

Perspective

Aging as a Potential Modulator of Extramedullary Hematopoiesis: A Hypothesis-Generating Niche–HSPC Competence–Trigger Model

Zoe Bezirgiannidou^{1,4}, Iliana Stamatou^{1*}, Konstantinos Liapis¹, Emmanouil Spanoudakis¹, Evdokia Mandala², Ioannis Kotsianidis¹, Timoleon-Achilleas Vyzantiadis³, Efthymia Vlachaki⁴

¹Department of Hematology, University General Hospital of Alexandroupolis, Medical School, Democritus University of Thrace, Alexandroupolis, Greece. ²Fourth Medical Department, Hippokration General Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece. ³Department of Microbiology, Hippokration General Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece. ⁴Second Department of Internal Medicine, Adult Thalassemia Unit, Hippokration General Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece.

[Received July 15, 2026; Revised September 8, 2026; Accepted September 11, 2026]

ABSTRACT: Extramedullary hematopoiesis (EMH) is a compensatory response to hematopoietic stress that occurs unevenly across disorders with ineffective erythropoiesis (IE). The mechanisms underlying differing EMH patterns despite similarly severe IE remain poorly understood. Thalassemia and congenital dyserythropoietic anemias frequently develop overt EMH, whereas myelodysplastic syndromes (MDS), despite severe IE, rarely show overt EMH. We propose the Niche–HSPC Competence–Trigger Model as a hypothesis-generating framework in which EMH expression reflects the interaction among erythropoietic trigger load, intrinsic hematopoietic stem and progenitor cell (HSPC) competence, and extramedullary niche fitness. Erythropoietic trigger load reflects the intensity and persistence of erythropoietic stress, particularly anemia and hypoxia, in disorders characterized by ineffective erythropoiesis. HSPC competence refers to the intrinsic capacity of HSPCs for proliferation and differentiation, whereas extramedullary niche fitness denotes the capacity of target tissues to support HSPC homing, engraftment, survival, expansion, and productive hematopoiesis; this capacity is shaped by stromal and vascular integrity, inflammatory and fibrotic remodeling, metabolic support, and iron availability. Within this framework, overt EMH is hypothesized to be favored when erythropoietic drive coincides with preserved HSPC competence and extramedullary niche fitness. Conversely, impaired HSPC competence or reduced niche fitness may limit overt EMH, while whether subclinical extramedullary hematopoietic activity occurs in MDS remains unresolved. We hypothesize that aging may modulate this balance by influencing both HSPC competence and niche fitness, thereby potentially contributing to the rarity of overt EMH in MDS. These hypotheses, including the contribution of aging and the possible occurrence of subclinical extramedullary hematopoietic activity, require experimental and clinical validation.

Keywords: Aging, Hematopoietic niche, Extramedullary hematopoiesis (EMH), Ineffective erythropoiesis, Myelodysplastic syndromes (MDS), Thalassemia

1. Introduction

Extramedullary hematopoiesis (EMH) represents a compensatory response to sustained hematopoietic stress, particularly in disorders characterized by severe ineffective erythropoiesis (IE) or bone marrow failure, in which chronic anemia and hypoxia may increase hematopoietic demand [1, 2]. Its occurrence, however, is

neither consistent nor proportional to the degree of erythropoietic demand. Thalassemia syndromes and congenital dyserythropoietic anemias (CDA) frequently develop clinically overt EMH, including paraspinal and other extramedullary tissue masses [3–5]. Splenic EMH may also occur in thalassemia and has also been described in CDA; however, splenomegaly alone is not specific for active EMH, because chronic extravascular hemolysis,

*Correspondence should be addressed to: Dr. Iliana Stamatou, Department of Hematology, University Hospital of Alexandroupolis, Democritus University of Thrace, Alexandroupolis, 68100, Greece. Email: iliana_o6@hotmail.com.

Copyright: © 2026 Bezirgiannidou Z. et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

reticuloendothelial hyperplasia, and increased splenic sequestration may also contribute to splenic enlargement [6]. By contrast, myelodysplastic syndromes (MDS), despite comparably severe IE, rarely develop clinically overt EMH [7]. This contrasting pattern suggests that erythropoietic drive alone is insufficient to determine EMH expression.

The bone marrow niche is a well-organized network of stromal, endothelial, and perivascular compartments that provides trophic support to hematopoietic stem cells (HSCs) and functions as a dynamic regulatory environment rather than a static scaffold [8, 9]. Progressive age-dependent deterioration of the bone marrow niche involves adipogenic remodeling, senescence-associated secretory phenotype (SASP) activation, transforming growth factor beta (TGF- β)–mediated fibrosis, and mitochondrial oxidative stress, collectively eroding the niche's capacity to sustain stress-responsive hematopoiesis [10–13]. This deterioration is likely to be more advanced in older patients with MDS, whereas younger individuals with thalassemia and CDA may retain greater functional niche reserve despite disease-related abnormalities, potentially contributing to greater EMH permissiveness [5, 8, 14–16].

Building on established concepts of stress erythropoiesis, splenic niche formation, and extramedullary hematopoietic microenvironments [17, 18], we propose the Niche–HSPC Competence–Trigger Model as an integrative, hypothesis-generating framework linking cumulative erythropoietic exposure, disease-specific hematopoietic stem and progenitor cell (HSPC) competence, and aging-associated changes in extramedullary niche fitness to the contrasting EMH phenotypes observed in thalassemia/CDA versus MDS.

2. The Niche–HSPC Competence–Trigger Model: A Hypothesis-Generating Framework

2.1 Core Components of the Niche–HSPC Competence–Trigger Model

The Niche–HSPC Competence–Trigger Model conceptualizes EMH expression as the result of the interaction among three components: erythropoietic trigger load, intrinsic HSPC competence, and extramedullary niche fitness (Fig. 1), while aging is proposed as a potential modulator of EMH expression through its effects on HSPC competence and extramedullary niche fitness.

Erythropoietic trigger load refers primarily to the intensity and persistence of physiological stimuli that activate stress erythropoiesis, particularly anemia and hypoxia, often arising in the context of ineffective erythropoiesis [2, 17, 19]. Ineffective erythropoiesis is

characterized by increased erythroid precursor activity that fails to generate adequate red blood cell output, thereby sustaining anemia, hypoxia, and erythropoietic stress. Hypoxia enhances erythropoietic signaling through erythropoietin (EPO), a principal mediator of the erythropoietic response [19]. Trigger load should not, however, be regarded as a single-time-point measure of anemia or stress signaling; its biological impact is also shaped by the duration and timing of exposure and by therapeutic interventions, including transfusions, erythropoiesis-stimulating agents, and disease-directed treatments. In this model, inflammation is considered a context-dependent modifier of erythropoietic stress rather than a primary component of trigger load [11, 20].

Intrinsic HSPC competence refers to the cell-intrinsic capacity of HSPCs to proliferate and differentiate under hematopoietic stress. This capacity is disease-specific and may be influenced by inherited abnormalities, acquired clonal lesions, and age-associated changes in progenitor function [15, 21, 22]. Accordingly, HSPC competence is considered a variable determinant of the cellular response to erythropoietic stress.

Extramedullary niche fitness refers to the capacity of a target tissue—such as the spleen, liver, or paraspinal tissue—to support HSPC homing, engraftment, survival, expansion, differentiation, and functional hematopoietic output. This capacity depends on adequate stromal and vascular support and appropriate chemokine and growth-factor signaling, and may be impaired by local inflammatory, fibrotic, or metabolic dysfunction [18]. Bone marrow niche dysfunction may influence the preceding stages of HSPC retention and mobilization, but bone marrow and extramedullary niches are biologically distinct and should not be considered interchangeable [8, 9, 18]. Thus, marrow stress may promote HSPC mobilization, but overt EMH additionally requires successful establishment of hematopoiesis within a permissive extramedullary niche [8, 9, 18].

Accordingly, overt EMH is hypothesized to be favored when sustained erythropoietic trigger load coincides with preserved intrinsic HSPC competence and a permissive extramedullary niche. Conversely, impaired HSPC competence, reduced extramedullary niche fitness, or both may limit overt EMH despite substantial erythropoietic drive. This framework provides a conceptual basis for interpreting the contrasting EMH phenotypes observed in thalassemia/CDA and MDS, as discussed in the following section.

2.2 Why Similar Erythropoietic Stress Produces Different EMH Phenotypes

The contrasting EMH phenotypes of thalassemia/CDA and MDS illustrate how the three components of the

Niche–HSPC Competence–Trigger Model may interact differently across diseases [4, 5, 7]. In thalassemia and CDA, erythropoietic stress develops early in life, before age-related niche deterioration occurs [3, 5, 8]. Although chronic disease and, particularly in transfusion-dependent thalassemia, progressive iron overload and associated oxidative stress may impair stromal and vascular niche integrity over time [16], these insults occur within a comparatively younger bone marrow microenvironment

that may retain greater functional reserve. Within the proposed framework, this younger biological context may also be associated with greater capacity to establish permissive extramedullary niches, thereby contributing to the greater propensity for clinically apparent EMH, particularly paraspinal and other extramedullary tissue masses [3–5]. Splenomegaly may coexist with EMH, but its presence is not specific for active extramedullary hematopoiesis [6].

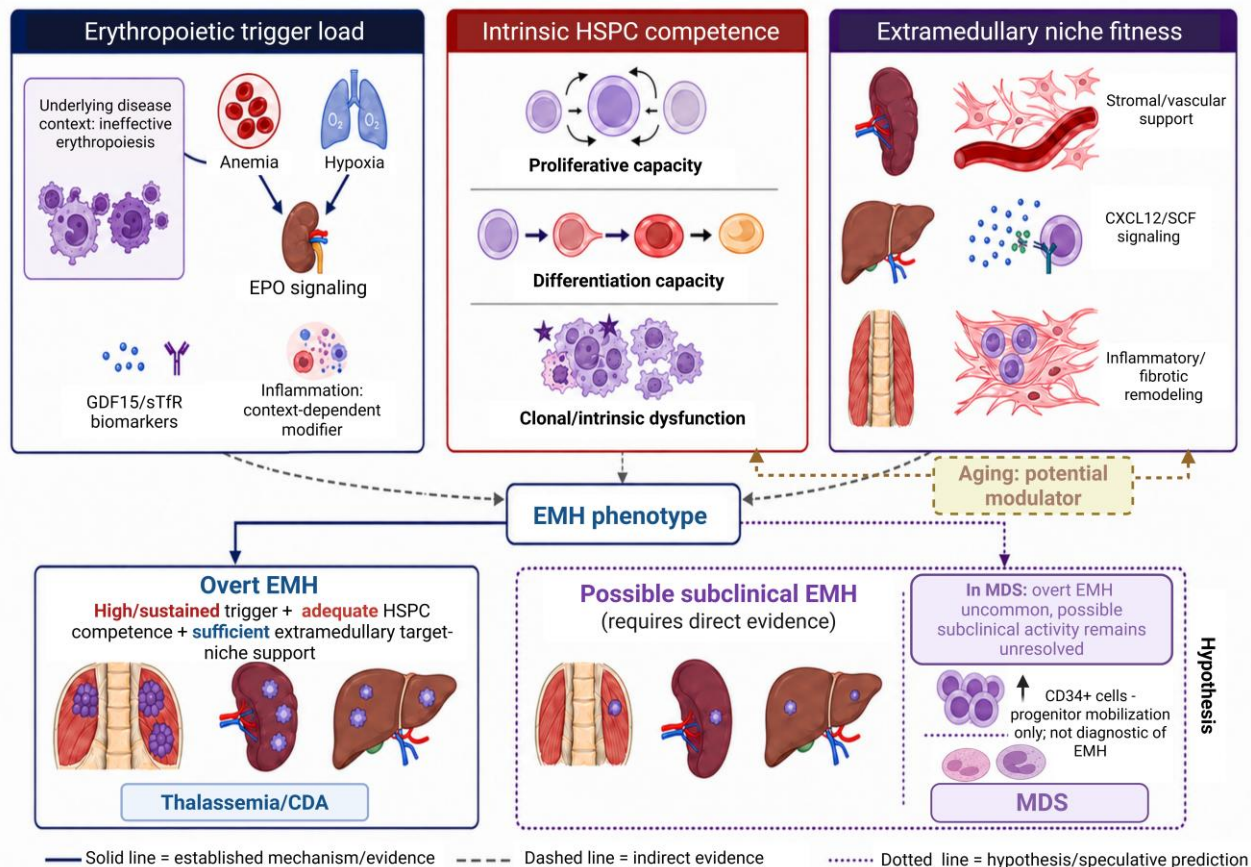


Figure 1. The Niche–HSPC Competence–Trigger Model of EMH. EMH expression is proposed to reflect the interaction among erythropoietic trigger load, intrinsic HSPC competence, and extramedullary niche fitness. Preserved HSPC competence and niche fitness are proposed to favor overt EMH (thalassemia/CDA), whereas impaired HSPC competence and/or niche fitness may limit overt EMH; whether subclinical extramedullary hematopoietic activity occurs in MDS remains unresolved and requires direct validation. Abbreviations: EMH, extramedullary hematopoiesis; EPO, erythropoietin; CD34⁺, CD34-positive cells; GDF15, growth differentiation factor 15; sTfR, soluble transferrin receptor; HSPC, hematopoietic stem and progenitor cell; MDS, myelodysplastic syndromes; CDA, congenital dyserythropoietic anemias; SCF, stem cell factor; CXCL12, C-X-C motif chemokine ligand 12. Figure created by I.S. with BioRender.com.

In MDS, by contrast, the clonal hematopoietic population arises mainly in older adults within an already altered bone marrow microenvironment, in which stromal dysfunction, inflammatory reprogramming, and fibrotic remodeling have progressively reduced the capacity to support stress-responsive hematopoiesis [12, 14, 23, 24]. Unlike thalassemia, where erythropoietic stress occurs before significant niche aging, MDS imposes a double

burden: intrinsic clonal HSPC defects combined with age- and disease-associated bone marrow microenvironmental dysfunction [25, 26]. We hypothesize that these abnormalities, together with potentially reduced fitness of extramedullary target niches, may contribute to the low propensity for overt EMH in MDS despite substantial erythropoietic drive [7, 18]. As summarized in Table 1, thalassemia/CDA and MDS show distinct biological

profiles within the proposed framework, with differences in age at presentation, HSPC clonality, marrow niche characteristics, and erythropoietic and iron-regulatory

responses that are associated with markedly different patterns of EMH expression.

Table 1. Conceptual comparison of biological features relevant to the proposed Niche–HSPC Competence–Trigger Model in thalassemia/CDA and MDS.

Feature	Thalassemia/CDA	MDS
Typical age at presentation	Younger to middle-aged patients	Predominantly older adults (>65 years)
Bone marrow niche support (CXCL12/SCF)	Relatively greater functional reserve, although disease- and iron-related stromal dysfunction may occur	Reduced or dysfunctional niche support, with impaired stromal CXCL12/SCF output
BMAT expansion	Potentially less BMAT expansion	Increased BMAT and adipogenic remodeling
Stromal senescence/SASP	Likely lower burden earlier in life; oxidative stress may contribute	Prominent inflammatory/senescence-associated niche remodeling
ECM remodeling/fibrosis	Potentially less prominent	Progressive, often TGF-β-linked
ROS–DNA damage axis	Potentially less pronounced than in aged marrow	Elevated oxidative/mitochondrial stress
HSPC clonality	Predominantly non-clonal hematopoiesis	Clonal HSPCs with intrinsic dysfunction
GDF15/EPO axis	High, with adaptive coupling to stress erythropoiesis	High, but often uncoupled from effective output
Hepcidin regulation	Often suppressed via erythroid signaling	May remain inappropriately elevated despite EPO-driven demand
EMH prevalence	Common; often clinically overt	Overt EMH is rare; evidence for subclinical EMH remains limited
Splenomegaly	Frequent but not specific for active EMH	Infrequent

Abbreviations: BMAT, bone marrow adipose tissue; CXCL12, C-X-C motif chemokine ligand 12; SCF, stem cell factor; SASP, senescence-associated secretory phenotype; ECM, extracellular matrix; ROS, reactive oxygen species; DNA, deoxyribonucleic acid; HSPC, hematopoietic stem and progenitor cell; GDF15, growth differentiation factor 15; EPO, erythropoietin; TGF-β, transforming growth factor beta; EMH, extramedullary hematopoiesis; MDS, myelodysplastic syndromes; CDA, congenital dyserythropoietic anemias.

Temporal exposure and therapeutic interventions further modify EMH expression. In thalassemia/CDA, the prolonged course of ineffective erythropoiesis provides a longer window for the establishment and expansion of extramedullary hematopoietic sites, although cumulative erythropoietic exposure is substantially modified by treatment. In transfusion-dependent thalassemia, regular transfusion programs maintain relatively higher pre-transfusion hemoglobin levels, thereby suppressing endogenous EPO-driven erythropoiesis and reducing effective trigger load [6]. Conversely, although MDS is characterized by a shorter duration of erythropoietic stress, transfusion support is generally more individualized and symptom-driven, allowing periods of deeper anemia and potentially stronger hypoxic and erythropoietic signaling. Erythropoiesis-stimulating agents may further enhance erythropoietic drive in selected patients with MDS [27]. Thus, erythropoietic exposure in MDS may be shorter in duration but

intermittently more intense or less effectively suppressed than in regularly transfused congenital anemias. Shorter disease duration alone is therefore unlikely to explain the rarity of overt EMH in MDS.

2.3 Overt and Subclinical EMH: Definitions and Validation Requirements

Overt EMH refers to established extramedullary hematopoiesis with macroscopic or clinically detectable extramedullary expansion, typically demonstrated by characteristic imaging findings, such as paraspinal or other extramedullary hematopoietic masses, with histological confirmation when required [1, 18]. Splenomegaly may coexist with EMH but is not, by itself, evidence of active extramedullary hematopoiesis [6].

By contrast, “subclinical EMH” denotes objectively demonstrable hematopoietic activity outside the bone marrow in the absence of clinically apparent

manifestations. Evidence may include characteristic imaging abnormalities, histological or immunophenotypic demonstration of hematopoietic tissue, lineage-specific progenitor activity within an extramedullary site, molecular or spatial evidence of local lineage-specific hematopoiesis, or demonstrable functional extramedullary hematopoietic output [18, 28]. Increased circulating CD34⁺ cells indicate progenitor mobilization but do not establish extramedullary engraftment or hematopoiesis, while the absence of splenomegaly does not exclude subclinical EMH [28, 29]. Functional splenic erythropoiesis has historically been demonstrated by ferrokinetic studies, including in a small number of patients with MDS, whereas more recent human studies have identified stress-responsive extramedullary HSPC reservoirs [28, 30]. Nevertheless, direct evidence for subclinical EMH in MDS remains limited.

Experimental and human observations also raise the possibility of an earlier state of extramedullary hematopoietic priming, involving extramedullary HSPC localization or recruitment and activation of local stress erythropoiesis programs before demonstrable hematopoietic output is established [28, 31–33]. Such findings should be regarded as potential precursors of EMH rather than as EMH itself. Whether this priming occurs in human MDS and precedes subclinical EMH remains a testable hypothesis requiring tissue-level molecular, spatial, cellular, or functional validation.

3. Biological Rationale Supporting the Niche–HSPC Competence–Trigger Model

3.1 Erythropoietic Triggers, Biomarkers, and Modifiers

Ineffective erythropoiesis (IE) reflects abnormalities in erythroid maturation, including premature apoptosis, differentiation arrest, and morphologic dysplasia [2, 27]. Although the underlying mechanisms differ across diseases, IE in thalassemia results primarily from unpaired globin-chain precipitation, oxidative stress, and dysregulated autophagy, whereas CDA arises from inherited defects in erythroid maturation and MDS from clonal abnormalities that disrupt erythroid differentiation [3, 5, 14, 27]. Stress erythropoiesis (SE) is an EPO-driven increase in erythroid production that occurs in response to anemia, hypoxia, or acute blood loss [17, 19]. Several mediators coordinate stress erythropoiesis. In murine experimental models, growth differentiation factor 15 (GDF15) and bone morphogenetic protein 4 (BMP4) support stress erythroid progenitor expansion and splenic niche formation [31, 33]. EPO acts as the principal regulator of this response and is produced via hypoxia-

inducible factor 2 α (HIF-2 α)–dependent mechanisms [19]. In clinical settings, circulating GDF15 primarily serves as a biomarker of ineffective erythropoiesis, whereas soluble transferrin receptor (sTfR) primarily reflects erythropoietic activity and iron demand [34, 35].

Inflammation exerts context-dependent and potentially opposing effects on stress erythropoiesis: inflammatory cytokines may augment stress signaling, while interleukin-6 (IL-6) can increase hepcidin through signal transducer and activator of transcription 3 (STAT3) signaling; tumor necrosis factor alpha (TNF- α) and other inflammatory mediators can impair erythroid maturation and promote stromal and fibrotic remodeling, thereby reducing hematopoietic support [11, 12, 14, 26].

Hepcidin, induced by iron overload and inflammation via bone morphogenetic protein 6 (BMP6)–SMAD and IL-6–STAT3 signaling, reduces iron availability via ferroportin downregulation [20, 36]. During SE, erythroferrone (ERFE) suppresses hepcidin to facilitate iron mobilization for hemoglobin synthesis [27, 36]. However, in chronic inflammatory states such as aging and MDS, hepcidin may remain inappropriately elevated despite increased EPO-driven erythropoietic demand. Persistently elevated hepcidin under these conditions restricts iron availability and acts as a functional ‘brake’ on SE and EMH [14, 20, 27, 36].

3.2 Intrinsic HSPC Competence in Aging and MDS

Aging is accompanied by cell-intrinsic alterations in HSCs, including reduced regenerative capacity, altered lineage output with myeloid bias, and progressive transcriptional and epigenetic remodeling [22, 37]. In MDS, these age-associated changes coexist with acquired clonal abnormalities in HSPCs that disrupt normal differentiation and erythroid output, while mutant HSPCs may acquire a relative competitive advantage within an inflammatory bone marrow environment [14, 21]. Within the proposed model, these intrinsic abnormalities may limit the capacity of HSPCs to translate a strong erythropoietic drive into productive extramedullary hematopoiesis, independently of—or in addition to—extramedullary niche dysfunction. However, whether aged or MDS-derived HSPCs have intrinsically impaired trafficking or engraftment within human extramedullary tissues remains unproven and should be regarded as a testable hypothesis.

3.3 Age-Associated Bone Marrow Niche Dysfunction

3.3.1 Structural and Molecular Determinants of Niche Fitness

Adult bone marrow HSCs are maintained within specialized microenvironments formed by closely interacting stromal and vascular compartments. Perisinusoidal stromal niches enriched in Leptin receptor-positive (LepR⁺) stromal cells, including C-X-C motif chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells, maintain HSCs through CXCL12 and stem cell factor (SCF) signaling [8, 9, 38].

The CXCL12–C-X-C motif chemokine receptor 4 (CXCR4) axis is the principal retention signal for HSCs within the bone marrow. Under conditions of hematopoietic stress, reduced CXCL12-mediated retention may promote HSPC mobilization from the bone marrow into the circulation; subsequent trafficking and homing to extramedullary tissues require additional local signals [8, 9, 18]. SCF produced by CAR cells and other stromal populations delivers critical signals through the KIT (c-KIT, CD117) receptor and supports the survival, proliferation, and expansion of stress erythroid progenitors (SEPs) [17, 38, 39]. During stress erythropoiesis, SCF, BMP4, and GDF15 support stress erythroid progenitor expansion and contribute to the establishment and maintenance of splenic stress-erythropoietic niches in experimental models [17, 31, 33]. Angiopoietin-1 (ANGPT1) contributes to vascular niche stability through tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (TIE2) signaling and preservation of endothelial integrity. Age-associated dysregulation of angiopoietin–TIE signaling may contribute to vascular dysfunction [8, 9, 40].

Murine studies provide direct evidence that splenic stress erythropoiesis depends on a specialized local microenvironment [31, 32]. Tlx1-expressing splenic stromal/progenitor cells provide CXCL12 and SCF and can promote HSPC recruitment and splenic EMH, whereas EPO-induced BMP4 signaling in splenic macrophages and GDF15 support stress erythroid progenitor expansion and maintenance of the splenic stress-erythropoietic niche [31, 33].

3.3.2 Structural Remodeling

Niche aging is characterized by progressive loss of CXCL12 and SCF support, associated with functional remodeling and altered composition of CAR/LepR⁺ stromal populations [8, 41]. Concurrently, age-related changes in stromal heterogeneity, marked by dysfunction of perivascular subsets and the development of inflammatory stromal states, further erode niche supportive capacity [8, 41, 42]. Together, these alterations weaken HSC retention, disrupt perivascular architecture, and reduce the niche's ability to respond effectively to hematopoietic stress [41, 43]. Bone marrow adipose tissue (BMAT) accumulation is a hallmark of niche aging,

reflecting an age-related shift of mesenchymal stromal cells (MSCs) toward adipogenic differentiation and disrupting marrow architecture and niche composition [10]. These processes are further reinforced by cellular senescence and SASP within the aging niche, collectively leading to progressive niche dysfunction [37, 42].

3.3.3 Inflammaging and Inflammatory Remodeling

Accumulation of senescent niche cells promotes SASP, a persistent pro-inflammatory state that progressively erodes niche function [37]. Among its key mediators, IL-6 activates STAT3 signaling, upregulates hepcidin expression, and thereby contributes to functional iron deficiency, impaired erythropoiesis, and myeloid-biased HSC differentiation [11, 20]. TNF- α contributes to chronic inflammatory signaling and alters HSC fate and self-renewal, promoting myeloid-biased differentiation [11, 37]. Interleukin-1 β (IL-1 β) further enhances niche inflammation and contributes to age-associated stromal remodeling [42, 44], while TGF- β promotes extracellular matrix remodeling and fibrosis, reducing niche plasticity and regenerative capacity [12, 45].

3.3.4 Metabolic Exhaustion

With aging, reactive oxygen species (ROS) levels increase in both HSCs and stromal niche populations [13, 37]. Major contributors include mitochondrial electron transport chain (ETC) dysfunction, mitochondrial DNA damage, and dysregulated oxidative metabolism, each promoting excess ROS production [13, 22]. Increased ROS induces DNA damage and p53 pathway activation, leading to cell-cycle arrest or apoptosis in susceptible HSC populations [13, 22]. Over time, this contributes to HSC exhaustion and myeloid-biased differentiation [22, 37, 42]. Oxidative stress also promotes cellular senescence mediated through the p53–p21 and p16–Rb cell cycle regulatory axes, while stromal dysfunction further reduces the supportive capacity of the niche [13, 37, 42].

Type 2 diabetes, obesity, and metabolic syndrome may further contribute to age-associated niche dysfunction [46]. Through sustained hyperglycemia, insulin resistance, and adiposity-driven inflammation, these conditions may exacerbate processes associated with impaired stromal and vascular niche integrity, including oxidative stress, mitochondrial dysfunction, SASP activation, and BMAT expansion [10, 37].

Although these age-associated alterations are best characterized in the bone marrow, experimental studies of splenic stress erythropoiesis demonstrate that extramedullary hematopoiesis likewise depends on a specialized local supportive microenvironment [31–33]. It

is therefore biologically plausible, but not established, that analogous age-associated changes in splenic or other extramedullary niches may reduce their capacity to support HSPC engraftment and expansion, particularly in MDS.

4. Future Directions and Validation of the Niche–HSPC Competence–Trigger Model

4.1 Validating the Niche–HSPC Competence–Trigger Model

As a hypothesis-generating framework, the Niche–HSPC Competence–Trigger Model gives rise to experimentally testable predictions. Because no validated composite measure of niche fitness currently exists and most candidate biomarkers are indirect, validation will require integrated molecular, imaging, tissue-level, and functional assessment rather than isolated measurements.

Circulating biomarkers alone cannot fully capture niche fitness, as imaging and tissue-level approaches provide a complementary and, in some cases, more direct readout of microenvironmental integrity [8, 37]. In MDS, discordance between markers of erythropoietic drive, including EPO and GDF15, and limited imaging evidence of splenic EMH could be explored as a candidate indicator of discordance between erythropoietic drive and EMH expression [7, 47]. Magnetic resonance imaging–derived proton density fat fraction (MRI-PDFF) provides a noninvasive quantitative measure of marrow fat content, while diffusion-weighted MRI–derived apparent diffusion coefficient (ADC) provides additional information on marrow cellularity and BMAT distribution [48]. However, both MRI-PDFF and ADC are indirect and non-specific measures of niche status and cannot, in isolation, establish niche dysfunction or extramedullary hematopoietic activity. Finally, bone marrow biopsy immunophenotyping, including assessment of LepR⁺, CXCL12⁺, and CD146⁺ stromal populations alongside aging and vascular markers, links assessment of bone marrow niche features directly to tissue findings [8, 9, 38].

Functional and ex vivo assays address a question that circulating biomarkers cannot resolve: whether reduced hematopoietic output reflects niche failure, HSPC-intrinsic dysfunction, or both [49]. Bone marrow-derived stromal cultures provide a practical assessment of niche function by evaluating stromal support and the production of niche-associated factors such as CXCL12 and SCF, while erythroblastic island assays evaluate macrophage–erythroid interactions and niche–HSPC co-culture systems allow direct quantification of stromal support under controlled conditions [16, 49].

Recent advances in spatial transcriptomics, single-cell multi-omics, and multiplex tissue imaging provide

complementary approaches for investigating niche organization at the tissue level [50, 51]. Applied initially to bone marrow and subsequently to human splenic tissue, where feasible, these technologies may provide a more direct readout of niche integrity, identify age-associated alterations in stromal and vascular compartments, and determine whether impaired extramedullary niche establishment contributes to the absence of overt EMH or to possible subclinical extramedullary activity in human MDS.

A staged validation pathway would involve, first, analytical standardization and reproducibility assessment of quantifiable circulating, cellular, tissue-level, and imaging-derived measures; second, their integrated evaluation within a multimodal framework combining biomarkers of erythropoietic activity and niche function with histological and immunophenotypic quantification, functional assays, and magnetic resonance imaging or other imaging evidence of marrow and extramedullary hematopoietic activity; third, prospective validation of these composite patterns in independent disease cohorts; and only thereafter, investigation of possible clinical utility.

4.2 The Niche–HSPC Competence–Trigger Map

To facilitate conceptual interpretation of the proposed framework, the Niche–HSPC Competence–Trigger Map integrates three interacting components: erythropoietic trigger load, intrinsic HSPC competence, and extramedullary niche fitness (Fig. 2). Because these components are measured across different biological compartments and currently lack standardized weighting and validation, they cannot yet be combined into a reliable composite score. Conceptually, effective erythropoietic pressure encompasses the intensity and duration of anemia, hypoxia, and ineffective erythropoiesis and is dynamically shaped by inflammation, transfusions, erythropoiesis-stimulating agents, and disease-directed treatments. Concurrently, niche fitness is inferred from complementary stromal, vascular, adipogenic, fibrotic, and senescence-related observations rather than from a single biomarker.

Within this conceptual framework, overt EMH is predicted to occur in conditions such as thalassemia and CDA, where sustained erythropoietic pressure coexists with a permissive niche and adequate HSPC competence. Conversely, impaired HSPC competence, a functionally non-permissive extramedullary niche, or both may limit the development of overt EMH despite high erythropoietic pressure. The Niche–HSPC Competence–Trigger Map should therefore be regarded as a research-oriented conceptual representation rather than a clinical classification system. Its purpose is to facilitate

hypothesis generation and future experimental investigation rather than to serve as a validated biomarker, quantitative index, or clinical instrument.

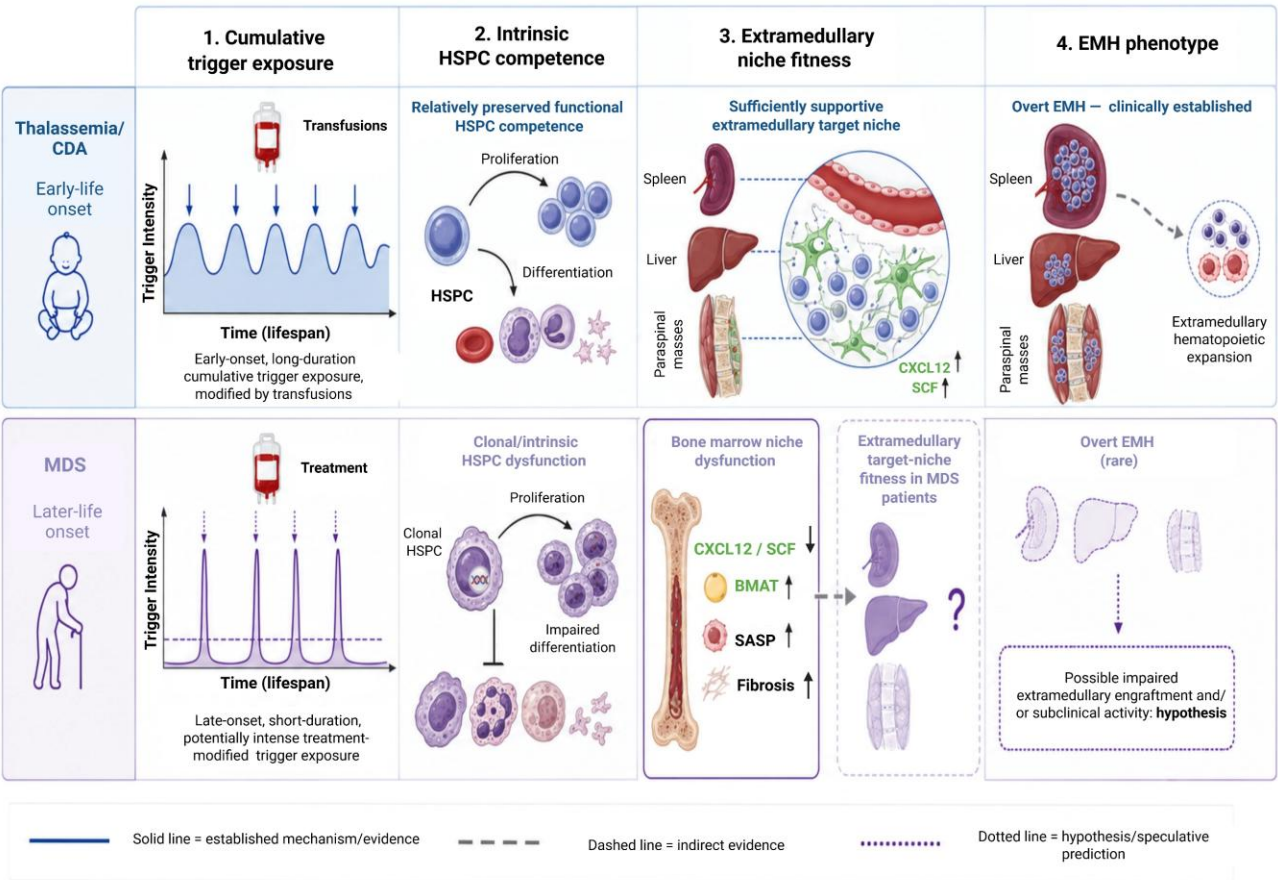


Figure 2. Temporal and disease-specific application of the Niche–HSPC Competence–Trigger Model. The phenotypic expression of extramedullary hematopoiesis is proposed to reflect the interaction among cumulative erythropoietic trigger load, intrinsic HSPC competence, and target-niche fitness. In thalassemia and congenital dyserythropoietic anemias, early-life onset and prolonged erythropoietic stress generate a large cumulative trigger load, while relatively preserved stromal and vascular support may permit successful extramedullary engraftment and overt EMH. In myelodysplastic syndromes, later-life onset, treatment-modified erythropoietic exposure, clonal HSPC dysfunction, and age- and disease-associated bone marrow niche abnormalities may coexist; whether analogous impairment of extramedullary target-niche fitness contributes to the rarity of overt EMH, or whether subclinical extramedullary activity occurs, remains unresolved. Transfusions, disease-directed therapy, HSPC competence, and niche integrity act as dynamic modifiers of EMH expression. **Abbreviations:** BMAT, bone marrow adipose tissue; CDA, congenital dyserythropoietic anemias; CXCL12, C-X-C motif chemokine ligand 12; EMH, extramedullary hematopoiesis; HSPC, hematopoietic stem and progenitor cell; MDS, myelodysplastic syndromes; SASP, senescence-associated secretory phenotype; SCF, stem cell factor. Figure created by I.S. with [BioRender.com](#).

4.3 Future Research Priorities

Several important questions remain unresolved, including the reversibility of niche dysfunction, the optimal timing of niche-targeted intervention, whether splenic HSPC engraftment is impaired in human MDS and, if so, which mechanisms underlie this impairment, and the influence of metabolic and patient-specific factors on niche function [7, 43, 46].

Future studies should prospectively validate the Niche–HSPC Competence–Trigger Model in MDS cohorts by integrating erythropoietic trigger markers, niche-supportive signals, splenic imaging, marrow adiposity, circulating CD34⁺ cells, and tissue-level biomarkers. Candidate biomarkers and measurable parameters for future evaluation are summarized in Table 2. Standardization of assays for circulating and tissue-level niche biomarkers, including CXCL12, SCF, ANGPT1, GDF15, hepcidin, inflammatory cytokines, and

BMAT-related imaging measures, will be essential. The development of *ex vivo* niche–HSPC co-culture systems, stromal functional assays, and spatial and multi-omics approaches will further help distinguish HSPC-intrinsic dysfunction from microenvironmental failure and facilitate investigation of the splenic microenvironment in human MDS.

Table 2. Candidate biomarkers and measurable parameters for future evaluation of the Niche–HSPC Competence–Trigger Model.

Biomarker/measurable parameter	Interpretation within the Niche–HSPC Competence–Trigger Model
CXCL12	Low levels may indicate reduced stromal supportive signaling; early compensatory increases may occur
SCF/KITLG	Reduced levels may indicate reduced stromal supportive signaling; assays lack standardization
GDF15	Primarily a biomarker of ineffective erythropoiesis
sTfR	Marker of erythropoietic activity and iron demand; not a direct trigger
EPO	Elevated EPO with limited erythroid output suggests trigger–output mismatch, consistent with IE
Hepcidin	High hepcidin despite increased EPO-driven erythropoietic demand indicates inadequate hepcidin suppression and functional iron restriction (‘iron brake’)
IL-6, TNF-α	Elevated cytokine levels may reflect inflammatory/SASP-related signaling and may impair niche support
TGF-β1	Elevated levels suggest fibrotic stromal/ECM-remodeling
CD34+ cells	Marker of progenitor mobilization, not diagnostic of EMH
Reticulocytes	Low reticulocytes despite high EPO/GDF15 indicate erythroid output blockade

Abbreviations: CXCL12, C-X-C motif chemokine ligand 12; SCF, stem cell factor; KITLG, KIT ligand; GDF15, growth differentiation factor 15; sTfR, soluble transferrin receptor; EPO, erythropoietin; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; TGF-β1, transforming growth factor beta 1; CD34+, CD34-positive cells; IE, ineffective erythropoiesis; EMH, extramedullary hematopoiesis; SASP, senescence-associated secretory phenotype; ECM, extracellular matrix.

Potential therapeutic implications should be interpreted cautiously, and considered only after appropriate validation, as improving microenvironmental support could theoretically enhance both residual normal hematopoiesis and malignant clonal fitness [26]. The timing of intervention may be critical, with niche-restorative approaches potentially being most effective before irreversible stromal failure, fibrosis, or advanced clonal dominance has developed. Finally, the proposed model should continue to be refined by incorporating patient-specific modifiers, including age, metabolic status, inflammatory burden, prior therapy, transfusion history, iron overload, and disease subtype.

5. Conclusions

The Niche–HSPC Competence–Trigger Model proposes that EMH phenotypes are shaped by the interaction among erythropoietic trigger load, intrinsic HSPC competence, and extramedullary niche fitness. This framework may help explain why sustained ineffective erythropoiesis in thalassemia/CDA is often accompanied by overt EMH, whereas MDS, despite substantial

erythropoietic stress, rarely produces macroscopic extramedullary expansion. In this context, aging is considered a potential modulator rather than a solitary cause, acting through cumulative effects on HSPC function and niche support, including inflammatory and fibrotic remodeling, while therapeutic interventions may further modify erythropoietic exposure. The model remains hypothesis-generating and should not be interpreted as a validated mechanistic pathway or clinical classification system. Its value lies in defining testable questions, particularly whether subclinical extramedullary hematopoietic activity occurs in MDS.

Author Contributions

Z.B. and E.V. conceived the idea and designed the study. Z.B. performed the literature review and investigation, curated the data, and wrote the original draft, including the development of the Niche–HSPC Competence–Trigger Model and the preparation of the tables. I.S. contributed to the methodology, visualization, preparation of figures and tables, and writing—review and editing. E.V. supervised the study, critically revised the

manuscript for important intellectual content, and approved the final version. K.L., T.-A.V., E.M., E.S., I.K., I.S., Z.B., and E.V. contributed to the literature review. All authors reviewed and approved the final version of the manuscript.

Acknowledgments

The authors thank Konstantinos Chouchos for his valuable technical assistance, including troubleshooting related to document processing, formatting, and reference management.

Conflict of interest statement

All authors declare that they have no relevant conflicts of interest.

Originality statement

This manuscript contains original material that has not been published or submitted to another journal. All authors agree to the submission of this manuscript to *Aging and Disease*.

References

- [1] Yang X, Chen D, Long H, Zhu B (2020). The mechanisms of pathological extramedullary hematopoiesis in diseases. *Cell Mol Life Sci*, 77:2723–2738.
- [2] El Hoss S, Lizarralde-Iragorri MA, Maciel TT, Hermine O (2026). Erythropoiesis in health and disease: Distinguishing defective and ineffective erythropoiesis. *Hemasphere*, 10:e70297.
- [3] Oikonomidou PR, Rivella S (2018). What can we learn from ineffective erythropoiesis in thalassemia? *Blood Rev*, 32:130–143.
- [4] Chapchap EC, Silva MMA, Baroni RH, Araujo A da S, de Assis RA, Loggetto SR, et al. (2023). Extramedullary haematopoiesis in patients with thalassemia: a cross-sectional description of its prevalence, clinical features and survival. *Hematol Transfus Cell Ther*, 46:S143.
- [5] Roy NBA, Babbs C (2019). The pathogenesis, diagnosis and management of congenital dyserythropoietic anaemia type I. *Br J Haematol*, 185:436–449.
- [6] Farmakis D, Porter J, Taher A, Cappellini MD, Angastiniotis M, Eleftheriou A, et al. (2022). 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. *Hemasphere*, 6:e732.
- [7] Wang C, Shi Y (2022). Extramedullary Hematopoiesis in Myelodysplastic Syndromes: A Systematic Literature Review. *Hemato*, 3:569–573.
- [8] Nakatani T, Nagasawa T (2025). Bone marrow niches for hematopoietic stem cells in homeostasis and aging. *Exp Hematol*, 144:104749.
- [9] Pereira AL, Galli S, Nombela-Arrieta C (2024). Bone marrow niches for hematopoietic stem cells. *Hemasphere*, 8:e133.
- [10] Fan Y, Elkhalek M, Zhang Y, Liu L, Tian Q, Chueakula N, et al. (2025). Bone marrow adipocytes: key players in vascular niches, aging, and disease. *Front Cell Dev Biol*, 13:1633801.
- [11] Pietras EM (2017). Inflammation: a key regulator of hematopoietic stem cell fate in health and disease. *Blood*, 130:1693–1698.
- [12] Valletta S, Thomas A, Meng Y, Ren X, Drissen R, Sengül H, et al. (2020). Micro-environmental sensing by bone marrow stroma identifies IL-6 and TGFβ1 as regulators of hematopoietic ageing. *Nat Commun*, 11:4075.
- [13] Miwa S, Kashyap S, Chini E, von Zglinicki T (2022). Mitochondrial dysfunction in cell senescence and aging. *J Clin Invest*, 132:e158447.
- [14] Villaume MT, Savona MR (2025). Pathogenesis and inflammaging in myelodysplastic syndrome. *Haematologica*, 110:283–299.
- [15] Aprile A, Gulino A, Storto M, Villa I, Beretta S, Merelli I, et al. (2020). Hematopoietic stem cell function in β-thalassemia is impaired and is rescued by targeting the bone marrow niche. *Blood*, 136:610–622.
- [16] Crippa S, Rossella V, Aprile A, Silvestri L, Rivi S, Scaramuzza S, et al. (2019). Bone marrow stromal cells from β-thalassemia patients have impaired hematopoietic supportive capacity. *J Clin Invest*, 129:1566–1580.
- [17] Paulson RF, Hariharan S, Little JA (2020). Stress erythropoiesis: definitions and models for its study. *Exp Hematol*, 89:43–54.e2.
- [18] Rivera-Torruco G, Muench MO, Valle-Rios R (2024). Exploring extramedullary hematopoiesis: unraveling the hematopoietic microenvironments. *Frontiers in Hematology*, 3:1371823.
- [19] Semenza GL (2023). Regulation of Erythropoiesis by the Hypoxia-Inducible Factor Pathway: Effects of Genetic and Pharmacological Perturbations. *Annu Rev Med*, 74:307–319.
- [20] Weiss G, Ganz T, Goodnough LT (2019). Anemia of inflammation. *Blood*, 133:40–50.
- [21] Chen L, Bian Y, Pronk E, van Dijk C, van Tienhoven TVD, Hoogenboezem RM, et al. (2025). An inflammatory T-cell-stromal axis contributes to hematopoietic stem/progenitor cell failure and clonal evolution in human myelodysplastic syndrome. *Nat Commun*, 16:10041.
- [22] Ye L, Tian C, Li Y, Pan H, Hu J, Shu L, et al. (2024). Hematopoietic aging: Cellular, molecular, and related mechanisms. *Chin Med J (Engl)*, 137:1303–1312.
- [23] Cong X, Peng X, Chen X, Wang N, Zhang L, Li L (2026). Bone marrow microenvironment reprogramming in myelodysplastic neoplasms: from pathological mechanisms to targeted therapeutic strategies. *Front Immunol*, 17:1726707.
- [24] Pronk E, Raaijmakers MHGP (2019). The mesenchymal niche in MDS. *Blood*, 133:1031–1038.

- [25] Friedrich C, Kosmider O (2022). The Mesenchymal Niche in Myelodysplastic Syndromes. *Diagnostics*, 12:1639.
- [26] Li AJ, Calvi LM (2017). The microenvironment in myelodysplastic syndromes: Niche-mediated disease initiation and progression. *Exp Hematol*, 55:3–18.
- [27] Cazzola M (2022). Ineffective erythropoiesis and its treatment. *Blood*, 139:2460–2470.
- [28] Mende N, Bastos HP, Santoro A, Mahbubani KT, Ciauro V, Calderbank EF, et al. (2022). Unique molecular and functional features of extramedullary hematopoietic stem and progenitor cell reservoirs in humans. *Blood*, 139:3387–3401.
- [29] Cesana C, Klersy C, Brando B, Nosari A, Scarpati B, Scampini L, et al. (2008). Prognostic value of circulating CD34+ cells in myelodysplastic syndromes. *Leuk Res*, 32:1715–1723.
- [30] Beguin Y, Fillet G, Bury J, Fairon Y (1989). Ferrokinetic study of splenic erythropoiesis: relationships among clinical diagnosis, myelofibrosis, splenomegaly, and extramedullary erythropoiesis. *Am J Hematol*, 32:123–128.
- [31] Millot S, Andrieu V, Letteron P, Lyoumi S, Hurtado-Nedelec M, Karim Z, et al. (2010). Erythropoietin stimulates spleen BMP4-dependent stress erythropoiesis and partially corrects anemia in a mouse model of generalized inflammation. *Blood*, 116:6072–6081.
- [32] Oda A, Tezuka T, Ueno Y, Hosoda S, Amemiya Y, Notsu C, et al. (2018). Niche-induced extramedullary hematopoiesis in the spleen is regulated by the transcription factor Tlx1. *Sci Rep*, 8:8308.
- [33] Hao S, Xiang J, Wu DC, Fraser JW, Ruan B, Cai J, et al. (2019). Gdf15 regulates murine stress erythroid progenitor proliferation and the development of the stress erythropoiesis niche. *Blood Adv*, 3:2205–2217.
- [34] Prahasanti K, Ashariati A, Herawati L, Romadhon PZ, Mahdi BA, Dzakiyah AZ, et al. (2025). Ineffective Erythropoiesis Markers in β -Thalassemia: A Systematic Review. *J Clin Med*, 15:308.
- [35] Beguin Y (2003). Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. *Clin Chim Acta*, 329:9–22.
- [36] Nemeth E, Ganz T (2021). Hepcidin-Ferroportin Interaction Controls Systemic Iron Homeostasis. *Int J Mol Sci*, 22:6493.
- [37] Matteini F, Mulaw MA, Florian MC (2021). Aging of the Hematopoietic Stem Cell Niche: New Tools to Answer an Old Question. *Front Immunol*, 12:738204.
- [38] Aoki K, Kurashige M, Ichii M, Higaki K, Sugiyama T, Kaito T, et al. (2021). Identification of CXCL12-abundant reticular cells in human adult bone marrow. *Br J Haematol*, 193:659–668.
- [39] Ji P (2020). Finding erythroid stress progenitors: cell surface markers revealed. *Haematologica*, 105:2499–2501.
- [40] Hayashi S, Rakugi H, Morishita R (2020). Insight into the Role of Angiopoietins in Ageing-Associated Diseases. *Cells*, 9:2636.
- [41] Du L, Freitas-Cortez MA, Zhang J, Xue Y, Veettil RT, Zhao Z, et al. (2025). Periarteriolar niches become inflamed in aging bone marrow, remodeling the stromal microenvironment and depleting lymphoid progenitors. *Proc Natl Acad Sci U S A*, 122:e2412317122.
- [42] Mitchell CA, Verovskaya EV, Calero-Nieto FJ, Olson OC, Swann JW, Wang X, et al. (2023). Stromal niche inflammation mediated by IL-1 signalling is a targetable driver of haematopoietic ageing. *Nat Cell Biol*, 25:30–41.
- [43] Ramalingam P, Gutkin MC, Poulos MG, Tillery T, Doughty C, Winiarski A, et al. (2023). Restoring bone marrow niche function rejuvenates aged hematopoietic stem cells by reactivating the DNA Damage Response. *Nat Commun*, 14:2018.
- [44] Caiado F, Manz MG (2024). IL-1 in aging and pathologies of hematopoietic stem cells. *Blood*, 144:368–377.
- [45] Tominaga K, Suzuki HI (2019). TGF- β Signaling in Cellular Senescence and Aging-Related Pathology. *Int J Mol Sci*, 20:5002.
- [46] McGraw KL, Larson DR (2024). Implications for metabolic disturbances in myelodysplastic syndromes. *Semin Hematol*, 61:470–478.
- [47] Park S, Kelaidi C, Meunier M, Casadevall N, Gerds AT, Platzbecker U (2020). The prognostic value of serum erythropoietin in patients with lower-risk myelodysplastic syndromes: a review of the literature and expert opinion. *Ann Hematol*, 99:7–19.
- [48] Karampinos DC, Ruschke S, Dieckmeyer M, Diefenbach M, Franz D, Gersing AS, et al. (2018). Quantitative MRI and spectroscopy of bone marrow. *J Magn Reson Imaging*, 47:332–353.
- [49] Kumar S, Geiger H (2017). HSC Niche Biology and HSC Expansion Ex Vivo. *Trends Mol Med*, 23:799–819.
- [50] Cooper RA, Thomas E, Sozanska AM, Pescia C, Royston DJ (2025). Spatial transcriptomic approaches for characterising the bone marrow landscape: pitfalls and potential. *Leukemia*, 39:291–295.
- [51] Bandyopadhyay S, Duffy MP, Ahn KJ, Sussman JH, Pang M, Smith D, et al. (2024). Mapping the cellular biogeography of human bone marrow niches using single-cell transcriptomics and proteomic imaging. *Cell*, 187:3120–3140.e29.
- [52] Huang H, Xu C, Gao J, Li B, Qin T, Xu Z, et al. (2020). Severe ineffective erythropoiesis discriminates prognosis in myelodysplastic syndromes: analysis based on 776 patients from a single centre. *Blood Cancer J*, 10:83.
- [53] Geyh S, Rodríguez-Paredes M, Jäger P, Koch A, Bormann F, Gutekunst J, et al. (2018). Transforming growth factor β 1-mediated functional inhibition of mesenchymal stromal cells in myelodysplastic syndromes and acute myeloid leukemia. *Haematologica*, 103:1462–1471.
- [54] Pierro F, Fazio M, Murdaca G, Stagno F, Gangemi S, Allegra A (2025). Oxidative Stress and Mitochondrial Dysfunction in Myelodysplastic Syndrome: Roles in Development, Diagnosis, Prognosis, and Treatment. *Int J Mol Sci*, 26:6415.
- [55] Maryanovich M, Zahalka AH, Pierce H, Pinho S, Nakahara F, Asada N, et al. (2018). Adrenergic nerve degeneration in bone marrow drives aging of the hematopoietic stem cell niche. *Nat Med*, 24:782–791.
- [56] Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, et al. (2007). High levels of GDF15 in thalassemia

suppress expression of the iron regulatory protein
hepcidin. Nat Med, 13:1096–1101.