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REVIEW ARTICLE

Myelodysplastic syndrome

Transforming growth factor (TGF)-β pathway as a therapeutic target in lower risk myelodysplastic syndromes

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Abstract

The transforming growth factor (TGF)-β superfamily comprises more than 30 soluble growth factors that play a central role in erythropoiesis and are part of a tightly regulated myelosuppressive negative feedback loop under physiologic conditions. TGF-β receptor activation and phosphorylation trigger a regulatory circuit of activating and inhibitory SMAD proteins and increased activation of the TGF-β signaling pathway either by a loss of negative feedback or constitutive activation has been associated with the myelosuppression and ineffective erythropoiesis in myelodysplastic syndromes (MDS). Anemia is the predominant cause of morbidity and quality of life impairment in patients with lower-risk (LR)-MDS, and there are very limited therapy options for these patients after failure of erythropoiesis stimulating agents (ESAs). Targeting the aberrant TGF-ß signaling pathway has therefore been investigated as a promising therapeutic approach to resolve the ineffective erythropoiesis in LR-MDS. In this article, we provide a brief overview of the TGF-β signaling cascade in hematopoiesis under physiologic conditions and its role in MDS pathogenesis. We also review preclinical and clinical data for the activin receptor type IIA ligand traps sotatercept and luspatercept that have recently shown promising results in overcoming the myelosuppressive effects of TGF-β signaling alterations to improve hematopoiesis in transfusion-dependent, non-del(5q) LR-MDS patients. Additional potential targets within the TGF-β pathway have also been identified in preclinical experiments and may provide further therapeutic options. Finally, combining different TGF-β pathway inhibitors or using them in combination with ESAs or the immunomodulator lenalidomide might have synergistic effects as well.

Introduction

Myelodysplastic syndromes (MDS) comprise a spectrum of clonal bone marrow disorders that are characterized by peripheral blood cytopenias and dysplastic features secondary to ineffective hematopoiesis as well as a variable risk of progression to acute myeloid leukemia (AML) [1–3]. Given the heterogeneity in MDS symptom burden and AML progression risk, treatment approaches vary for individual patients based on risk stratification tools such as the revised International Prognostic Scoring System (IPSS-R) [2, 4, 5]. The IPSS-R is based on the severity of peripheral blood cytopenias, bone marrow blast percentage and an assessment of the cytogenetic risk group [5].

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While for patients with intermediate- or high-risk MDS (IPSS-R score ≥ 3.5) aggressive treatment with chemotherapy (e.g. hypomethylating agents (HMAs)) or allogeneic hematopoietic stem cell transplant is recommended [4, 6, 7], supportive care with blood transfusions, iron chelation, and growth factor support (erythropoietin, granulocyte colony-stimulating factor, and thrombopoietin) alone might be sufficient for patients with lower-risk MDS (LR-MDS) and no high-risk cytogenetic features (e.g. mutations in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1)* [3, 8, 9]. However, even LR-MDS is a very heterogeneous disease that can be further subdivided based on cytogenetic abnormalities such as deletion 5q [del (5q)], clinical symptoms (anemia or thrombocytopenia predominance) and morphologic features such as the presence of ringed sideroblasts which have implications on treatment approach in these patients [2, 3, 10]. Potential treatment options in addition to supportive transfusions and growth factor support include lenalidomide, which has been shown to be especially efficacious in MDS with del(5q), immunosuppressive therapy (IST),

HMAs, and transforming growth factor beta (TGF-β) pathway inhibitors [11–14].

Anemia due to ineffective hematopoiesis and an increased rate of apoptosis of progenitor cells is the main presenting symptom of LR-MDS and is treated supportively with red blood cell (RBC) transfusion and erythropoiesis-stimulating agents (ESA) as needed [15, 16]. Although RBC transfusions and ESAs can improve quality of life substantially [15, 17, 18], chronic anemia and iron overload can have severe long-term effects especially on the cardiovascular system and efforts to overcome the ineffective hematopoiesis are highly warranted [19]. In addition, a substantial subset of patients already has elevated erythropoietin (EPO) levels at baseline and therefore fails to respond to exogenous ESA therapy [2, 20]. Alterations in TGF-β signaling have been identified as key mediators of apoptosis and ineffective erythropoiesis in MDS and targeting this pathway has yielded promising results recently [12, 21, 22].

The TGF-β superfamily comprises more than 30 soluble growth factors that are part of a negative feedback loop that regulates hematopoietic stem cell (HSC) proliferation and differentiation [23–25]. TGF-β signaling is mediated by a regulatory circuit of activating and inhibitory SMAD proteins that can either promote gene transcription and erythroid differentiation (SMAD2/3) or inhibit proliferation (SMAD6/7) [25–27]. In this review, we outline the role of TGF-β signaling in MDS as well as preclinical and clinical data for TGF-β pathway inhibitors in the treatment of low-risk, non-del(5q) MDS.

TGF-β signaling in LR-MDS

Erythropoiesis is a tightly orchestrated process that is mediated by the simultaneous suppression of TGF-β signaling and the pro-survival and pro-differentiation effects of EPO [15, 25]. More than 30 soluble growth factors such as bone morphogenetic proteins (BMPs), growth differentiation factor 11 (GDF11) and activin comprise the TGF-β superfamily which play a central role in hematopoietic stem cell (HSC) proliferation and differentiation and are part of a myelosuppressive negative feedback loop under physiologic conditions [23–25]. TGF-β is an essential factor in the bone marrow niche that is produced locally by various cells including megakaryocytes and nonmyelinating Schwann cells and is stored in the bone matrix [25, 28, 29]. Binding of various ligands leads to TGF-β receptor activation and phosphorylation of the receptor which in turn activates a regulatory circuit of activating and inhibitory SMAD proteins [25–27]. These phosphorylated SMAD proteins act as transcription factors that can either promote gene transcription and erythroid differentiation (SMAD2/3) or inhibit proliferation (SMAD6/7) [25]. However, the effect of SMAD signaling is highly context dependent and is affected by cross-regulation with other regulatory circuits within the bone marrow niche [25, 27, 30]. One of these additional regulatory factors in hematopoietic stem cells is the ubiquitous nuclear protein Transcriptional Intermediary Factor 1γ (TIF1γ) that competes with SMAD4 for binding to phosphorylated SMAD2/3. While the complex of SMAD2/3-SMAD4 inhibits HSC proliferation by suppressing gene transcription in conjunction with various DNA binding cofactors, binding of TIF1γ to SMAD2/3 can stimulate erythroid differentiation by promoting access to otherwise repressed genes [30].

While downstream TGF-β signaling is tightly regulated under physiologic conditions, several alterations in this pathway have been identified in early stages of MDS. For example, several inhibitory factors in the TGF-β pathway such as SMAD7 and SKI have been shown to be diminished early in MDS leading to a constitutive activation of the TGF-β pathway and suppression of hematopoiesis [23, 31–33]. In addition, activin A and GDF11 were shown to be elevated in MDS patients compared to healthy controls and the increase in GDF11 levels was associated with erythroid hyperplasia and ineffective erythropoiesis in a mouse model of MDS [34]. In addition to canonical TGF-β receptor signaling via SMAD, activation of the TGF-β receptor also leads to the cross-activation of various other signaling pathways such as NF-κB via the TAK1-MEK-AKT pathway, the RAS/MAPK/ERK pathway, and the PI3K/mTOR pathway. These signaling pathways either lead to activation of various nuclear transcription factors or modulate the function of different SMADs. A detailed discussion of these pathways would be beyond the scope of this review and has been published elsewhere [35, 36]. Figure 1 illustrates the TGF-β signaling pathway and identifies potential therapeutic targets in MDS.

Of note, while genetic mutations affecting the TGF-β pathway have been identified in various solid tumors and occasionally lymphoid malignancies, loss-of-function mutations are uncommon in myeloid neoplasms [37–39]. Furthermore, it has instead been suggested that abnormal RNA and DNA splicing and differential gene expression rather than genetic mutations are the underlying mechanism for TGF-β signaling abnormalities in MDS [23, 25]. The importance of abnormal splicing is further supported by the finding that spliceosome mutations such as *SF3B1, SRSF2, U2AF1*, and *ZRSR2* are common in MDS and have been associated with ineffective erythropoiesis [40–45]. Mutations in *SF3B1* have been identified in up to 81% of patients with refractory anemia with ringed sideroblasts and the presence of this mutation has been linked to a favorable outcome compared to patients without *SF3B1* mutations [40, 46]. Therefore, the presence of spliceosome mutations

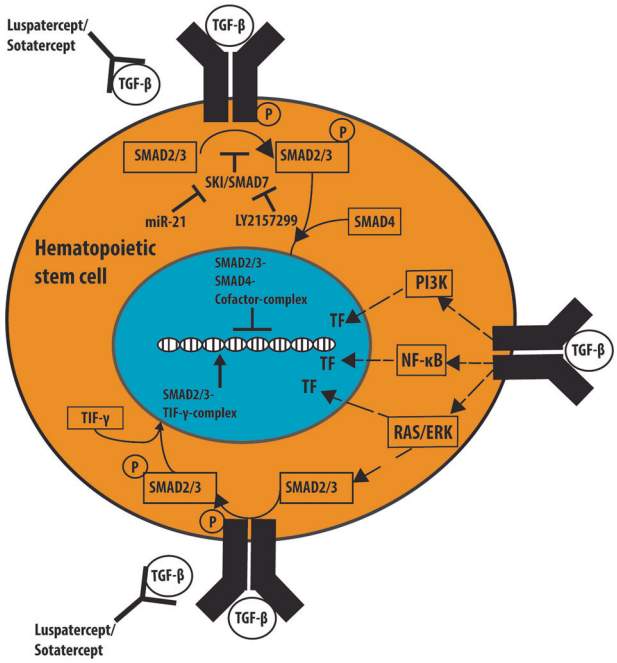

Fig. 1 Overview of TGF-β signaling pathway and potential therapeutic targets. The TGF-β receptor type I (TGF-βRI) which is also known as ALK5 is activated by various ligands of the TGF-β superfamily which leads to receptor phosphorylation. The binding of ligands such as activin to the receptor can be inhibited by the ligand traps luspatercept and sotatercept. TGF-βRI also has kinase activity and phosphorylates SMAD2/3 which can be inhibited by SKI or SMAD7 which have both been described as potential therapeutic targets for miR-21 and galunisertib (LY2157299), respectively. The effects of the SMAD signaling cascade are context-dependent and depend on whether phosphorylated SMAD2/3 binds to either SMAD4 or TIFγ. While binding of SMAD2/3 to SMAD4 and translocation to the nucleus inhibits hematopoietic stem cell proliferation in conjunction with various DNA-binding cofactors, the complex of TIFγ-SMAD2/3 stimulates erythroid differentiation. In addition to SMAD-signaling, TGF-βRI activation also cross-activates various other signaling pathways such as NF-κB via the TAK1-MEK-AKT pathway, the RAS/MAPK/ERK pathway, and the PI3K/mTOR pathway which leads to phosphorylation and activation of various transcription factors (TF) that regulate gene expression in the nucleus. ERK is a downstream kinase in the RAS pathway that can also activate SMAD2/3 via phosphorylation

may have important therapeutic implications with higher response rates to TGF-β pathway-directed treatments and can potentially aid in treatment selection for individual patients [40, 44].

TGF-β pathway inhibitors in the treatment of MDS

Since various in-vitro studies have implicated TGF-β signaling abnormalities in the ineffective hematopoiesis in MDS, multiple attempts to overcome TGF-β-mediated myelosuppression have been successfully performed [23, 31, 34, 47]. While the complexity of the TGF-β pathway offers various potential targets, enhancing the inhibitory effect of SMAD7 or inhibiting activin receptor-mediated signaling are the two most commonly studied approaches. SMAD7 deficiency which is mediated by increased levels of microRNA-21 (miR21) leading to impaired SMAD7 gene transcription can be successfully targeted in-vitro by inhibition of miR21 [32]. However, this strategy has not been tested in humans yet.

In addition, inhibiting the kinase activity of the TGF-β receptor and thereby reducing phosphorylation of SMAD2/3 by the small molecule inhibitor galunisertib (LY2157299) has been successfully tested in in-vitro models [31, 33]. Following these successful preclinical studies, galunisertib has been tested in a phase II study in 41 patients with very low-, low-, and intermediate-risk MDS by IPSS-R with the majority of patients being classified as refractory cytopenia with multilineage dysplasia (66.7%) or refractory anemia with ringed sideroblasts (20.5%) [48]. Preliminary data showed that galunisertib was overall well tolerated with 30.8% of patients experiencing treatment-emergent adverse events ≥grade 3. Galunisertib led to erythroid hematologic improvement in 26% of patients with 4 patients becoming transfusion-independent without improvements in platelet or neutrophil counts. There was no correlation between a particular cytogenetic or morphologic MDS subpopulation and response to galunisertib [48]. A phase II/III randomized, placebo-controlled, triple blinded trial (NCT02008318) of this agent has completed patient accrual for treatment of LR-MDS. Table 1 provides an overview of completed clinical trials of TGF-β pathway-targeted therapy in MDS.

Luspatercept (ACE-536) is a recombinant fusion protein consisting of a modified activin receptor type IIB and the Fc domain of human immunoglobulin (Ig) G1 that structurally resembles various TGF-β receptor ligands such as GDF11 and activin B and therefore inhibits TGF-β receptor signaling by acting as a ligand trap [12, 15]. In preclinical studies, the murine analog of luspatercept (RAP-536) promoted erythropoiesis and reduced anemia in a mouse model by inhibiting GDF11-mediated SMAD2/3 activation [34]. In addition, a phase I clinical trial in 24 healthy postmenopausal women with a baseline hemoglobin of 11.0–14.5 g/dl showed dose-dependent increases in hemoglobin levels without clinically relevant adverse events [49]. Notably, mean baseline hemoglobin was 13.2 g/dl and 83.3% of patients receiving the highest dose of luspatercept (0.25 mg/kg) had an increase in their hemoglobin of ≥1 g/dl. Response durations were 14 and 21 days in study subjects who received a single and two doses, respectively [49]. Importantly, effects of luspatercept were independent of EPO as it is affecting the later stages of erythropoiesis and therefore appeared to be a feasible option for patients with

Table 1 Completed clinical trials of TGF-β pathway inhibition in MDS

Drug	Target/mechanism	Phase (ref)	*N*	Patient characteristics	Outcomes
Sotatercept	Recombinant fusion protein of activin receptor type IIA+Fc domain of human IgG1	II [22]	74	Low-/intermediate-I risk MDS or CMML, transfusion-dependent, refractory to ESA, non-del(5q)	36 (49%) HI-E, 20 (27%) RBC transfusion independence; patients with ≥15% ringed sideroblasts higher response rates than <15% ringed sideroblasts (59% vs. 22%; $p = 0.008$).
Luspatercept	Recombinant fusion protein of activin receptor type IIA+Fc domain of human IgG1	II [12]	52	Low-/intermediate-I risk MDS or CMML; anemia+/− transfusion dependence	63% HI, 38% transfusion independence; higher response rates in patients with ≥15% ringed sideroblasts or *SF3B1* mutation
Galunisertib (LY2157299)	Inhibition of TGF-β receptor type I kinase activity	II/III	140	IPSS-R very low, low, or intermediate risk MDS	Trial completed

ESA-refractory anemia and elevated EPO levels at baseline [49]. This led to various clinical trials of luspatercept for various diseases characterized by ineffective erythropoiesis such as myelofibrosis, thalassemias, and MDS [12, 50, 51]. A phase 2 trial of luspatercept (PACE-MDS) has been recently published and the results from the large phase III study (MEDALIST trial; NCT02631070) have been presented but not yet published in a peer-reviewed journal (see below) [12, 21].

In an open-label phase II clinical trial (PACE-MDS) that tested luspatercept in 58 anemic patients with low or intermediate-I risk MDS or non-proliferative chronic myelomonocytic leukemia (CMML), 63% and 38% of patients achieved erythroid hematologic improvement (HI-E) and transfusion independence, respectively (51 and 42 evaluable patients, respectively) [12]. Notably, response rates to luspatercept were higher in patients with positive spliceosome mutation status (*SF3B1, SRSF2, U2AF1*, and *ZRSR2)* and ≥15% ringed sideroblasts. While response rates tended to decrease with higher serum EPO levels, even patients with an elevated EPO level at baseline showed a response to luspatercept (response rates of 58% in patients with EPO < 200 IU/l compared to 43% in patients with EPO > 500 IU/l) which is of great therapeutic relevance as these patients usually do not respond to exogenous ESAs and treatment options are otherwise limited. However, in a multivariate analysis of factors associated with RBC-TI lower serum EPO levels were statistically significantly associated with a higher rate of RBC-TI (<500 IU/L vs ≥500 IU/L; $p = 0.02$).

Importantly, luspatercept was very well tolerated with only three grade 3 treatment-related adverse events (one case each of myalgia, increased blast cell count and general physical health deterioration) [12]. While only treatment-related adverse events were reported in the phase II (PACE-MDS) study, data from the randomized, placebo-controlled phase III MEDALIST trial (NCT02631070) trial showed a rate of treatment-emergent adverse events grade 3–4 of 42.5% which was comparable to placebo (44.7%) [21]. These findings probably indicate that most adverse events are disease related. However, it has to be kept in mind that PACE-MDS was a single arm study and the final data from the randomized, placebo-controlled phase III study of luspatercept have only been recently presented as an oral presentation in the American Society of Hematology (ASH) annual meeting in December 2018 and not published yet (MEDALIST trial; NCT02631070) [21].

In the MEDALIST trial, the investigators randomized 229 RBC transfusion-dependent, non-del(5q) patients with very-low, low-, or intermediate-risk MDS by IPSS-R and ≥15% ringed sideroblasts or ≥5% with *SF3B1* mutation in a 2:1 ratio to receive either luspatercept or placebo. In addition, patients included had <5% bone marrow blast

percentage, were refractory to ESA (or had elevated serum EPO levels at baseline) and had not received any disease-modifying therapy such as HMAs or immunomodulators before [21]. The authors reported that 37.9% of patients in the luspatercept group met the primary endpoint of RBC-TI for more than 8 weeks compared to 13.2% in the placebo group ($p < 0.0001$). A response rate of 13.2% to placebo is substantially higher compared to other trials in LR-MDS that tested lenalidomide versus placebo which reported RBC-TI rates of up to 5.9% in the placebo group [52]. This unusually high response rate in the placebo arm might also reflect the lower minimum transfusion frequency required for eligibility in this trial compared to other trials. Responses to luspatercept appeared to be sustained with a median duration of RBC-TI of 30.6 weeks in the luspatercept and 13.6 weeks in the placebo arm, respectively and ~40% of patients treated with luspatercept maintained transfusion independence for 12 months [21].

While RBC-TI is the ideal outcome in these patients, it is also encouraging that HI-E as defined by a reduction in RBC transfusion burden by ≥4 RBC units/8 weeks or an increase in mean hemoglobin of ≥1.5 g/dL achieved in weeks 1–24 [53] was achieved by 52.9% of patients in the luspatercept and 11.8% in the placebo arm, respectively [21]. Notably, subgroup analyses showed that response rates for the primary endpoint were higher for patients with lower transfusion burdens and higher platelet counts at baseline. In addition, understanding that limited sample size limits definitive conclusions, subgroup analyses showed that luspatercept was not superior to placebo in patients requiring ≥6 units of RBCs per 8 weeks and in patients with a platelet count of $<100 \times 10^9$/L [21]. This suggests that luspatercept is more effective if given early in the disease course and in patients with less severe cytopenias while patients with more advanced disease may profit more from other disease-modifying agents such as HMAs or IST. Luspatercept was well-tolerated and there were no new safety signals compared to the phase II study [12, 21].

Importantly, the MEDALIST trial only included patients with ≥15% ringed sideroblasts or who were positive for a *SF3B1* mutation with 5% or more ringed sideroblasts, which have both been previously shown to be a predictive marker for response to luspatercept in the phase 2 trial [12, 21]. While responses in patients without *SF3B1* mutations or <15% ringed sideroblasts were observed with luspatercept in the phase 2 trial, the response rate is lower which underlines the importance of individualizing therapeutic approaches in MDS [12].

It is also important to note that a variety of treatment modalities including IST, lenalidomide, and low-dose HMA are available for patients with LR-MDS [10, 54] and that comparison to placebo in the MEDALIST trial instead of an active comparator arm precludes assessment of the efficacy of luspatercept compared to other therapeutic options.

Cross-trial comparisons should always be interpreted cautiously. Lenalidomide yielded RBC-TI in 56.1% in del(5q) LR-MDS, but only in 26.9% in non-del(5q) LR-MDS [52, 55]. While there is a lack of large randomized trials evaluating IST in LR-MDS, we have previously reported a large retrospective, multicenter study showing a RBC-TI rate of 30% with antithymocyte globulin (ATG) and cyclosporine, the most commonly used IST regimen in LR-MDS [11]. While RBC-TI rates up to 50% with HMA in LR-MDS have been reported, recent prospective trials of azacitidine showed RBC-TI rates of only 16–20% in LR-MDS even in combination with EPO; decitabine yielded a RBC-TI rate of 32% in one trial [56–61]. Direct head-to-head comparisons of luspatercept with either IST, HMA or lenalidomide are therefore warranted to further evaluate the role of luspatercept in the treatment of LR-MDS. However, given the molecular and cytogenetic heterogeneity of MDS with lenalidomide being only approved for MDS with del(5q) and luspatercept being shown to be most effective in patients with ≥15% ringed sideroblasts or who are positive for a *SF3B1* mutation, designing such trials will be difficult.

A recent placebo-controlled phase III trial of lenalidomide versus placebo in RBC-transfusion-dependent LR-MDS patients has shown that lenalidomide is effective in patients without del(5q) as well (RBC-TI 26.9% in the lenalidomide vs. 2.9% in the placebo group), which makes a trial comparing lenalidomide and luspatercept an interesting option [52]. Although the median duration of RBC-TI with lenalidomide in that trial was 31 weeks, there was no improvement in quality of life scores over placebo, and there was a high incidence of lenalidomide induced neutropenia and thrombocytopenia. It remains to be seen if luspatercept has a modifying effect on the progression rate to AML as well, which has been demonstrated for lenalidomide [55]. Table 2 provides an overview of ongoing clinical trials of luspatercept in MDS.

Similar to luspatercept, sotatercept (ACE-011) is a recombinant fusion protein of the activin receptor type IIA and the Fc domain of human IgG1 that inhibits SMAD2/3 activation by neutralizing various TGF-β receptor ligands including GDF11 [22, 62]. Several clinical trials showed its safety and erythropoiesis promoting effects in both healthy volunteers and patients with anemia post-chemotherapy or with various bone marrow disorders such as multiple myeloma and MDS [22, 62–64]. In a recent open-label, single arm phase II study (NCT01736683) of 74 low- or intermediate-1-risk, RBC-transfusion dependent patients with MDS and CMML, 36 (49%) and 20 (27%) patients achieved HI-E and RBC-TI, respectively [22]. Comparable

Table 2 Selected ongoing trials of TGF-β pathway inhibition in MDS

Drug	Target/mechanism	Phase	NCT	Patient characteristics	Intervention
Luspatercept	Recombinant fusion protein of activin receptor type IIA+Fc domain of human IgG1	II	NCT02268383	Low or intermediate-1 risk MDS	Open-label extension of NCT01749514 study of luspatercept
		III	NCT02631070 MEDALIST	Very low, low, or intermediate risk MDS in RBC transfusion-dependent patients with ring sideroblasts with ESAs failure or unlikely to respond to ESAs	Luspatercept vs. placebo
		III	NCT03682536	IPSS-R very low, low, or intermediate risk MDS, ESA-naïve, RBC transfusion-dependent patients	Luspatercept vs. epoetin alfa

to luspatercept, patients with ≥15% ringed sideroblasts achieved statistically significant higher response rates than patients with <15% ringed sideroblasts (59% vs. 22%; $p = 0.008$). Similar to luspatercept, higher serum EPO levels tended to be associated with a lower response rate (64% response rate for EPO <200 IU/l vs. 33% in patients with EPO >500 IU/l). However, this difference did not meet statistical significance ($p = 0.096$) which might be due to the small sample size in the sotatercept trial [22]. Interestingly, 97% of patients in the study had previously been treated with ESAs and 44% of the patients who had previously failed ESA therapy responded to sotatercept. This is likely due to different mechanisms of actions of ESAs and sotatercept along the erythropoietic differentiation process as EPO and its analogs promote the early stages of erythroid proliferation and survival while TGF-β pathway targeting therapies restore the later stages of erythropoiesis that are EPO-independent [15, 65].

Treatment-related adverse events grade ≥3 were comparable between luspatercept and sotatercept and occurred in ~5% of patients [12, 22]. Comparable to the 43% of patients with treatment-emergent grade 3–4 adverse events treated with luspatercept in the MEDALIST trial (which were not significantly different compared to the placebo arm), treatment-emergent grade 3–4 adverse events were reported in 25 (34%) out of 74 patients treated with sotatercept with a single grade 5 adverse event (subdural hematoma) [21, 22]. Although comparisons across studies are always limited due to different inclusion criteria, it is important to note that the response rates to sotatercept are similar to treatment with both lenalidomide and HMAs [52, 66, 67]. However, lenalidomide is not approved in the United States and Europe for treatment of non-del(5q) LR-MDS. In addition, rates of cytopenia are higher for treatment with lenalidomide and HMAs than with sotatercept which can be an important consideration when selecting treatment options for LR-MDS patients [22, 52, 68]. Given the absence of a comparator arm, additional, placebo-controlled or multi-arm studies are needed to determine the role of sotatercept in the management of LR-MDS. However, currently the manufacturer will not develop sotatercept further for treatment of MDS [10]. Other agents such as imetelstat and roxadustat are also in late clinical trial development for LR-MDS [10].

Future directions

Further studies are needed to fully elucidate the complex and intertwined TGF-β pathway and its crosstalk with other factors that influence hematopoiesis in the bone marrow niche. Even the mechanisms of action of both sotatercept and luspatercept are still incompletely understood. Given the heterogeneity of MDS, careful patient selection based

on genetic (e.g. spliceosome mutations) and morphologic features (presence of ringed sideroblasts) is warranted to optimize response rates. With the broader availability of more sensitive diagnostic techniques such as next-generation sequencing, mutational testing may be incorporated more broadly into an increasingly individualized treatment concept for MDS patients [69].

Recent studies have also shown that chronic inflammation with higher levels of the pro-inflammatory cytokine interleukin (IL)-6 might contribute to the myelosuppression and clonal expansion in MDS by induction of miR21 [70–72]. A recent in-vitro study could also show that miR21 mediates repression of SMAD7 and SKI activity which both act as suppressors of TGF-β signaling under physiologic conditions [23]. Targeting miR21 to enhance the expression of the TGF-β pathway inhibitors SMAD7 and SKI could therefore be another option with a different mechanism of action than luspatercept and sotatercept. Interestingly, higher expression of miR21 and SKI was observed in MDS with del(5q) which has been shown to be associated with alterations in the TGF-β signaling pathway and microRNA profile [23, 73]. As only patients without del(5q) were included in the luspatercept and sotatercept trials, targeting miR-21 might be a potential therapeutic option in these patients. In addition, patients with higher levels of IL-6 may also be candidates for miR-21-directed therapy. However, there are no clinical trials testing this approach so far. Targeting multiple components of the TGF-β signaling cascade might also have synergistic effects.

Finally, the combination of TGF-β pathway-targeted therapy with ESAs may have synergistic effects to improve anemia as they are affecting different stages of erythropoiesis. While the early stages of erythroid proliferation and survival are dependent on EPO, TGF-β pathway targeting therapies restore the later stages of erythropoiesis that are EPO-independent and the combination may therefore improve anemia more than either drug alone [15, 65]. Combinations of sotatercept and lenalidomide have only been tested in multiple myeloma so far with the rationale that both can improve osteolytic bone lesions and anemia in these patients (NCT01562405). Lenalidomide has been shown to have osteoclastic side effects by increasing the secretion of activin A by bone marrow stromal cells which can be reversed by combination with sotatercept which acts as an activin A-neutralizing antibody [74, 75]. One of the various immunomodulatory effects of lenalidomide is the downregulation of inflammatory markers such as IL-6 which has been associated with myelosuppression and activation of the TGF-β pathway [74, 76]. While no clinical trial data have been published yet, combining lenalidomide and sotatercept in MDS may also yield synergistic effects with regards to improving anemia.

Conclusions

For lower-risk MDS patients with anemia predominance the main focus of treatment is to reduce symptom burden by minimizing transfusion needs and the sequelae of the resulting iron overload. ESAs remain the mainstay of therapy for these patients. However, given the elevated baseline EPO levels, a substantial proportion of patients is refractory to exogenous ESAs and additional treatment options are needed. The ineffective hematopoiesis in MDS has been linked to chronic activation of the myelosuppressive TGF-β signaling pathway. Recently, the recombinant activin receptor type II ligand traps luspatercept and sotatercept have been shown to yield hematologic improvement in transfusion-dependent, non-del (5q) LR-MDS patients. These drugs were especially active in patients with a higher percentage of ring sideroblasts and in patients harboring a *SF3B1* mutation, which again underlines the heterogeneity of MDS and the importance of careful patient selection. However, further results from the MEDALIST trial are awaited and other potential targets in the TGF-β signaling cascade such as miR-21, LY2157299 or combination with other treatment modalities such as lenalidomide and ESAs warrant further investigation as well.

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Compliance with ethical standards

Conflict of interest A.M.Z. received research funding (institutional) from Celgene, Acceleron, Abbvie, Otsuka, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Trovagene, Incyte, Takeda, and ADC Therapeutics. A.M.Z. had a consultancy with and received honoraria from AbbVie, Otsuka, Pfizer, Celgene, Agios, Boehringer-Ingelheim, Novartis, Acceleron, Astellas, Daiichi Sankyo, Ariad, Cardinal Health, Beyond Spring, Seattle Genetics, and Takeda. A.M.Z. received honoraria from and was a speaker for Takeda (past). A.M.Z. is one of the MEDALIST investigators. None of these relationships were related to the development of this manuscript. The other author declares that he has no conflict of interest.

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