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REVIEW ARTICLE

Myelodysplastic syndrome

The role of TGFβ in hematopoiesis and myeloid disorders

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Abstract

The role of transforming growth factor-β (TGFβ) signaling in embryological development and tissue homeostasis has been thoroughly characterized. Its canonical downstream cascade is well known, even though its true complexity and other non-canonical pathways are still being explored. TGFβ signaling has been described as an important pathway involved in carcinogenesis and cancer progression. In the hematopoietic compartment, the TGFβ pathway is an important regulator of proliferation and differentiation of different cell types and has been implicated in the pathogenesis of a diverse variety of bone marrow disorders. Due to its importance in hematological diseases, novel inhibitors of this pathway are being developed against a number of hematopoietic disorders, including myelodysplastic syndromes (MDS). In this review, we provide an overview of the TGFβ pathway, focusing on its role in hematopoiesis and impact on myeloid disorders. We will discuss therapeutic interventions with promising results against MDS.

Introduction: TGFβ

The transforming growth factor β (TGFβ) signaling pathway has been investigated for >30 years. This has led to progressive understanding of the different steps involved in the function of this superfamily of cytokines and receptors, as well as its diverse effects on multiple cellular types [1, 2]. TGFβ ligands act as a communication network between cells and also the extracellular matrix, affecting the transcriptional status of multiple targets. TGFβ signaling has been preserved among metazoan organisms through evolution and has an important role in embryologic development and tissue homeostasis [3]. Specifically, TGFβ growth factors are involved in embryonic stem cell differentiation, organ and body axis generation, and controlling diverse cellular events such as proliferation, adhesion, metabolism, and apoptosis [4, 5]. In addition to its regulatory function on multiple tissues, TGFβ signaling has an important role in certain pathological conditions including cardiovascular and fibrotic diseases, immunological dysregulation, and oncogenesis [6–9].

The superfamily of TGFβ ligands comprises 33 structurally similar proteins, secreted mainly as homodimers and carrying a cysteine-knot motif [10]. These ligands can be classified into two subfamilies: TGFβ and bone morphogenic protein (BMP), which are functionally opposed. The TGFβ subfamily includes TGFβ1, TGFβ2, and TGFβ3, activins A and B, nodal, myostatin, and different members of the growth and differentiation factor (GDF) group. The BMP subfamily includes >10 different proteins and also the anti-Müllerian hormone (AMH) [5]. Despite the large number of ligands, there are fewer receptors and downstream intracellular signaling molecules, the last of which are denominated mothers against decapentaplegic homologs (SMADs). As summarized in Fig. 1, once a receptor is activated, downstream signaling leads to activation of SMADs, which in turn reach the nucleus, interact with different transcriptional machinery, and promote diverse cellular responses.

An overview of the TGFβ pathway

Receptor binding

Before targeting the surface receptor, the activity of TGFβ ligands is regulated by multiple variables [11], including the amount and type of TGFβ ligand, as well as the amount and

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type of receptors in the target cell. In addition, the existence of extracellular ligand traps and antagonistic ligands modulate signal intensity. For example, the BMP antagonist gremlin 1 (GREM1) inhibits BMP signaling in some types of colorectal carcinoma, maintaining a more mesenchymal or stem cell phenotype in these neoplasms, which may be associated with a self-renewing tumoral niche [12]. Another regulatory element is the need for accessory receptors to activate the principal receptor, such as β-glycan for TGFβ1, TGFβ2, and TGFβ, or CRIPTO for GDF1, GDF3, and Nodal [13, 14]. Moreover, these co-receptors may also act as membrane inhibitors, such as for Activin signaling [13].

Activation of the receptor

Once the ligand binds the TGFβ receptor, the signal is transmitted downstream to the nucleus. The TGFβ receptor is an heterotetrameric complex formed by the assembly of two pairs of components with serine/threonine kinase activity: the type I receptor component, which propagates the signal, and of which there are five subtypes, and the type II receptor component, which activates type I receptor, and of which there are seven subtypes [15]. Every TGFβ ligand has binding selectivity to diverse receptors according to the combination of type I and type II receptor components that determines the cellular sensitivity of each particular TGFβ ligand type [5]. As an example, the type 1 receptor TGFBR1 assembled with the type 2 receptor TGFBR2 can be activated only by TGFβ ligands of the TGFβ family, whereas TGFBR1 assembled with the activin type 2 receptor (ACVR2) can be activated by myostatin (also known as GDF8) or GDF11 [16, 17]. In addition, a number of molecules can interfere with the receptor to prevent its activation. Such molecules include FK506 binding protein 12 (FKBP12), which locks the TGFRB1 in an inactivated state, or BMP and activin membrane-bound inhibitor homolog (BAMBI), which prevents the activation of the receptor [15]. Upon ligand-receptor binding, type II component-mediated phosphorylation of the type I component leads to subsequent SMAD protein activation and downstream signaling [18].

Cytoplasmic pathways

Canonical TGFβ signaling is dependent upon SMAD proteins. There are eight SMAD proteins, predominantly located in the cytoplasm, including six effectors (SMAD1–5 and SMAD8) and two inhibitors (SMAD6 and 7) [19]. Structurally, SMAD proteins have two globular domains separated by a linker region: one (MH1) binds the DNA and the other (MH2) interacts with diverse proteins, such as other SMADs, transcription factors, or histone modifying enzymes [20]. Type I receptors triggered by TGFβ-like ligands (TGFBR1, ACVR1B, and ACVR1C) activate SMAD2 and 3 (SMAD2/3), whereas type I receptors triggered by BMP-like ligands (BMPR1A, BMPR1B, ACVR1A, and ALK1) activate SMAD1, 5, and 8 (SMAD1/5/8) [5]. These activated SMADs are also known as R-SMADs and are phosphorylated at the linker region, allowing their further assembly with SMAD4 in order to form trimeric structures (two R-SMADs and SMAD4). Phosphorylation of R-SMAD is controlled by regulatory proteins such as SMAD anchor for receptor activator (SARA) or endofin [21]. Once the trimeric structure is formed, this complex can translocate to the nucleus and interact with other DNA regulators to modify transcriptional activity [22]. Inhibitory SMADs (SMAD6 and 7) prevent downstream activation via TGFβ receptor ubiquitination, assembly with SMAD4, or R-SMADs phosphorylation blockade [23]. Signaling from TGFβ in order to activate SMADs is regulated by clathrin-associated sorting protein Disabled-2 (Dab2), which contains an N-terminal phsphotyrosine binding domain (PTB) or phosphotyrosine interacting domain (PID) and a C-terminal proline-rich domain (PRD). Both the PTB and PRD domains are required for TGFβ signaling and phosphorylation of SMADs [24]. Epigenetic downregulation of Dab2 has been associated with a functional switch in TGFβ signaling leading to tumor promoter instead of tumor-suppressor effects [25].

In addition to the canonical SMAD activation pathway, TGFβ receptors can also activate non-canonical signaling cascades, including the mitogen-activated protein kinase (MAPK) pathway that includes c-Jun amino terminal kinase (JNK), p38 MAPK, or extracellular regulated kinases (ERKs). Other pathways such as phosphatidylinositol-3 kinase and AKT (PI3K-AKT) and Rho-like GTPases are also triggered by the TGFβ receptor [26]. Although the SMAD pathway is considered the central TGFβ cascade, these alternative signaling routes may establish a crosstalk with the SMAD pathway, modulating its activation and its final output [2]. The detailed mechanisms and influence of this interrelationship in different physiological and pathological contexts are still unclear.

Nuclear interactions

R-SMADs and SMAD4 traffic between cytoplasm and nucleus is controlled by contact with nuclear pore complex proteins. However, oligomerization of R-SMADs with SMAD4 requires specific importing factors to enter the nucleus [27]. After reaching the nucleus, the R-SMAD/SMAD4 complex interacts with diverse transcription factors to modulate genetic expression patterns. Specifically, the different R-SMADs assemble with SMAD4 to bind to different DNA sequences, although this poorly explains the different transcriptional output of each TGFβ ligand [28]. The presence or absence of certain transcription factors also

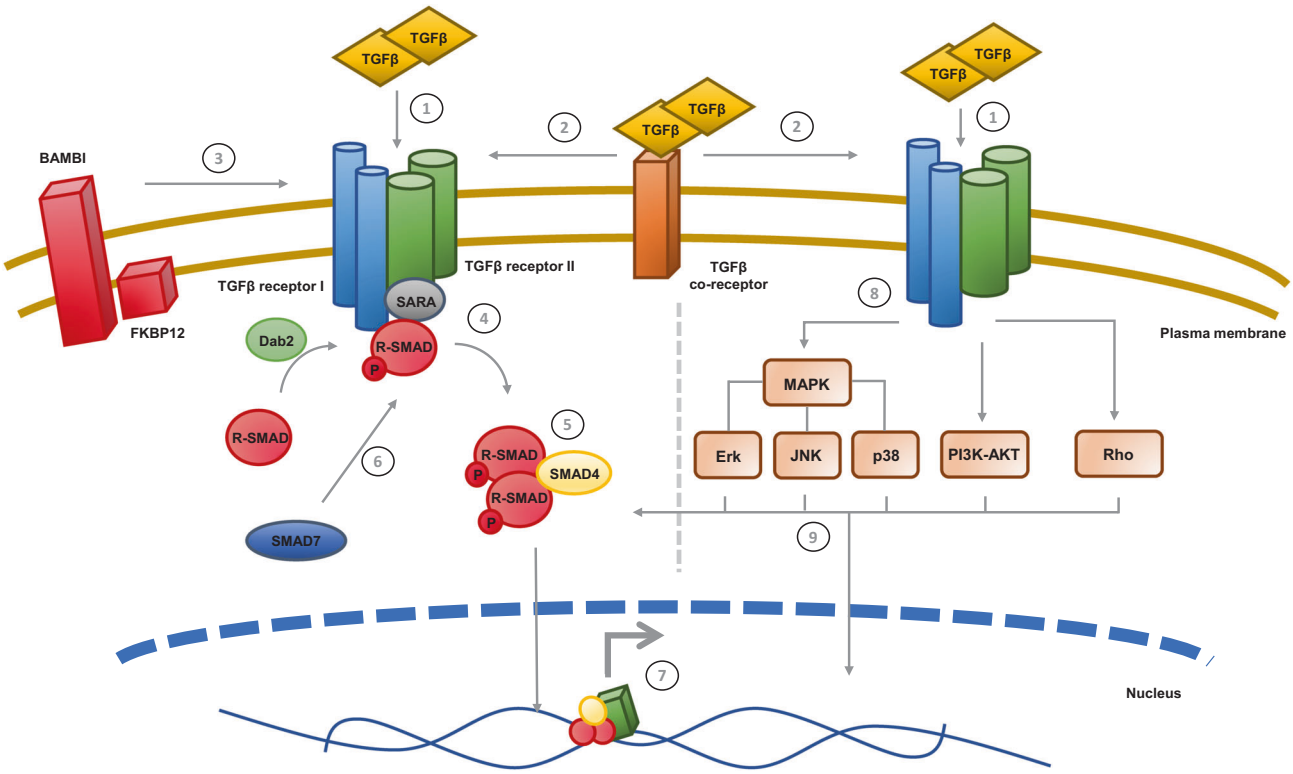

Fig. 1 TGFβ ligands are released at the extracellular space, triggering the TGFb receptor in a dimeric structure (1). Some TGFb receptors need the presence of co-receptors (such as betaglycan) to facilitate the activation (2). After being activated, the TGFβ receptor I phosphorylates R-SMAD proteins (SMAD2/SMAD3 or SMAD 1/SMAD5/SMAD8), activating the canonical pathway (4). Some proteins enhance this phosphorylation (SARA), but inhibitors (e.g., BAMBI or FKBP12) can prevent it (3). SMAD7 is an inhibitory SMAD that prevents the downstream activation of the SMAD pathway (6). After being phosphorylated, R-SMAD binds to SMAD4 (5) and translocates to the nucleus, recruiting diverse transcriptional factors and enhancing specific genetic expression (7). Besides the canonical pathway, the TGFb receptor can activate non-SMAD, non-canonical signaling pathways, such as MAPK, PI3K-AKT, or Rho pathways (8) that can modulate SMAD downstream regulation and modify transcriptional patterns (9)

influences transcriptional changes in a specific cell. For example, activated SMAD2/3 bind different transcription factors depending on the cell lineage: Oct4 in embryonic stem cells, Myod1 in myotubes, and PU.1 in pro-B cells [29]. After R-SMAD binds to DNA in conjunction with the transcription factors, the transcriptional machinery is recruited in order to initiate transcription. Moreover, activated R-SMAD can also recruit chromatin and DNA modifiers, such as histone acetyl transferases to stimulate transcription, and histone deacetylases to repress specific regions [30]. For instance, R-SMAD can bind to tripartite motif containing 33 (TRIM33) at the cytoplasm instead of SMAD4, and can then bind and open the chromatin harboring master differentiation genes, which normally is kept silent due to the presence of histone H3 with specific methylatios and acetylation.

TGFβ in hematopoiesis

TGFβ signaling is crucial for diverse biological processes in multicellular organisms, influencing both embryological development and maintenance of adult homeostasis (Fig. 2). Although the BMP subfamily includes important regulators of hematopoietic stem cell progenitor (HSCP) development from embryonic stem cells, their clear function in hematopoiesis has not been fully elucidated [31]. Even though hematopoiesis includes myeloid and lymphoid development, TGFβ ligands participate in a high number of processes within lymphoid development, including cellular lineage determination and immunological regulation. The specific mechanisms are beyond the scope of this review, and have been described in previous publications [5, 7, 32].

Embryogenesis and HSCP

Hematopoietic tissue derives from the embryonic mesoderm and its anatomical location changes during embryological development (from yolk sac to bone marrow going through the aorta gonad mesonephros, placenta, and fetal liver). BMP family ligands have been demonstrated to directly participate in the differentiation of hematopoietic progenitors from the mesodermal layer [33]. However, in

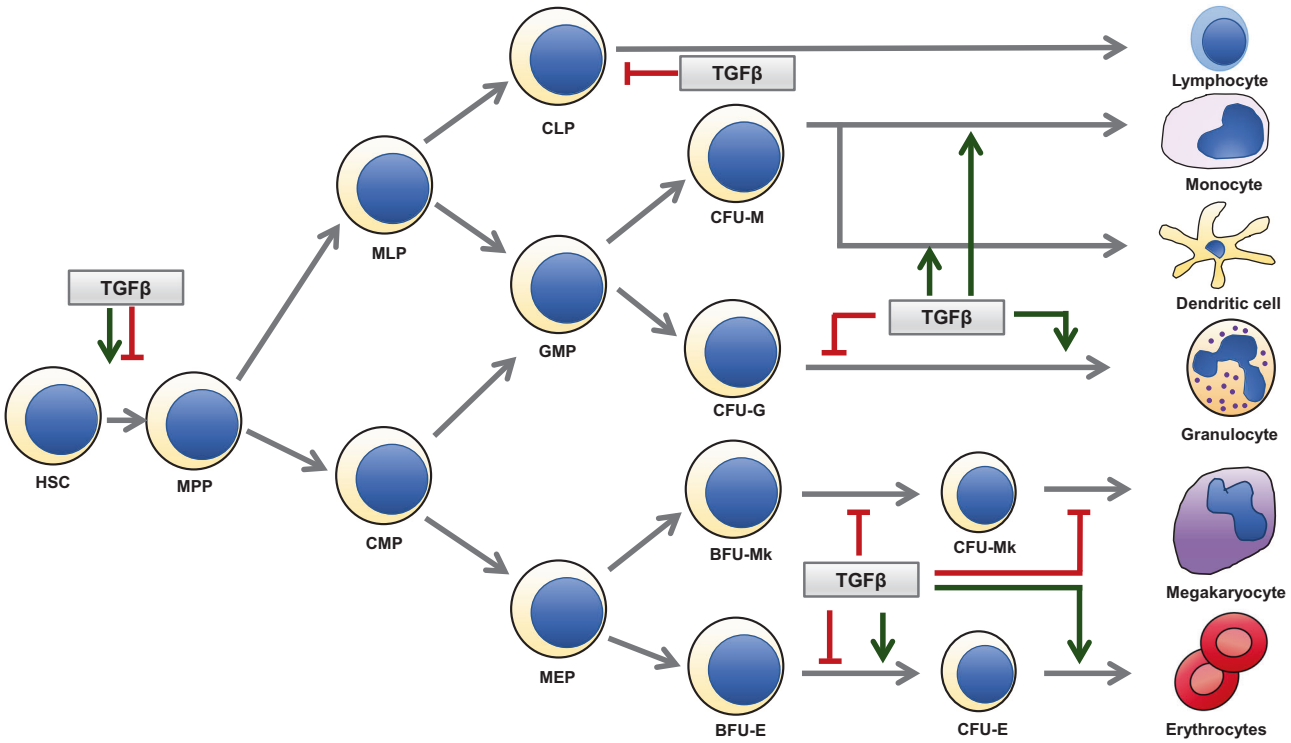

Fig. 2 Role of TGFβ in the different hematopoietic lineages. HSC hematopoietic stem cell, EMkP erythroid-megakaryocyte progenitor, GMP granulocyte-monocyte progenitor, CFU-M colony- forming unit monocyte. CFU-G colony-forming unit granulocyte

adulthood, the role of BMP on HSCP is not as well understood as that of the TGFβ subfamily ligands.

TGFβ exerts an inhibitory stimulus to the HSCP in terms of differentiation and proliferation [31, 33–35]. Specifically, TGFβ maintains quiescence of HSCP, preventing their commitment to a specific lineage. In vitro experiments exposing HSCP to high levels of TGFβ1 showed decreased proliferation without induction of differentiation or apoptosis [36]. This hibernation status may be achieved due to the upregulation of p57 cyclin-dependent kinase inhibition as a result of TGFβ signaling [37]. Furthermore, blocking TGFβ signaling in cultured cells using antibodies or antisense oligonucleotides caused HSCP to resume the cell cycle [38].

In addition, different biologic effects can be observed according to the amount of TGFβ that HSCP are exposed to. As such, although the presence of a high concentration of TGFβ maintains the HSCP in a dormant status, low doses of TGFβ1 could have stimulatory effects, specifically in the subset of HSPC with a higher tendency of generating myeloid descendants (myeloid-biased HSCP) [39]. Moreover, TGFβ signaling has an important role in hematopoietic lineage determination, controlling the transcriptional program of specific hematopoietic cells. It has been demonstrated that activated SMAD1 colocalizes with different lineage transcription factors at specific genome sites depending on the committed precursor: GATA 1 and GATA 2 in erythroid progenitors, C/EBPα in myeloid progenitors, and PU.1 in lymphoid progenitors [29]. In addition, recent studies suggest different HSCP subtypes may respond differently to TGFβ with myeloid-biased HSCs being stimulated and lymphoid-biased HSCP growth being inhibited by TGFβ [39–41]. This is of particular importance considering myeloid-biased hematopoiesis with impaired lymphoid potential is not only characteristic of the normal aging process, but also observed in myeloid malignancies such as myelodysplastic syndromes (MDSs). In fact, TGFβ is known to be implicated in the aging process of non-hematopoietic tissues [42].

Bone marrow microenvironment

The bone marrow niche includes a diversity of cells that interact with each other and with non-cellular components to regulate bone metabolism, blood production, and hematopoietic stem cell homeostasis [43]. In this environment, TGFβ ligands, together with other cytokines, work as signaling molecules involved in niche regulation. The most common ligand in the bone marrow microenvironment is TGFβ1, which is usually trapped in the extracellular matrix in its inactive latent form, bound to latent-associated peptide (LAP) [44]. Surprisingly, Yamoussoukro et al. observed that active TGFβ1 was located predominantly in Schwann cells of the autonomic nerves of the bone marrow, which

also had an enhanced expression of integrin-αvβ8 [45]. This adhesion molecule could be the key to binding LAP, exposing inactive TGFβ1 to metalloproteinases, and releasing active TGFβ1 to the bone marrow. Furthermore, the same study demonstrated that experimental denervation of the bone marrow provoked a decrease of these glial cells and therefore enhanced the proliferative capacity of HSCP, with a further reduction of the absolute HSCP cells [45]. Also, TGFβ-mediated effects in mesenchymal cells is mediated by Dab2. Loss of Dab2 has been associated with enlarged early endosomal antigen1-positive endosomes and inhibition of endosomal recycling [46].

In addition to latent TGFβ1, megakaryocytes are an important source of TGFβ1 and participate in maintaining its role in the microenvironment. SMAD2/3 activation has been demonstrated to be increased in megakaryocytes, translating increased activity in TGFβ1 signaling. Moreover, megakaryocyte knock down in HSCP culture was associated with a reduction of CD34+ cells, suggesting that TGFβ1 from megakaryocytes may have an important role in HSCP long-term maintenance due to its capacity to induce quiescence [47]. In addition, TGFβ and BMP are known to have a crucial role in bone remodeling by modulating the migration of mesenchymal cells in order to maintain bone homeostasis and promote healing [48].

Erythroid development

The earliest erythroid-committed cells are the burst-forming unit erythroid progenitors (BFU-Es), which are defined based on their ability to produce colonies in specific cultures with "bursts" of differentiating cells inside, expressing CD34 and CD45 and lacking CD36 [49]. Although they exhibit a low proliferative rate, "early" BFU-Es maintain a higher self-renewal capacity than "late" BFU-Es [50]. Erythroid-committed cells can be further differentiated into additional states, including the colony-forming unit erythroid progenitors (CFU-Es), which have reduced proliferative activity and rapidly differentiate to more mature erythroid progenitors (erythroblasts), losing their organelles, reducing their size, and inducing production of hemoglobin.

TGFβ signaling has an essential role in erythropoiesis, with a dual effect depending on the cellular differentiation stage. BFU-Es respond to TGFβ1 with proliferation arrest, increasing the length of mitotic G_1 phase, but also produce a differentiating response from early BFU-Es to late BFU-Es and CFU-Es [51]. Similarly, TGFβ1 reduces mitotic activity and forces differentiation of CFU-Es to more mature stages, working synergistically with erythropoietin (Epo) [51]. This response may be explained by different responses to activated SMAD2/3, as demonstrated by He et al. [52]. Using in vitro short hairpin RNA gene silencing techniques, they observed that the interaction of SMAD2/3 with SMAD4 inhibited proliferation of erythroid progenitors, while SMAD2/3 interaction with TRIM33 was followed by activation of erythroid maturation.

TGFβ1 has been the most studied TGFβ ligand in hematopoiesis, while the specific actions of other TGFβ family molecules remain less understood. TGFβ2 may have a proliferative effect on erythropoiesis and activin A might be an enhancer in erythropoiesis differentiation increasing the presence of Epo receptors and decreasing GATA 2 expression [53, 54]. The role of other TGFβ ligands are less known, but BMP4 could have a positive effect on erythroid differentiation and its downstream blockade has been shown to impair erythropoiesis [54, 55].

In summary, the overall response of erythroid progenitors to TGFβ ligands (mainly TGFβ1) results in reduced erythropoiesis due to the inhibition of their mitotic activity, although the remaining progenitors suffer from rapid differentiation to mature forms [31, 35]. In fact, the use of TGFβ inhibitors enhance erythropoiesis without critically impairing their maturation, and this has led to targeting this pathway in clinical situations involving ineffective erythropoiesis [50, 56–58].

Granulocytic/monocytic development

Granulocytes and monocytes derive from a common progenitor (CFU granulocyte/macrophage [CFU-GM]), which also derives from a CFU granulocyte/erythrocyte/monocyte/macrophage. It has been observed that TGF-β ligands also control proliferation and maturation of this lineage, but its role is highly dependent on the activity of other cytokines and opposed effects of these ligands have been described in vitro [34, 59]. At early CFU-GM stages, TGFβ1 signaling has been proposed as an inhibitory input [34]. However, at more mature stages, it seems to have a stimulatory effect, enhancing proliferation and differentiation synergistically with GM colony-stimulating factor (GM-CSF) [60]. Data from murine models further support the synergy between GM-CSF and TGFβ1 in enhancing the growth and differentiation of granulocytic precursors [59]. Moreover, upregulation of GM-CSF receptors in murine granulocytic progenitors triggered by the effect of the TGFβ1 has also been described [61]. It is thought that TGFβ2 could have the same effects as TGFβ1, and that TGFβ3 could have an inhibitory role only at early stages [62].

Dendritic cell development

Dendritic cells develop from a common monocytic-dendritic progenitor, known to be a specialized antigen-presenting cell that can be located in many tissues [63]. It has been observed that TGFβ1 is required for their differentiation, as demonstrated by the observation that TGFβ1

knockout mice exhibit a lack of dendritic cells [64, 65]. Moreover, TGFβ1 upregulates the expression of *FLT3*, *IRF8*, and *IRF4* in multipotent hematopoietic progenitors, which are known to be part of the dendritic cell lineage expression pattern [66]. Also, TGFβ1 accelerates the differentiation of conventional dendritic cells from the common dendritic progenitors [67].

Megakaryocytic development

Committed megakaryocytic progenitors are also classified as BFU and CFU-megakaryocytes (BFU-Mks and CFU-Mks, respectively). Early BFU-Mk tend to expand, enhanced by regulators like thrombopoietin (TPO), Kit ligand (also known as stem cell factor; SCF), IL3, IL6, or IL11. Subsequently, CFU-MK differentiate to megakaryocytes and initiate platelet formation via a process named endomitosis [68]. As in other lineages, TGFβ1 acts as an inhibitory input in early development stages, preventing proliferation of megakaryocytic progenitors. However, at late stages it also acts as a negative signal, including inhibition of endomitosis and prevention of platelet production [69, 70].

Despite this inhibitory role in megakaryopoiesis, megakaryocytes, and platelets remain one of the most important sources of TGFβ1 in the bone marrow, with TGFβ1 being stored inside α-granules and being released in response to diverse signals. It is thought that this signaling could act as a negative feedback to control the differentiating process and platelet production.

TGFβ in myeloid malignancies

TGFβ signaling has been described as an involved pathway in some neoplastic diseases of the bone marrow, summarized in the following sections (see also Table 1).

MDSs

MDS are clonal diseases of the hematopoietic stem cell, characterized by bone marrow dysplastic features and cytopenias due to impaired hematopoiesis. The TGFβ pathway has been shown to be involved in the pathophysiology of these diseases [43, 71]. Upregulation of TGFβ signaling has been proposed as one of the causes of ineffective hematopoiesis. Thus, its inhibition could be a target against MDS cytopenia. Increased levels of activated SMAD2 and inhibition of SMAD7 has been described in MDS cells, suggesting downstream TGFβ pathway signaling activation [72]. In those cells, microRNA-21 (miR-21) is highly expressed, preventing the expression of the downstream inhibitory ligand SMAD7, and therefore inhibiting erythropoiesis [73]. Due to these observations, TGFβ signaling inhibitors have been tested to determine if this process could be reverted, with exciting results. Zhou et al. demonstrated enhanced erythropoiesis in vitro in MDS cells as a result of TGFβ receptor I (TGFRI) inhibition, and could observe similar results in vivo using an MDS murine model [74]. Further studies evaluating MDS animal models have also shown increased expression of GDF11 in bone marrow and spleen, leading to inhibition of erythroid proliferation [57]. Use of inhibitors of TGFβ signaling to improve anemia in MDS patients is now a reality, with some TGFβ receptor inhibitors currently in advanced clinical development (see below "Novel agents in TGFβ signaling in myeloid malignancies") [57, 58].

Acute leukemia

Acute leukemias represent the most aggressive HSCP-derived neoplasms and include myeloid (AML) and lymphoid (ALL) subtypes. In contrast to solid tumors, TGFβ signaling alterations have not been determined to be the central role in leukemogenesis, but still play critical roles in certain situations [75]. Imai et al. described two SMAD4 mutations in AML that might explain its insensitivity to TGFβ ligands. In addition, Jakubowiak et al. demonstrated SMAD inhibition through a potential interaction between SMAD3 and the chimeric transcription factor AML1/ETO (currently known as RUNX1/RUNXT1) present in t(8;21) AML [76]. Interaction between SMAD3 and the fusion product (AML1/EVI1) in t(3;21) AML has also been described [77].

Acute promyelocytic leukemia (APL) is characterized by rearrangement of the promyelocytic leukemia gene (*PML*) and the retinoic acid receptor alpha gene (*RARα*). Wild-type PML protein is predominantly localized in the nucleus but can also be present in the cytoplasm, where it enhances the interaction between inactivated SMAD proteins (forming a complex with SARA) and the TGFβ receptor, leading to activation of the SMAD pathway through phosphorylation. Antagonism between PML/RARα and cytoplasmic PML has been described in APL, blocking SMAD phosphorylation and therefore downregulating TGFβ signaling [78]. Pathway gene enrichment studies demonstrated low TGFβ signaling gene enrichment in t(8;21) and t(15;17) AML. However, this enrichment is less clear in other forms of leukemia with recurrent translocations, such as inv(16) AML involving CBF/MYH11 [79].

The TGFβ pathway is also involved in some of the pathogenic features of ALL. In pediatric T-cell ALL (T-ALL), low or absent levels of SMAD3 have been described, and may explain T-ALL insensitivity to TGFβ signaling. There was no association between loss of SMAD3 and T-ALL induction in SMAD3 knockout mice

Table 1 Role of TGFβ in different acute leukemia and other myeloid malignancies

Diseases	Role of TGFβ	Biological effects
MDS	■ Increased TGFβ signaling	■ Ineffective erythropoiesis
	■ Downstream of SMAD7 signaling	
AML	■ Insensitivity to the inhibitory TGFβ effects	■ Loss of TGFβ inhibitory effect
	■ Interaction between AML1/ETO chimeric protein and SMAD3 in AML with t(8;21)	■ Suppressed TGFβ signaling
	■ Downregulation of SMAD pathway due to interaction with PML/RARα in acute promyelocytic leukemia	■ Suppressed TGFβ signaling
ALL	■ Low or absent SMAD3 in pediatric T-ALL	■ Insensitivity to TGFβ signaling
	■ SMAD3 inhibition by TEL/AML1 chimeric protein in B-ALL with t(12;21)	■ Decreased *CDKN1* expression
CML	■ TGFβ expression by CML hemangioblasts with subsequent enhanced MMP9 and s-ICAM expression	■ Immune evasion and peripheral mobilization
	■ TGFβ insensitivity due to AKT activation and FOXO3a kidnapping	■ Loss of TGFβ inhibitory effect
	■ TGFβ regulation of the dormant CML cells	■ Quiescence maintenance
ET	■ Decreased SMAD4 expression in megakaryocytes	■ Loss of TGFβ inhibitory effect
MF	■ Possible role of TGFβ in MF due to its capacity of enhance collagen synthesis	■ Fibrosis enhancement

MDS myelodysplastic syndrome, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *CML* chronic myeloid leukemia, *ET* essential thrombocytosis, *MF* idiopathic myelofibrosis, *TGFβ* transforming growth factor-β

[80]. However, the homozygous loss of cyclin-dependent kinase inhibitor 1B (CDKN1 or $p27^{Kip1}$) along with the loss of one SMAD3 allele promoted transformation to T-ALL in mice [80]. In addition, the chimeric protein TEL/AML1 resulting from t(12;21) in a subset of B-cell ALL (B-ALL) is hypothesized to inhibit SMAD3 and bind to DNA co-repressors to inhibit *CDKN1* gene transcription [81]. Finally, the human T-cell leukemia virus 1 (HTLV1) carries a transcriptional activator factor (TAX) that activates viral genome transcription and also induces constitutive activation of the JNK pathway, avoiding the DNA–SMAD3 union [82]. This prevents the activation of the TGFβ transcriptional pattern [82].

Chronic myeloid leukemia

The *BCR/ABL* fusion gene induces a constitutively activated tyrosine kinase (TK) that maintains leukemic cells in a persistent proliferative status, in chronic myeloid leukemia (CML). Compared with controls, in vitro CML hemangioblasts have been demonstrated to express higher levels of TGFβ1, which acts as a paracrine and autocrine factor enhancing expression of MMP9, subsequently increasing s-ICAM and sKitL production [83]. Interestingly, s-ICAM1 prevents tumor recognition by T-lymphocytes, and sKitL enhances peripheral mobilization of CML cells. Exposure of these cells to imatinib leads to a decreased in TGFβ1 levels [84]. In addition, AKT activation and FOXO3a arrest appear to be responsible for CML cell resistance to TGFβ inhibition by preventing transcriptional response to TGFβ signaling [85]. In addition, Naka et al. described possible TGFβ regulation of dormant CML cells via downregulation of AKT and induction of FOXO3a, maintaining these cells in a quiescent status and preventing their response to TK inhibitors [86].

Philadelphia-negative myeloproliferative neoplasms

Megakaryocytic insensitivity to TGFβ1 inhibition has been described as in essential thrombocythemia and might explain the proliferative advantage of this disease [87]. This resistance can be explained by decreased expression of SMAD4 in CFU-Mk, impairing appropriate signaling of TGFβ ligands [88]. Moreover, TGFβ1 has been identified as an important cytokine involved in primary myelofibrosis (PMF). In a study evaluating transplanted mice with *TGFβ1* null or wild-type progenitors, transfection with a retrovirus that enhanced TPO expression and induced medullary fibrosis led to lower reticulin fibrosis in *TGFβ1* null progenitors, compared with wild type [89]. In addition, another study used two PMF murine models (ectopic TPO expression and hypomorphic *GATA1* mutated mice) detected higher levels of extracellular TGF-β1 in mutated mice, associated with development of PMF [90]. TGFβ1 is known to play a role in collagen synthesis [90].

Novel agents in TGFβ signaling in myeloid malignancies

As the TGFβ pathway has been described as a key alteration in some blood diseases (mainly MDS), a number of

Table 2 Summary of the remarkable results of the latest study of each novel drug

Novel drug	Mechanism of action	Remarkable results in myeloid malignancies
Luspatercept (ACE − 536)	Ligand trap (modified extracellular human activin receptor IIB domain linked to an IgG1 Fc region)	Phase II study in low/intermediate risk MDS (NCT01749514), $n = 58$
		• 65% of HI-E in low transfusion burden patients
		• 62% of HI-E in high transfusion burden patients
		• 77 vs 40% of HI-E in *SF3B1* mutation-positive and -negative patients, respectively
		Phase III study in very low-, low-, or intermediate-risk MDS (NCT02631070), $n = 299$
		• 37.9 and 28.1% of patients achieving transfusion independence for ≥ 8 and ≥ 12 weeks, respectively
Sotatercept (ACE − 011)	Ligand trap (extracellular human activin receptor IIB domain linked to an IgG1 Fc region)	Phase II study in low/intermediate risk MDS (NCT01736683), $n = 74$
		• 58% of HI-E in low transfusion burden patients
		• 47% of HI-E in high transfusion burden patients
		• 59 vs < 15% of HI-E in patients with and without > 15% ring sideroblasts, respectively
Galunisertib (LY2157299)	TGFβ receptor I kinase activity inhibitor (also known as ALK5)	Phase II study in low/intermediate risk MDS (NCT02008318), $n = 41$
		• 26% of HI-E in low transfusion burden patients
		• 38% of HI-E in high transfusion burden patients
		• No correlation between responses and MDS subtypes
Vactosertib (TEW-7197)	TGFβ receptor I kinase activity inhibitor	Phase I/II study in low/intermediate risk MDS (NCT03074006)
		• Initiated January 2018. Currently ongoing

HI-E hematological improvement-erythroid defined by the International Working Group, *MDS* myelodysplastic syndrome

inhibitor molecules have been tested to demonstrate their effect on these disorders, as briefly summarized below (see also Table 2). As will be discussed below, modulation of TGFβ signaling by activin II receptor traps has led to improvement of ineffective hematopoiesis in conditions such as β-thalassemia and MDSs. Although the detailed mechanisms of action are still not fully understood, these agents act as ligand traps inhibiting the interaction between TGFβ ligands and their receptor [91]. GDF11 is known to contribute to immature erythroblast pool expansion and inhibition of terminal erythropoiesis [58]. By trapping activin and other ligands such as GDF11, signaling through TGFβ receptors and activation of SMAD2/3 decreases leading to increased terminal differentiation of erythroid precursors. In vivo pre-clinical studies using these agents have shown decrease in BFU-Es and CFU-Es in bone marrow after 48 h of exposure to these compounds followed by progressive maturation of basophilic erythroblasts to poly and orthocromatophilic erythroblasts and reticulocytes within 72 h of treatment [57, 92]. Prolonged exposure to these agents (7 days) induces proliferation of BFU-Es and CFU-Es. This suggests acute exposure to TGFβ and activin ligand traps with subsequent decreased SMAD2/3 signaling may unblock differentiation arrest, while continued exposure leading to longer periods of SMAD2/3 inhibition may induce proliferation of early erythroid progenitors, particularly in the presence of EPO. In addition, activin receptor ligand traps may also modulate erythropoiesis via the microenvironment. By neutralizing TGFβ family members, these compounds can modulate SMAD signaling within stromal cells leading to differential secretion of proteins, which inhibit (VEGF, OSM, BMP2, and MMPs) or stimulate (IGFBPs, angiotensin II, BMP6, CCL8, and CC13) different stages of erythropoiesis [93].

Although the biological mechanisms responsive for ineffective hematopoiesis in MDS are unclear, current data suggest many of the recurrent genomic abnormalities characteristic of MDS can be functionally linked to this phenomenon (Fig. 3). For instance, RPS14 haploinsufficiency, observed in MDS with deletion 5q (del5q), causes a block in erythroid differentiation dependent on P53-mediated apoptosis at the transition from polychromatic to orthochromatic erythroblasts via S100A8 and S100A9 [94]. Similarly, damage-associated molecular pattern molecules, whose levels increase in ageing bone marrow, induce TNF and IL6 upregulation in myeloid-derived suppressor cells in models of del(5q) inhibiting erythroid colony formation, and differentially affect terminal erythropoiesis through

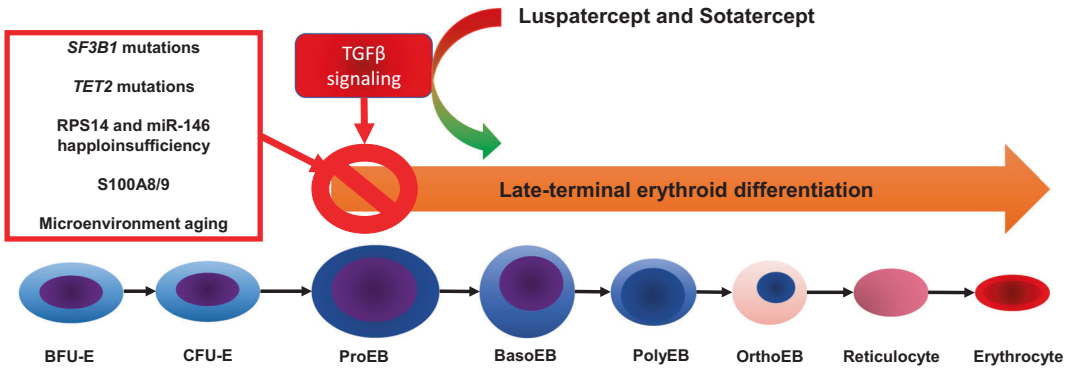

Fig. 3 Ineffective erythropoiesis in myelodysplastic syndromes and effects of TGFβ modulation. Genomic, epigenetic and microenvironment factors induce functional abnormalities in hematopoietic stem cells and progenitors, leading to early erythroid apoptosis and ineffective erythropoiesis with the absence of late-terminal erythroid differentiation. This is further enhanced by SMAD2/3 signaling via TGFβ activation. Inhibition of TGFβ signaling may partially restore the differentiation block and confer increased fitness to erythroid precursors, leading to late-terminal erythroid differentiation and hematological improvement. BFUE burst-forming unit erythroid progenitor, CFU-E colony-forming unit erythroid progenitor, ProEB proerythroblast, BasoEB basophilic erythroblast, PolyEB polyorthochromatic erythroblast, OrthoEB orthochromatic erythroblast

reactive oxygen species-induced caspase-3 activation and apoptosis [95]. Mutations in *SF3B1*, present in 20–30% of patients with MDS and >70% of those with MDS with ring sideroblasts, are known to induce ineffective hematopoiesis with abnormal late-stage erythroblasts through deregulated splicing of genes involved in hematopoiesis, iron homeostasis, and mitochondrial metabolism [96], as well as through P53 activation [97]. Loss of TET2, observed in up to 20% of patients with MDS who harbor loos of function mutations in *TET2*, cooperates with *SF3B1* mutations to cause more severe erythroid dysplasia and maturation defects [98] and can contribute to ineffective erythropoiesis [99].

Despite this heterogeneity, maturation arrest and cell death of erythroid precursors leading to abnormal late-state erythropoiesis seems to be at the center of anemia in MDS. It is possible that TGFβ modulation partially restores this differentiation blockade and increases erythroid fitness irrespective of the specific cellular processes leading to ineffective hematopoiesis.

Luspatercept (ACE-536)

Luspatercept is a recombinant protein that acts as a ligand trap, blocking the interaction between TGFβ family ligands (primarily GDF11 and GDF8) and their receptor. It is comprised of a modified extracellular domain of human activin receptor IIB that is linked to the Fc region of human IgG1 [100]. A study with the murine version (RAP-536) demonstrated that luspatercept increased hemoglobin levels in an MDS murine model and, subsequently, a phase 1 randomized double-blind placebo-controlled study with 40 postmenopausal women showed a dose-dependent increase of hemoglobin to >10 g/L in 83% of participants [57, 100]. In addition, a phase 2 open-label dose-finding study with long-term extension (NCT01749514 and NCT02268383) showed a 63% improvement in erythroid response, defined by the modified International Working Group-hematological improvement-erythroid criteria (HI-E; defined as >1.5 g/L hemoglobin increase for transfusion-independent patients or reduction of red blood transfusions in transfusion-dependent patients) in IPSS-defined low- or intermediate-risk MDS [101]. Patients presenting with >15% ring sideroblasts in the bone marrow and harboring an *SF3B1* mutation showed a stronger and more robust response to luspatercept (69 and 77% achieving HI-E, respectively). Related toxicities to this treatment were fatigue (7%), bone pain (5%), and diarrhea (5%), with grade 3 adverse events in 5% of patients (myalgia, general deterioration, and increased blast cell count). A phase 3 double-blind, randomized, placebo-controlled, multicenter study (MEDALIST) evaluating the activity of luspatercept for treatment of anemia in low or intermediate-I risk MDS (NCT02631070) has been completed. Results from this study were presented at the 2018 annual meeting of the American Society of Hematology. A total of 299 patients with very low-, low-, or intermediate-risk MDS by IPSS-R were included following a 2:1 randomization design [102]. The median age of the population was 71 (40–95) and 72 (26–91) years for the luspatercept and placebo arms, respectively. Both groups were homogeneous in terms of frequency of MDS with ring sideroblasts (94.8 vs 97.4%), red blood cell transfusion burden (43.1 vs 43.4% requiring ≥6 units/8 weeks), pre-transfusion hemoglobin (7.6 vs 7.6 g/dL) and *SF3B1* mutation (92.2 vs 85.5%). Of 153 patients receiving luspatercept, 58 (37.9%) achieved transfusion independence for ≥8 weeks compared with 10/76 (13.2%) with placebo (odds ratio (OR) 5.1, $p < 0.001$). In addition, patients receiving luspatercept were

more likely to achieve minor erythroid hematological improvement. The median duration of response was longer in the luspatercept arm (30.6 vs 13.6 weeks). No differences in treatment emergent adverse events were observed between arms.

In addition, luspatercept is also being tested in patients with β-thalassemia (transfusion dependent or independent), following two phase 2 studies (NCT01749540 and NCT01571635) showing promising results in terms of reducing the need for transfusions and increasing hemoglobin levels. Results from a phase 3 double-blind, randomized, placebo-controlled, multicenter study (BELIEVE) with patients with β-thalassemia (NCT02604433) were presented at the 2018 annual meeting of the American Society of Hematology [103]. A total of 336 patients were enrolled, with 224 receiving luspatercept and the remaining receiving placebo. The primary endpoint of the study (reduction in transfusion burden of ≥33%) was achieved in 21.4% of patients treated with luspatercept compared wichith 4.5% with placebo (OR 5.79, $p < 0.0001$). In addition, 70.5% of patients in the luspatercept arm achieved a ≥33% transfusion reduction over any consecutive 12 weeks compared with 29.5% with placebo ($p < 0.0001$) [104].

Sotatercept (ACE-011)

Sotatercept is another TGFβ ligand trap comprised of the extracellular domain of the human activin receptor IIA fused to the Fc domain of human IgG1. Initially designed for the treatment of bone loss diseases, it showed interesting results in pre-clinical studies in mice and non-human primates [105]. Due to its structure, sotatercept blocks TGFβ ligands, such as activins, GDF11, or BMP10, avoiding bone resorption and permitting late erythroid differentiation [92]. Using a murine chemotherapy-induced anemia model, the sotatercept murine analog (RAP-011) demonstrated less hemoglobin decrease and shorter anemia duration, compared with the control group [106]. In addition, sotatercept showed improvement in erythroid parameters in a β-thalassemia murine model and in an hepcidin overexpressing anemia murine model [58, 107]. A phase 1 randomized, double-blind, placebo-controlled, dose-escalation study (NCT00709540) was performed to assess safety and tolerability, as well as bone resorption and formation markers, in postmenopausal women. In this study, a significant dose-dependent increase of hemoglobin and hematocrit was observed [108]. Subsequently, an open-label randomized phase 2 parallel dose-ranging multicenter study (NCT01736683) assessed the hematological responses of sotatercept in transfusion-dependent patients with IPSS-defined low or intermediate-I risk, where 97% of patients received a previous treatment with erythropoiesis-stimulating agents [109]. Nearly half of the patients (49%) achieved erythroid improvement, and 27% acquired transfusion independence for at least 56 days. Interestingly, this study also showed a specific benefit in patients with >15% ring sideroblasts (69% of the patients in the study). Erythroid improvement was observed in 59% of the patients with >15% ring sideroblasts vs erythroid improvement of <15% in patients with <15% ring sideroblasts. Related toxicities of any grade were present in a 92% of the patients, with fatigue (26%), peripheral edema (23%), and diarrhea (22%) reported most frequently. Grades 3–4 adverse events were reported in 34% of the patients, although only 5% of them were considered treatment related by the investigator.

Sotatercept has been studied in phase 2 studies in β-thalassemia, chemotherapy-induced anemia in solid tumors, treatment-induced anemia in myeloma, and myelofibrosis with anemia, resulting in an improved erythroid response [110, 111]. Finally, a phase 2 study is currently ongoing with sotatercept in patients with pulmonary arterial hypertension (NCT03496207).

Galunisertib (LY2157299)

Galunisertib is a direct inhibitor of the kinase activity of the TGFβ receptor 1 (TGFBR1), preventing the downstream activation of the SMAD cascade triggered by TGFβ ligands [112]. It is currently being studied in the treatment of diverse solid neoplasms, such as pancreatic cancer and hepatocellular carcinoma [113]. Galunisertib was tested in a triple-blind, randomized, phase 2, parallel study (NCT02008318), in patients with MDS and up to intermediate risk, as defined by the R-IPSS. However, the results showed less efficacy than other TGFβ pathway drugs, with 26% of the patients achieving hematological improvement without any specific benefit to a ring sideroblast enriched patients subgroup [114].

Vactosertib (TEW-7197)

Vactosertib is a direct inhibitor of the kinase domain of TGFBR1. After demonstrating its antineoplastic efficacy in in vitro assays and in a murine melanoma model, a first-in-human phase 1 study was initiated, recruiting patients with advanced solid tumors (NCT02160106) [115]. In addition, a quadruple-blind, randomized, phase 1/2, parallel study (NCT03074006) in patients with up to intermediate risk according to the R-IPSS is ongoing. It is evaluating hematological improvement, maximum tolerated dose, bone marrow response, and quality of life during vactosertib treatment. Naka et al. also described an inhibitory effect of vactosertib in murine CML-initiating cells, blocking the

quiescence of this tyrosine kinase inhibitor-insensitive leukemia source [116].

Conclusions

The TGFβ pathway is a complex network of ligands, receptors, and intracellular signaling, which results in diverse effects depending on the cellular type and differentiation status, and is influenced by a variety of other signaling cascades. Given their ability to modify transcription, TGFβ ligands are involved in diverse processes, such as embryologic development, immunity regulation, healing and fibrosis, oncogenesis, and neoplasm progression. In hematological homeostasis, the TGFβ pathway plays an important regulatory role, mostly inhibitory, and is important for maintaining bone marrow self-renewal capacity. It is also involved in pathological situations such as myeloid neoplasms, where it can be inactivated to promote replication, or enhanced, to maintain an inhibitory microenvironment in non-leukemic cells and impair hematopoiesis. Unlike other signaling pathways, TGFβ complexity remains uncharacterized in hematological malignancies, but is gaining interest due to its potential as a therapeutic target through the pathway inhibitors described here. Novel drugs targeting this pathway are being tested in a variety of clinical trials and are showing promising activity with a good safety profile. Ongoing studies will confirm their role in the treatment of MDS and other myeloid disorders.

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Compliance with ethical standards

Conflict of interest GG-M declares support and an advisory role for Celgene Corporation and Acceleron Pharma. The remaining authors declare that they have no conflict of interest.

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