

REVIEW

Myelodysplasia is in the niche: novel concepts and emerging therapies

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Myelodysplastic syndromes (MDS) represent clonal disorders mainly of the elderly that are characterized by ineffective hematopoiesis and an increased risk of transformation into acute myeloid leukemia. The pathogenesis of MDS is thought to evolve from accumulation and selection of specific genetic or epigenetic events. Emerging evidence indicates that MDS is not solely a hematopoietic disease but rather affects the entire bone marrow microenvironment, including bone metabolism. Many of these cells, in particular mesenchymal stem and progenitor cells (MSPCs) and osteoblasts, express a number of adhesion molecules and secreted factors that regulate blood regeneration throughout life by contributing to hematopoietic stem and progenitor cell (HSPC) maintenance, self-renewal and differentiation. Several endocrine factors, such as erythropoietin, parathyroid hormone and estrogens, as well as deranged iron metabolism modulate these processes. Thus, interactions between MSPC and HSPC contribute to the pathogenesis of MDS and associated pathologies. A detailed understanding of these mechanisms may help to define novel targets for diagnosis and possibly therapy. In this review, we will discuss the scientific rationale of 'osteohematology' as an emerging research field in MDS and outline clinical implications.

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DEFINITION AND COMPOSITION OF THE OSTEO-HEMATOPOIETIC NICHE

Hematopoietic stem and progenitor cells (HSPCs) reside within the so-called 'bone marrow niches', which are defined as cellular and molecular microenvironments that cooperate with cell intrinsic mechanisms to maintain and regulate stem cell functions.¹ The complexity of the niche is attributed to the fact that it simultaneously contains stem cells, precursor cells and terminally differentiated cells. HSPC represent precursors for osteoclasts (OCs) responsible for bone resorption, whereas mesenchymal stem and progenitor cells (MSPCs) progressively differentiate to give rise to mature osteoblasts (OBs) that produce the bone matrix.¹ Below, we will summarize the role of bone cells and their respective progenitors, in particular, in the maintenance of the HSPC niche.

Mesenchymal stem cells and their progeny

Despite the established concept of the niche, there are still controversies concerning the composition and relevance of various compartments of the HSPC niche. OBs are considered key factors of the osteo-hematopoietic niche as transplanted HSPC often, but not always, lodge in close proximity to the bone trabeculae.² Indeed, depletion of MSPCs³ or osteoblastic progenitors⁴ results in a decreased number of bone marrow HSPC. Similarly, the dependence of HSPC engraftment on the calcium-sensing receptor,⁵ as well as the ability of OB to produce several key cytokines and adhesion molecules, support the essential role of the osteoblastic lineage in maintaining hematopoiesis.

More recent studies have focused on dissecting the osteogenic HSPC niche. This has led to the identification of intermediate filament protein nestin-expressing MSPC and CXCL12-abundant reticular (CAR) cells, which are critical for niche homeostasis.⁶ Most of the nestin⁺ cells (60%) are found adjacent to HSPC both within and outside the endosteal region³ and highly express important genes for HSPC maintenance, such as CXCL12, interleukin-7 (IL-7), angiopoietin-1 and osteopontin. Deletion of *CXCL12* from osterix-expressing stromal cells, which include CARs and OBs, results in constitutive HSPC mobilization,⁷ indicating that they have a critical role in maintaining HSPC self-renewal and differentiation. Nestin⁺ MSPCs, CAR cells, as well as recently described stem cell factor-expressing LepR⁺ cells are clearly important components of the HSPC niche, but the extent to which they may overlap is still under investigation.⁸ Moreover, it remains to be clarified which cell type requires direct contact with HSPC within the niche and which component acts through paracrine signals.

OCs and their precursors

OCs represent polynuclear HSPC-derived cells of the monocytic/macrophagic lineage specialized in bone resorption. OCs modulate calcium and mineral balance, which affects the maintenance of HSPC.⁵ Functionally, OCs can degrade bone matrix and release numerous growth factors, including transforming growth factor β , into the bone marrow cavity. Moreover, OCs may directly modulate the mobilization of HSPC from the bone marrow to the circulation,⁹ both in normal and stress conditions, via cathepsin K-mediated cleavage of CXCL12. Experiments on osteopetrotic mice lacking OC demonstrated increased HSPC mobilization, thus

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indicating bone resorbing cells as important regulators of the hematopoietic system.¹⁰ Interestingly, zoledronic acid, which is known to suppress OC activity, supports HSPC indirectly by increasing the secretion of bone morphogenetic proteins (BMP)-2 and -6, which stimulate OB functions.¹¹ Importantly, niche formation is severely affected in the absence of OC with reduced capacity of MSPCs to differentiate into OB and attract hematopoietic progenitors.¹² This indicates that OC have a critical role in the initial steps of the osteo-hematopoietic niche formation.

The OC progenitors, monocytes, are responsible for the protection of the HSPC pool from exhaustion both in steady-state conditions and during stress.¹³ They can be recruited from the blood circulation into the marrow, where they differentiate into macrophages and reside within the niche. Monocytes and macrophages are the main cells found in the bone marrow niche after irradiation, where they protect the HSPC pool from exhaustion by producing prostaglandin E2 to maintain undifferentiated primitive HSPC; loss of macrophages results in the egress of HSPC to the bloodstream.¹⁴ Moreover, macrophages also indirectly support HSPC through the modulation of the CXCR4–CXCL12 axis. Prostaglandin E2, synthesized by macrophages, increases CXCL12 expression on nestin⁺ stromal cells¹³ and CXCR4 expression on primitive HSPC,¹⁵ thus improving maintenance and survival of HSPC in the bone marrow. Importantly, macrophages represent an important link between HSPC and bone cells. Indeed, beyond their effect on hematopoiesis, macrophages stimulate proliferation and mineralization of OB and enhance the bone-anabolic effect of parathyroid hormone (PTH).¹⁶

IMPACT OF INTRINSIC AND EXTRINSIC FACTORS ON THE OSTEO-HEMATOPOIETIC NICHE

The role of age

Functional properties of MSPCs are modulated by age and gender.¹⁷ In fact, Maijenburg *et al.*¹⁸ demonstrated that expression of MSPC surface markers, such as CD146, is age dependent. Interestingly, CD146 expression also depends on the proximity of MSPCs to certain bone compartments, with higher expression observed in perivascular niches, and lower levels in the osteoblastic niche.¹⁹ Moreover, a unique MSPC subset specific for fetal bone marrow has been identified. Currently, the reduced capacity of aged MSPCs to differentiate into OB is thought to be due to the deregulation of specific histone demethylases.²⁰ Growth rate and clonogenic potential are substantially higher in MSPCs from younger female donors compared with aging counterparts.^{17,21} It could be partially explained by an increase in adipocyte number, which exhibits suppressive influence on hematopoiesis in aged bone marrow.²⁰

Vice versa, coupling between HSPC functions and bone turnover during aging and development of osteoporosis is supported by the fact that HSPC are able to guide MSPCs toward the osteoblastic lineage through increased BMP-2 and -6 secretion, whereas this effect is diminished in aged mice.²² These observations suggest that the relative size and composition of niches are dynamic and require different types of MSPCs at different age periods.

The role of PTH and cytokines

The function of MSPCs and their progenies is modulated by several hormones (for example, PTH, estrogens) and local signals (for example, BMPs, Wnt). PTH is an approved polypeptide for the treatment of osteoporosis because it stimulates bone formation that results in increased bone mineral density with additional known effects on hematopoiesis.²³ Indeed, PTH can directly stimulate the proliferation and survival of HSPC, but it also partially exerts its effects through the OB²⁴ by increasing the production of Jagged 1 in OB, which in turn activates Notch receptors on nearby

HSPC. Moreover, PTH has also been shown to act through the insulin-like growth factor pathway, which is specifically upregulated in OB, but not MSPCs in response to PTH.²⁵ The effects of PTH on HSPC expansion are IL-6 dependent, as they are lost in IL-6-deficient mice. Blocking IL-6 also attenuated PTH anabolic action on OB.²⁶ Taken together, these data indicate that the effect of PTH through various molecules including IL-6 couples osteoblastic and hematopoietic activity.

At the local level, both the Wnt and BMP pathways are of crucial importance.²⁷ Canonical Wnt/ β -catenin signaling pathway, specifically via Wnt3a, promotes proliferation of MSPCs and suppresses their osteogenic differentiation.²⁸ At the same time, β -catenin inhibits formation of OB through increased osteoprotegerin expression.²⁹ Wnt5a, a member of the non-canonical pathway, has been shown to suppress MSPC proliferation, but to increase the number of alkaline phosphatase-positive OB.³⁰ This is further supported by the fact that Wnt inhibitory factor-1 acts as a negative regulator of OB differentiation in mouse MSPCs.³¹ The non-canonical Wnt pathway also has a role in forming OB–OC balance, as the upregulation of RANK by Wnt5a is a key step for osteoclastogenesis in response to receptor activator of nuclear factor kappa-B ligand (RANKL) secreted from OB.³² Importantly, lower Wnt-related transcripts in MSPCs are observed in old compared with young individuals, which may be a correlate of osteoporosis as an age-related disease.³³

BMP-6 is one of the most consistent and potent regulators of MSPC differentiation into OB as the lack of the *Bmp6* gene caused a reduction of bone formation because of the OB dysfunction.³⁴ Moreover, systemic administration of BMP-6 restores the bone capacity by promoting MSPC differentiation toward OB and decreasing HSPC differentiation toward OC.³⁵ Importantly, MSPCs can produce BMP-2, -4 and -6 in an autocrine manner that induce osteogenesis of marrow-derived MSPCs *in vitro*.³⁶

In addition, autonomic neuronal regulation also modulates the osteo-hematopoietic niche (as reviewed by del Toro and Mendez-Ferrer).³⁷

The effects of erythropoietin (Epo)

Epo receptor expression has long been thought to be restricted to the erythroid lineage. However, Epo can also directly stimulate the formation of bone by inducing OB differentiation and increasing mineral deposition.³⁸ Experiments *in vivo* revealed that Epo first activates osteoclastogenesis, which is later followed by osteoblastogenesis either directly or via HSPC-mediated BMP secretion. These seemingly opposing effects of Epo on bone loss and formation have been shown to be context dependent. In developing or in BMP-driven bone regeneration, Epo increases bone formation by increasing bone remodeling, whereas it increases bone resorption rather than bone formation in mature and old bones.³⁹ Although the molecular mechanisms remain poorly defined, recent data suggest that mammalian target of rapamycin signaling may have a role in Epo-mediated OBs and OCs formation.⁴⁰ The rapid loss of trabecular bone volume observed after Epo exposure in mice⁴¹ is believed to be driven by a release of oncostatin M from erythroblasts, which in turn induces OB to secrete RANKL thereby potentiating OC maturation. Moreover, zoledronic acid, which is a known molecule that inhibits OC formation and function, completely blocked Epo-induced decrease in bone, indicating the involvement of OC in this Epo-mediated process. Importantly, bone remodeling is crucial for a normal erythropoietic response to Epo, as bisphosphonate treatment also reduced the magnitude of the erythroid response to Epo.

Epo-releasing scaffolds showed induction of chemotaxis of MSPCs and their pro-osteogenic differentiation.⁴² MSPC motility was also increased after Epo exposure.⁴³ Furthermore, *in vivo* implementation of Epo loaded scaffolds showed that they

promote osteogenic activity. It is also important to mention that OBs are also capable of driving erythropoiesis by their ability to produce Epo.⁴⁴ Together these data demonstrate an intimate coupling between hematopoiesis and bone formation through Epo.

THE IMPACT OF THE OSTEO-HEMATOPOIETIC NICHE ON THE PATHOGENESIS OF MDS

The pathogenesis of myelodysplastic syndrome (MDS) is thought to be due to an altered phenotype of the HSPC compartment as a result of genetic instability and disease-modifying mutations. There are several well-described somatic genetic abnormalities, which are commonly, although not exclusively, found in MDS. At least one genomic alteration can be found in 78% of MDS patients, with genes involved in RNA splicing (SF3B1, SRSF2) to be the most common and the earliest in disease evolution.⁴⁵ Intriguingly, these early mutations could also influence the further development of the disease, as later cooperating mutations are shown to be different between these two genes, despite the fact that they are both involved in the same pathway. Finally, some data support the notion that the disease phenotype may be closely linked to the mutation profile. Indeed, SF3B1 is associated with the presence of ring sideroblasts, whereas SRSF2 mutation is more commonly observed in chronic myelomonocytic leukemia.⁴⁶

Although the most important event in MDS pathology appears to be a molecular defect in HSPC, evidence suggests that ineffective hematopoiesis may also result from abnormalities in the bone marrow microenvironment, including altered hematopoietic–stromal interactions and deregulated production of growth factors and hematopoietic modulators (Figure 1).

Preclinical models

Animal models represent powerful tools to model and study human diseases and are useful preclinical platforms. Several mouse models have been generated to represent MDS pathology. Most of them are connected with the mutations in HSPCs (*Evi-1* overexpression, *Npm1* hetero mice, *Nup98/HoxD3* transgenic mice, mutated *Aml1*, microRNAs-145 and 146a knockdown).^{47–50} NUP98-HOXD13 transgenic mice represent among others a very accurate preclinical model. The *NUP98* gene is found to be affected in chromosomal translocations commonly seen in MDS. Mice with this phenotype develop all of the key features of MDS, including peripheral blood cytopenias, bone marrow dysplasia, impaired differentiation of early hematopoietic progenitors and transformation to acute leukemia.⁵¹ The most recently developed mouse models have the merit of combining genetic events that are found in the HSPC of patients with MDS, such as concurrent loss of *Ezh2* and *Tet2*,⁵² deletion of *Asx1*⁵³ and haploinsufficiency of *SAMD9L* mimicking monosomy 7.⁵⁴ However, given the tremendous heterogeneity of MDS in patients, it is likely that no single genetic mouse model can recapitulate all the subtypes at once and therefore patient-specific models using xenografts would be highly valuable tool to study the biology of human MDS.

One noteworthy model of MDS/myeloproliferative neoplasm is connected with the deregulation of Wnt canonical pathway. After serial transplantations of HSPC with loss of the *APC* gene, which is a known negative regulator of Wnt pathway, mice developed an MDS/myeloproliferative neoplasm phenotype. *APC* is a tumor suppressor located on human chromosome 5q, which indicates that this model can be attributed to the well-known 5q-syndrome.⁵⁵

Another model represents microenvironment-induced myelodysplasia with *Dicer1* deletion in osteoprogenitors. Raaijmakers *et al.*⁵⁶ have provided evidence for the first time that a disturbance of the endosteal niche can result in MDS. Deletion of *Dicer1*—an RNase III endonuclease essential for microRNA biogenesis and

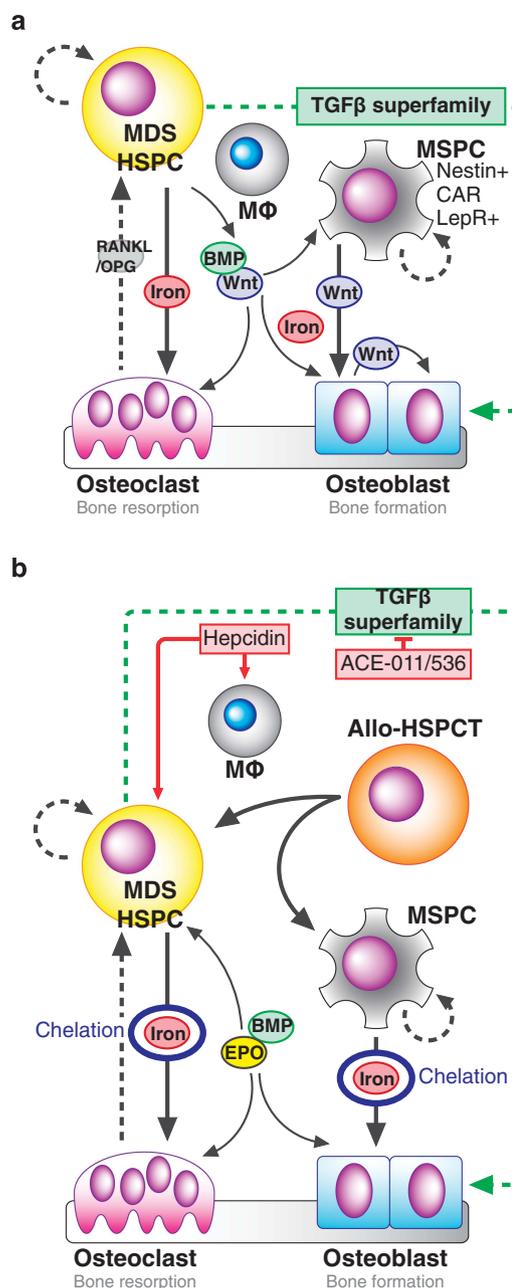


Figure 1. (a) Cellular and humoral components within the osteo-hematopoietic niche. Lines, differentiation/self-renewal, dash-lines, signaling pathways. (b) Potential therapeutic targets in modulating the osteo-hematopoietic niche of patients with MDS. Allo-HSPCT, allogeneic HSPCT; OPG, osteoprotegerin; TGFβ, transforming growth factor β; Wnt, Wnt signaling pathways.

RNA processing in mouse osteoprogenitors, but not in mature OB disrupts the integrity of hematopoiesis. OB number was significantly decreased, whereas OC number and function was not affected. These mice developed fatal neutropenia with hyperplastic bone marrow and dysmyelopoiesis, which is highly suggestive of MDS. When transplanted into wild-type recipient mice, these MDS cells were not able to propagate the disease, suggesting that this phenotype was not cell autonomous. Conversely, when *Dicer* knock-out mice were reconstituted with bone marrow cells from wild-type mice they developed MDS, thus demonstrating the essential role of the niche in instructing HSPC to acquire an MDS phenotype. Interestingly, MSPCs isolated from MDS patients also

exhibit a low expression of *Dicer1* and *DROSHA*—another RNA III endonuclease, as well as a global downregulation of microRNA expression.⁵⁷

Although genetic mouse models strongly argue in favor of a critical role of the bone marrow niche, translation of these findings to human MDS remains highly speculative. A recent study, however, has provided the first proof of concept that patient-derived stromal cells have a crucial role in the propagation of human MDS stem cells *in vivo*, thus establishing a robust xenograft model of lower-risk MDS.⁵⁸ Indeed the authors showed in a cohort of lower-risk MDS cases with different genetic makeup that co-injection of patient-derived CD34+ cells and the corresponding MSPCs into the bone marrow cavity of immunocompromised mice, lead to long-term and significant engraftment of *bone fide* MDS cells, as demonstrated by a strong myeloid bias and clonality tracking. In contrast, injection of CD34+ cells alone was highly inefficient; especially in the context of lower-risk MDS, as previously reported by others.⁵⁹ Interestingly, the properties of co-transfused MSPCs appear to be of importance, as patient-derived MSPCs were more efficient, than healthy age-matched MSPCs, in supporting MDS stem cells. Importantly, the authors also provided experimental evidence that a number of processes involved in cellular cross-talk were deregulated in MDS-MSPCs, some of which are instructively imposed by the diseased hematopoietic cells. Taken together, these data argue in favor of the view that stromal niche elements likely contribute to MDS pathogenesis, and that the bi-directional cross-talk may lead to yet to be defined stepwise events that may very well facilitate disease progression. Finally, the fact that specific MSPCs properties are important for MDS propagation, is also supported by the fact that cells with overexpression of CD146 (MCAM) exhibit an increased ability to support short-term (5–13 weeks) MDS engraftment and propagation (mostly higher risk cases), when compared with their cytokine-secreting counterparts.⁶⁰

Alterations of the cellular components of the niche in patients with MDS

Published data on the involvement of the cellular components of the 'niche' are summarized in Table 1. The majority have demonstrated alterations of niche cells. The first report revealed decreased OB and OC numbers and bone formation rate.⁶¹ Multiple data have shown that osteoprogenitors—MSPCs—exhibit normal morphology and frequency in the bone marrow of MDS patients,⁶² as well as undisturbed osteoblastic, adipocytic and chondrocytic differentiation potential *in vitro*. Similarly, MDS-MSPCs were reported to support the *in vitro* growth of MDS-derived HSPC.^{63,64} In contrast, other groups demonstrated reduced osteogenic differentiation and low ability to support hematopoiesis in long-term culture-initiating cells assays.^{65,66} A probable role of the chemokine CCL3 secreted by malignant cells in this process is suggested.⁶⁷

Antigen expression of adhesion molecules on the cell surface of MDS-MSPCs does not seem to differ from the normal counterparts, although some authors have postulated significantly lower expression level of CD90 and CD105.^{68,69} CD49b—an integrin involved in the interaction of MSPCs and collagen type I—is expressed at a higher level that can implicate changes in extracellular matrix synthesis and MSPC growth defects.^{63,70} MSPCs display a more widespread expression of CXCL12 in MDS, which may expose HSPC to increased contact-mediated signaling with CXCL12-expressing cells. Thus, these stromal cells fail to support trafficking of maturing hematopoietic cells to the microenvironment compartments.⁷¹ On the other hand, CXCR4 expression on HSPC has been found to be downregulated in MDS that could be successfully corrected by treatment with lenalidomide.⁷²

Various cytogenetic abnormalities have been shown to be present in MSPCs of up to 50% of patients with MDS, which are different from those detectable in the HSPC compartment.⁷³ Chromosomes 1 and 7 were shown to be more frequently involved in MSPC structural aberrations and a correlation between aberrations in MSPCs and overall survival has been published.⁷⁴ The presence of chromosomal abnormalities (mainly aneuploidy) in MDS-MSPCs is associated with the deregulated expression of *AURKA* and *AURKB* genes.⁷⁵ Thus, the presence of genetic abnormalities in MSPCs suggests that a genetic instability of the bone marrow microenvironment may facilitate the expansion of malignant cells and be, therefore, a particular mediator of leukemogenesis.

Other important cellular components, which can contribute to the disturbance of the hematopoietic niche, are monocytes and macrophages. In fact, monocytes from patients with MDS are derived from clonal HSPC and fail to upregulate matrix metalloproteinase-9 (*MMP-9*) gene expression in response to stromal signals.⁷⁶ Given the role of MMP-9 in facilitating the egress of cells from the bone marrow, it is reasonable to assume that non-responsive monocytes accumulate over time, whereas inducible levels of MMP-9 decline, thus resulting in hypercellularity in the bone marrow of patients with MDS. Macrophages can interrupt the normal interaction between MSPCs and hematopoietic progenitors in MDS through increased synthesis of tumor necrosis factor- α .⁷⁷ Moreover, macrophages represent target cells of iron deposition during transfusion-associated iron overload, which may further impair their function. Clearly, a better delineation of signaling pathways responsible for altered responses between stromal cells, macrophages and HSPC, as well as the activities that trigger these pathways in MDS are needed.

Impact of disturbed signaling within the osteo-hematopoietic niche

The issue of secreted cytokines and adhesion molecules in MDS is controversial, as some groups claim no difference in cytokine

Table 1. Overview of published data on the potential role of MSPCs and derivatives in the pathogenesis of MDS

PROs	CONS
<ul style="list-style-type: none"> ■ Cytogenetic aberrations in MDS-MSPCs^{62,63,74} ■ Lower expression of <i>Dicer1</i>, <i>DROSHA</i>,^{56,57} <i>AURKA</i>, <i>AURKB</i>⁷⁵ genes in MDS-MSPCs ■ Altered immunophenotype in MDS-MSPCs: decreased CD44 and CD49e,⁷⁰ CD90, CD104 and CD105⁶⁸ expression, increased CXCL12 expression⁷¹ ■ Impaired proliferation and differentiation capacity of MDS-MSPCs^{58,65,70} ■ Impaired cytokine production, including IL-32, by MDS-MSPCs⁵⁸ ■ Deregulation of Wnt signaling pathway in MDS-MSPCs^{82,89} ■ Impaired HSPC support by MDS-MSPCs^{65,66} 	<ul style="list-style-type: none"> ■ Normal cytogenetics in MDS-MSPCs^{64,66} ■ Normal structure, proliferation and differentiation potential of MDS-MSPCs^{62,63,64,66} ■ Normal HSC support by MDS-MSPCs^{62,63,64}

Abbreviations: HSPC, hematopoietic stem and progenitor cell; IL-32, interleukin-32; MDS, Myelodysplastic syndrome; MSPC, mesenchymal stem and progenitor cell.

status between MDS patients and healthy donors, whereas the others demonstrate profound deregulation of secretion of vascular endothelial growth factor, leukemia inhibitory factor and so on and highly upregulated N-cadherin expression in MDS-MSPCs.⁵⁸ An increased production of IL-1 β and stem cell factor was observed in response to tumor necrosis factor- α stimulation,⁷⁸ whereas tumor necrosis factor- α secretion itself induced conflicting data⁶³ with a proposal of its possible role together with interferon- γ in disease progression.⁷⁹

The impact of deregulated Wnt signaling in MSPCs on MDS pathogenesis remains largely elusive. Genes encoding known Wnt antagonists have been shown to be hypermethylated⁸⁰ in patients with MDS, suggesting a direct correlation between methylation status and risk of leukemia evolution.⁸¹ On the other hand, global gene expression profiling showed a significant downregulation of genes involved in canonical Wnt signaling, especially in the 5q-syndrome. This data led to the hypothesis that the imbalance between canonical and non-canonical Wnt signaling may contribute to the defective self-renewal of HSPC.⁸² This altered signaling is also suspected to have a role in iron regulation and appears to be an important factor in MDS pathogenesis, which requires further studies. Of note, deregulation of Wnt signaling not only affects HSPC, but also has an influence on the bone marrow niche.⁸³ As such, overexpression of various Wnt-inhibitors in OB exhibits a dual effect by altering the niche architecture with the reduction in trabecular bone and affecting hematopoietic progenitor cells,^{84–86} both impairing their localization and function within the bone marrow.⁸⁷ Depletion of iron can activate Wnt/ β -catenin pathway and induce osteoblastic differentiation of MSPCs.⁸⁸ However, it remains to be seen whether this has implications in the clinical setting, when iron chelation is administered to MDS patients. Importantly, activating mutation of β -catenin in OB, also found in patients with MDS, led to the increased synthesis of Notch ligand Jagged 1, which in turn activates Notch signaling in HSPC, leading to alteration of differentiation potential of hematopoietic progenitors and acute myeloid leukemia development.⁸⁹ This coupling of stromal and hematopoietic signaling pathways clearly highlights the need for developing new strategies aiming at disrupting this pathological niche-hematopoietic cell interaction.

Deregulation of the Epo signaling pathway may also be involved in hematopoietic niche alterations. The fact that Epo analogs induce erythroid responses in only up to 40% of unselected patients with lower-risk MDS⁹⁰ can be explained in part by the observation that most patients already have elevated Epo serum levels as a result of inefficient hematopoiesis. It is speculative to consider that the continuous and sometimes excessive elevation of endogenous Epo levels may lead to alterations of the bone microenvironment. Moreover, MDS HSPC exhibit an altered signaling in response to Epo in MDS,⁹¹ suggesting that HSPC-derived OC could also mediate this process. It is also conceivable that Epo may promote the production of other OB- or OC-stimulating proteins such as Wnt proteins or RANKL. Increased levels of circulating Epo and decreased sensitivity of erythroid progenitors to this factor have been found in Tfr2 $-/-$ mice, used as a model of iron overload. In fact, Tfr2 is a component of the Epo receptor complex,⁹² which may imply that Epo is involved in the process leading to iron overload in MDS patients.

The role of iron overload and disturbed iron homeostasis

The idea that iron metabolism is involved in the regulation and alteration of the bone marrow niche is based on several lines of evidence. First, iron overload is very common in MDS—both because of blood transfusions and inefficient erythropoiesis itself. Second, in general, iron overload is widely regarded as a risk factor for osteopenia and osteoporosis, as it has been shown to

inhibit osteoblastic differentiation and increase osteoclastic differentiation, potentiating bone resorption. Finally, osteoporosis is a very common complication of other diseases connected with iron overload, such as sickle cell anemia, thalassemia and hemochromatosis.

Defects in bone regulation in case of iron overload can be observed at various levels. Even in the most primitive non-cellular model, growth of hydroxyapatite crystals was inhibited because iron binds tightly phosphate groups and thus affects the nature of precipitates.⁹³

Several studies indicate adverse effects of iron overload on bone homeostasis. Precursors of OB are able to incorporate iron particles and inorganic iron, and both transferrin and ferritin were capable to inhibit alkaline phosphatase activity, a major enzyme required for proper OB function. Iron overload also suppressed OB-specific gene expression, such as osteocalcin, osterix and Runx2, in a dose-dependent manner. Thus, iron itself can inhibit essential OB functions such as bone formation.⁹⁴ These observations were confirmed at the organism level by creating an iron overload in *HFE* gene knock-out mice, representing the model of hereditary hemochromatosis. These mice exhibited a significant decrease in bone mineral density even when iron overload was mild.⁹⁵ However, changes in the functional capacity of the hematopoietic system were not investigated. The iron deterioration of OB activity can, at least partially, be mediated by ferritin and its ferroxidase activity, which inhibited the calcification, whereas mutant ferritin lacking ferroxidase activity failed to provide any inhibition.⁹⁶ The main component of the non-transferrin bound iron—labile plasma iron—is responsible for the formation of free radicals, which can lead to cell damage. The importance of oxidative stress in labile plasma iron-mediated toxicity was demonstrated in an experiment with iron-overloaded mice mimicking transfusional siderosis: increased reactive oxygen species (ROS) correlated with the severity of iron overload and thinning and alteration in the material properties of the bone. The prevention of bone abnormalities with the antioxidants supports the idea that oxidative stress is involved in the pathogenesis of the bone loss during iron excess.⁹⁷

Importantly, OC have also been found to be involved in the deterioration of the bone structure and function during iron overload.⁹⁷ Ferric application enhanced the formation of tartrate-resistant acid phosphatase-positive mononuclear cells from pre-OC cell lines in a dose-dependent manner,⁹⁸ whereas the treatment with iron chelators induced inhibition of osteoclastogenesis.⁹⁹ One of the mechanisms connected with these effects is also attributed to the formation of ROS.¹⁰⁰ Iron treatment alone or together with RANKL increased intracellular ROS in pre-OCs and therefore led to oxidative stress, which favored OC differentiation, whereas the treatment with antioxidants could neutralize these effects. ROS, induced by iron application, could also upregulate RANKL mRNA, but had little effect on osteoprotegerin, therefore again favoring OC development.⁹⁸

In the process of iron overload, an altered production of hepcidin has been postulated to have an important role. Although early data about rate of hepcidin excretion in patients with MDS were rather conflicting,^{101,102} recent analysis clearly showed that mean hepcidin levels are very heterogenous and depend on MDS subtype with the lowest level in refractory anemia with ringed sideroblasts and the highest in refractory anemia with excess blasts and chronic myelomonocytic leukemia.¹⁰³ These data are in concordance with an *in vitro* study showing a prominent variability in the ability of serum from MDS patients to suppress hepcidin expression in hepatocytes.¹⁰⁴

Thus, there are convincing studies highlighting a potential role of iron in the modulation of several components of the osteo-hematopoietic niche. However, whether iron overload or depletion is able to significantly alter the cross-talk of hematopoietic cells with the microenvironment in MDS has not been yet

investigated in details. Clearly, more research is required to assess this in preclinical and clinical studies.

PROPOSAL OF 'OSTEOHEMATOLOGY' AS A HOLISTIC APPROACH IN MYELODYSPLASIA

The existence of complex interactions between hematopoietic cells and their niche has been documented manifold and provides a rationale for developing a holistic approach, when it comes to treating pathologies of the osteo-hematopoietic niche, such as MDS. Concomitance of higher incidence of MDS in old individuals and age-related alterations of bone structure and function supports this idea. Further, multiple alterations of key signaling pathways are common in both types of cells, which proposes that a better understanding of the disease pathology may facilitate the development of effective therapeutic targets.

Potential therapeutic targeting of the osteo-hematopoietic niche

The proposal of osteohematology provides a new concept of possible concurrent medical impact on both hematopoietic and stromal cells in MDS (and other diseases like multiple myeloma). One of the best examples of an osteo-hematological approach can be demonstrated by means of allogeneic HSPC transplantation (HSPCT). Engraftment and maturation of donor-derived multipotent HSPC defines the success of this approach. The prerequisite for that is an appropriate milieu provided by a competent bone marrow microenvironment, which is chimeric following allogeneic HSPCT, containing recipient MSPC-derived cells and monocytes/macrophages, which may remain iron overloaded. Thus, when allogeneic stem cells are infused, they encounter a microenvironment, which is possibly impaired for a sustained period of time. This may in part explain the higher rates of graft failure and relapse as well as the prolonged time to stable engraftment seen in MDS patients mainly after HSPCT with reduced intensity conditioning. Given the long-term engraftment in this group of patients, irreversible MSPC defects seem rather unlikely and the problems are probably more related to disturbed cross-talks between hematopoietic cells and the bone microenvironment. On the other hand, allogeneic HSPCT may reprogram the microenvironment by modulating other HSPC-derived compartments including OCs. Comparative analyses on the functional capacity of the microenvironment in MDS before and after allogeneic HSPCT are not yet available.

Other therapeutic approaches, which are approved or currently in clinical studies, for MDS treatment are listed in Table 2. Two

hypomethylating agents—azacitidine and decitabine—which were originally thought to affect only the defective leukemic clone are now shown to have an influence also on the bone cells. As such, azacitidine inhibits the Wnt signaling pathway in MDS cells and may improve bone metabolism as well.¹⁰⁵ Further, hypomethylating agent could increase expression of BMP molecules in OB, thus potentially favoring the process of bone formation compared with bone resorption.¹⁰⁶

The most prominent example of potential simultaneous effect represents the activin receptor type II ligand trap sotatercept (ACE-011).¹⁰⁷ It represents a chimeric protein, which consists of extracellular domain of the ActRIIA receptor and Fc portion of the human immunoglobulin G antibody. Its main action is antagonizing activin and other ligands of the transforming growth factor β family and interfering with downstream signaling cascades, mainly the SMAD pathway. Activin levels correlate with bone lesions and in the preclinical studies ACE-011 increased bone formation, mineral density and strength of trabecular bone in monkeys.¹⁰⁸ Concurrent prevention of paclitaxel-induced anemia in murine models,¹⁰⁹ as well as observed increase in hemoglobin in patients, treated with this drug for postmenopausal osteoporosis¹¹⁰ and myeloma bone lesions,¹¹¹ led to the idea that ACE-011 could have an impact on erythropoiesis either directly or by modifying the functions of bone cells. However, it does not affect differentiation of erythroid progenitors or precursors directly, thus this effect is expected to be mediated by factors present within bone marrow niche.^{112,113} Indeed, stromal cells showed alterations in the expression of various important genes and cytokines in response to the drug.¹¹³

The analogous molecule ACE-536 (luspatercept) is a related compound targeting preferentially growth differentiation factor-8 and growth differentiation factor-11. It corrects the anemia associated with ineffective erythropoiesis in abovementioned NUP98-HOXD13 murine model.¹¹⁴ Importantly, both ACE-011 and ACE-536 promote maturation of late-staged Epo-independent erythroid precursors. Co-treatment with Epo induced synergistic responses, which provides a rationale for a combination therapy in patients with MDS-related anemia.¹¹² Interestingly, RAP-011 (murine ortholog of ACE-011) itself increased plasma levels of Epo, indicating that regulation of erythropoiesis by RAP-011 may not be limited to its activity within the bone marrow microenvironment.¹¹² These findings indicate that sotatercept/ACE-011 and ACE-536 may represent effective treatments for impaired erythropoiesis in MDS with concomitant alterations of the osteo-hematopoietic niche. Currently, two clinical trials are

Table 2. Currently available therapeutic options for patients with MDS and their potential impact on the functional properties of the osteo-hematopoietic niche

Agent	Mechanism
HMA (azacitidine/decitabine)	* Demethylation of Wnt-antagonist gene promoters and reduction of the non-phosphorylated β -catenin in HSPCs ¹⁰⁵
Lenalidomide	* Upregulation of BMP-2, -4 and -6 expression in osteoblasts ¹⁰⁶ * Inhibiting angiogenesis, suppressing the production of proinflammatory cytokines (for example, TNF α), inhibiting cytokinesis in MDS cell lines ⁷⁸ * Decrease of CXCL12 secretion by MSPCs, thereby detaching HSPC from their niche; enhancement of CD29 (integrin β 1) expression ⁶⁹ * Increase in expression of adhesion molecules in HSPCs; increase in CXCL12 and ICAM-1 secretion by MSPCs, thus improving their hematopoiesis-supporting capacity ⁷²
ACE-011/ACE-536	* Trap of important soluble factors (that is, activin, BMP-2, BMP-6) secreted by stromal cells ^{107,112} * Modulating of the SMAD signaling pathway, which leads to changes in the transcription of SMAD-regulated target genes ¹¹³
Iron chelation	* Activation of Wnt/ β -catenin pathway and induction of osteoblastic differentiation of MSPCs ¹¹⁷

Abbreviations: BMP, bone morphogenetic protein; HMA, hypomethylating agent; HSPC, hematopoietic stem and progenitor cell; ICAM-1, intercellular adhesion molecule-1; MDS, myelodysplastic syndrome; MSPCs, mesenchymal stem and progenitor cells; TNF α , tumor necrosis factor- α .

recruiting patients to evaluate the effect of activin receptor type II ligand trap on anemia in patients diagnosed with low- or intermediate-1 risk MDS (ACE-011/sotatercept—NCT01736683; ACE-536/luspatercept—NCT01749514) and preliminary data suggest clinical activity.¹¹⁵

Another approach to interfere with the osteo-hematopoietic niche in MDS may be through counteracting the phenomenon of iron overload. The mainstay of systemic iron overload treatment is iron chelation; however, treatment efficacy is compromised by suboptimal compliance and drug toxicity. Iron chelation seems to be important because of its role in improving hematopoiesis. Recent data suggest that iron chelation could even lead to the hematologic improvement in some patients.¹¹⁶ Various mechanisms have been proposed to mediate this effect, such as reduced oxidative stress and improved stromal support.¹¹⁷ Indeed, deferoxamine could partially attenuate MSPCs injury and inhibit signaling pathways induced by excessive iron. A role for iron chelation in the improvement of stromal cells function and their ability to support hematopoiesis is yet to be reported.

Novel agents, which influence the hepcidin-ferroportin axis, including exogenous hepcidin, transferrin, hepcidin analogs and signaling agonists, could provide an effective alternative. So far, in a mouse model of ineffective erythropoiesis the systemic transferrin application improved red blood cell survival and, most importantly, normalized the level of labile plasma iron.¹¹⁸ Furthermore, hepcidin and its analogs are attracting more attention as potential agents to treat iron overload and improve erythropoiesis. When *HFE* knock-out mice, representing parenchymal iron overload, were crossed with transgenic mice constitutively overexpressing hepcidin, iron accumulation was completely prevented.¹¹⁹ Synthetized minihepcidins showed an improved potency and longer action in comparison with natural hepcidin, its efficacy in iron depletion in mice raises the possibility of its potential usage in humans.

Another appealing approach, which couples iron homeostasis and bone physiology, is the treatment with BMP-6, which in cooperation with its receptor acts as an 'iron-sensing' system. Administration of BMPs, as well as upregulation of the BMP-6 signaling has shown not only *in vitro*, but also *in vivo* promising results in increasing hepcidin and reducing serum iron level.¹²⁰ Thus, members of this group of molecules, being at the same time the stimulators of the osteogenic differentiation of MSPCs, could be considered as the stimulants of hepcidin synthesis through targeting the BMP/SMAD signaling pathway.

In general, the regulation of hepcidin-ferroportin system could be an important approach in iron overload accompanying MDS, but the side effects, such as iron deficiency or excessive calcification should be avoided.

CONCLUDING REMARKS

The concept of 'osteoematology' provides a novel view onto the processes occurring within the bone marrow and raises new paradigms for pathomechanisms of hematological and skeletal diseases. MDS in particular, represents a disease with vivid interactions within the osteo-hematopoietic niche and the contribution of the niche has only recently been appreciated. Thus, treatment strategies need to be developed that not only target the leukemic cells, but also the signaling pathways connecting both sides to provide a holistic and effective approach to this disease.

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EB, LCH and UP drafted the manuscript. All other authors critically read and edited the paper. EB and MR contributed equally.

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