorable response to G-CSF and EPO combination if the RBC transfusion need was < 2 units per month [66]. This scoring system was validated in another prospective study, in which MDS patients with one or both of these positive predictors showed erythroid response rates of 14% versus 61%, respectively (Gotlib et al., 2005). The predictive model and scoring system were derived for patients treated with a therapeutic trial of 12-week duration, and 39% of patients with a high predictive score still fail to achieve an erythroid response (Bowen 2006). A recent Dutch study demonstrated that flow cytometric analysis of myeloblasts in the bone marrow samples can be used in predicting response to growth factor treatment in MDS patients. In a cohort of 46 MDS patients (low- and intermediate-risk) the predictive model and scoring system were associated with an erythroid response to G-CSF and EPO combination. However, according to the predictive model and scoring system, aberrant phenotype of myeloblasts was highly associated with treatment failure among patients with the greatest response probability (Westers et al., 2010).

It has been suggested that burst forming unit-erythroid (BFU-E)/colony forming unit-erythroid (CFU-E) has less response rate to EPO in non-responder MDS patients, and non-responders have less circulating BFU-E. The ERK1/2 pathway which is required for cell proliferation and differentiation has less activity after EPO administration in non-responder MDS patients, and may be a predictor of response to EPO in MDS patients (Frisan et al., 2010). As mentioned in this article, G-CSF and EPO are effective but costly therapeutic measures in low risk MDS patients. Most MDS patients are elderly with limited marrow

Table 1. Predictive Model and Scoring System for Erythroid Response to G-CSF and EPO Combination in MDS Patients

Variable	Score					
	-3	-2	-1	0	+1	+2
Serum EPO (U/I)	>500				100-500	<100
RBC transfusion (U/month)	>2				<2	

Predictive Score	Response Group	
	Type	% Responders (no. of patients)
> + 1	High	74 (22/29)
+1	Intermediate	23 (7/31)
< -1	Low	7 (3/34)

*G-CSF, granulocyte colony-stimulating factor; EPO, erythropoietin; RBC, red blood cell, From Hellström-Lindberg et al.(87) with permission from John Wiley and Sons

reserve, and considerable differences in both response and toxicity of biosimilars. Issues related to quality of life, overall life expectancy, cost and benefit, disease free period, and clinical efficacy should be considered when the clinician is making any therapeutic decision (Lyman et al., 2002).

Thrombopietic stimulating agents

Thrombocytopenia is common in MDS, and 33% of patients receive at least one platelet transfusion in the course of their disease. Platelets develop intrinsic functional defect in MDS, which contributes to the bleeding tendency in these patients. Thrombocytopenia is more common in RAEB subgroups, but its overall prevalence in MDS is 40-65% in different studies. It has been shown that about 26% of patients have mild spontaneous bleeding, less than 10% present with serious bleeding, and about 66% finally develop thrombocytopenia. Bleeding is the major cause of morbidity and mortality in those patients who transform to AML, and is cause of death in about 20% of patients. Platelet count has direct relation with survival, and severe thrombocytopenia is associated with shorter transformation time to AML. Platelet transfusion has the risk of infection transmission, platelet alloimmunization, transfusion-related acute lung injury, and febrile non-hemolytic reactions (Kantarjian et al., 2007; Kantarjian et al., 2010; Sekerers et al., 2011). Thrombopietin has a large molecule with 23% homology with erythropoietin, and its half-life is about 20 to 40 hours. Unlike erythropoietin, there is no "sensing" system for TPO; it is continuously produced by liver, and clears from blood by platelets, megakaryocytes, and reticuloendothelial system (Kutar 2009; Stasi et al., 2010). Binding of TPO to its receptor starts tyrosine phosphorylation of Stat3, Stat5, and Jak2, and enhances platelet progenitor cells production and survival (Miyakawa et al., 1995; Miyakawa et al., 1996). Thrombopietic growth factors (TPOs) are the novel therapeutic agents with uncommon side effects. The first generation of thrombopietic agents was recombinant proteins with the same amino acid structure as TPO, rhTPO. It was removed from market due to development of neutralizing antibody against endogenous TPO. The second generation of thrombopietic growth factors includes three general classes: TPO peptide mimetics, TPO nonpeptidemimetics, and TPO agonist antibodies. The second generation of TPOs does not have any sequence homology with endogenous TPO, is generally well-tolerated, and used in clinical trials of MDS patients (Kuter 2007; Perugini et al., 2009).

Romiplostim is a TPO peptide mimetic which activates TPO receptor on nonlymphoid CD34+ hematopoietic cells, induces proliferation and maturation of megakaryocytes and platelet production, and increases cell viability with inhibiting the apoptosis of megakaryocytes. A TPO nonpeptide mimetic, eltrombopag, stimulates TPO receptor at a site different from endogenous TPO. Eltrombopag is highly species specific, and its effect is additive to TPO (Kuter 2007; Perugini et al., 2009; Ikeda 2009).

Administration of TPO in MDS is in its early stages, and most usages have been reported in case rEPOrts, and phase I/II clinical trials. Pegylated recombinant TPO was used for more than 450 days in a Japanese patient with RARS MDS, and patient's platelet count improved from 7 x 109/l at the beginning to 45 x 109/l on day 450 without any adverse effect (Kizaki et al., 2009).

In an open-label, sequential-cohort, dose-escalation study of romiplostim in 44 patients with thrombocytopenia due to lower risk MDS, half of the patients had durable platelet responses and incidence of severe bleeding and platelet transfusions decreased. However, 2 of 44 patients (5%) progressed to AML and increased blasts were seen transiently during bone marrow evaluations in 4 patients (9%) (Kantarjian et al., 2010). Romiplostim was used in an open label study of 28 low risk MDS patients, and 65% of patients showed complete or major platelet response and need of platelet transfusion resolved in 61% of patients. Nevertheless, 2 of 28 patients (7%) developed an increased blast percentage during the study (Sekers et al., 2011). In a phase II randomized, placebo controlled study of romiplostim in 40 low or intermediate risk MDS patients this peptibody TPO mimetic reduced the incidence and number of platelet transfusions. But 2 patients in the romiplostim arm and 1 in the placebo arm progressed to AML (Kantarjian et al., 2010). Eltrombopag increased megakaryocytic progenitor cell proliferation, and platelet production in an ex vivo study of the effect of this non-peptidyl small molecule on megakaryopoiesis of low risk MDS patients (Mavroudi et al., 2011). Thrombocytopenia is a common side effect of hypomethylating agents, and platelet transfusion is the only management measure. It has been suggested that adding TPO reduces the risk of bleeding, and is well-tolerated in MDS patients receiving hypomethylating agents. Patients receiving combination of romiplostim and decitabine in a randomized, double-blind, placebo-controlled study of 29 lower risk MDS had higher platelet counts, and less need for platelet transfusion (Greenberg et al., 2009). In a randomized study, adding romiplostim to lenalidomide in low or intermediate risk myelodysplastic syndrome increased response rate from 8% in placebo group to 36% in patients receiving 500 μg of romiplostim and lenalidomide regardless of the baseline del (5q) status (Lyons et al., 2009). Thrombocytosis, thrombosis, stimulation of tumor cell growth, and increase in bone marrow fibrosis are major possible side effects of TPO therapy but rebound thrombocytopenia after stopping of TPO mimetics is a common side effect. Most of these studies have done on only small highly-selected patient groups and suffer from lack of control group. More double blind large-sample size studies are needed to provide us with the potential clinical benefits of these agent and a better characterization of side effects and safety profile of TPO mimetics in MDS patients.

Conclusion

There has been enormous progress in our understanding of the pathobiology and signaling pathways of MDS in recent years, which has led to the identification of specific subtypes with distinct clinical behavior and different therapeutic modalities. However, MDS continues to be a challenging disease to treat due to its complex and

heterogeneous nature and biology. Patients with lower risk MDS can benefit from supportive care including growth factors. G-CSF is an effective therapeutic modality in MDS patients, and it can be used for the management of refractory symptomatic cytopenias. G-CSF and EPO combination can improve outcomes in appropriate MDS patients such as lower risk MDS and RARS patients. Growth factors such as G-CSF and EPO have created a significant opportunity for improvement in the care of patients with MDS; however, there are still uncertainties around use of TPO mimetics in MDS patients. Further trials will determine additional effective agents, and answer critical questions regarding the optimal timing and duration of existing therapies for MDS patients.

Acknowledgment

The authors wish to thank Kathleen Kruger for her technical assistance. All authors do not have any conflict of interest to disclose.

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