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Haematopoietic stem cell activity and interactions with the niche

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Abstract

The haematopoietic stem cell (HSC) microenvironment in the bone marrow, termed the niche, ensures haematopoietic homeostasis by controlling the proliferation, self-renewal, differentiation and migration of HSCs and progenitor cells at steady state and in response to emergencies and injury. Improved methods for HSC isolation, driven by advances in single-cell and molecular technologies, have led to a better understanding of their behaviour, heterogeneity and lineage fate, and of the niche cells and signals that regulate their function. Niche regulatory signals can be in the form of cell-bound or secreted factors and other local physical cues. A combination of technological advances in bone marrow imaging and genetic manipulation of crucial regulatory factors has enabled the identification of several candidate cell types regulating the niche, including both non-haematopoietic (e.g. perivascular mesenchymal stem and endothelial cells) and HSC-derived (e.g. megakaryocytes, macrophages and regulatory T cells), with better topographical understanding of HSC localization in the bone marrow. Here, we review advances in our understanding of HSC regulation by niches during homeostasis, ageing and malignancy, and discuss their implications for the development of therapies to rejuvenate aged HSCs or niches or to disrupt self-reinforcing malignant niches.

Introduction

Haematopoiesis is the process by which the cellular constituents of blood are continually replenished throughout the lifetime of an organism. The haematopoietic system consists of various populations of highly specialized cells that have unique functions, such as oxygen transport and immune defence¹. It is estimated that an adult human generates approximately $4\text{--}5 \times 10^{11}$ haematopoietic cells per day². The continuous production of many blood cell types requires a highly regulated, yet highly responsive, system. Within the mammalian haematopoietic organization, rare haematopoietic stem cells (HSCs) sit at the top of the hierarchy. In adults, HSCs are found primarily in the bone marrow (BM) and are characterized by their ability to self-renew and produce various progenitors that proliferate

Author contributions

The authors contributed equally to all aspects of the article.

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The authors declare no competing interests.

and differentiate into mature blood cells³. HSCs are essential to replenish the haematopoietic system after transplantation into marrow-ablated recipients^{4,5} or after injury or infection^{6–9}. By contrast, committed progenitors have limited self-renewal ability and exhibit restricted lineage differentiation potential, and exhaust within a few weeks after transplantation³. At steady state, most HSCs are quiescent, which protects them from genotoxic insults^{10–13}, whereas the bulk of haematopoiesis is ensured by downstream progenitors^{14–16}. Several studies using single-cell transplantation and *in vitro* differentiation have challenged the classical hierarchical differentiation tree of haematopoietic progenitors, instead revealing lineage-restricted progenitors (restricted to one or two lineages) that may bypass multipotent progenitors and are generated directly from HSCs^{17–19}.

To ensure haematopoietic homeostasis throughout life, the balance between differentiation and self-renewal needs to be tightly regulated: excessive differentiation or insufficient self-renewal depletes the HSC pool, whereas insufficient differentiation or unrestrained self-renewal can lead to myeloproliferative diseases or leukaemia. HSC activity is regulated by an intricate interplay of cell-intrinsic factors, such as transcriptional and epigenetic regulators and metabolic pathways, and cell-extrinsic cues, including long-range humoral and neural signals or local cues from the BM microenvironment, which is referred to as the stem cell niche.

The concept of the ‘niche’ was proposed by R. Schofield in 1978 (REF²⁰) and refers to the regulatory unit that maintains and directs HSC self-renewal and differentiation. Building on this and other early observations, as well as new functional genetic tools and advancements in imaging methods and the discovery of new markers for HSCs and niche cells, have enabled a better understanding of the HSC microenvironment. The HSC niche is now viewed as a complex multicellular network that provides molecular cues and physical interactions that are essential for HSC localization, maintenance and differentiation. The field has grown rapidly in recent years; a search of PubMed using ‘haematopoietic stem cell niche’ as a search term retrieves >2,000 articles since Schofield’s seminal 1978 article²⁰; >85% of these articles were published in the past 10 years. Although distinct BM niche constituents have been identified, predominantly in mice, and include both HSC progeny and non-haematopoietic cell types²¹, the exponential growth of knowledge has led to a paradoxical situation in which almost all cellular constituents of the BM have been proposed to contribute to the niche, many of which act in redundant ways. The situation is further complicated by the fact that the HSC pool itself is functionally and molecularly heterogeneous^{6,19,22–29}, raising the possibility that distinct ‘specialized’ niches exist for distinct subpopulations of HSCs^{30–32}.

HSCs are the basis of bone marrow transplantation — a curative therapy for haematological diseases involving the replacement of an individual’s haematopoietic and immune systems with transplanted donor bone marrow^{33,34}. The number of available donor HSCs is often insufficient for marrow reconstitution, necessitating the *ex vivo* expansion of HSCs, which remains a major challenge. As the HSC population can expand substantially in its native niche, an understanding of the mechanisms of HSC maintenance is a prerequisite for the development of protocols to successfully expand HSC populations *ex vivo* for transplantation. Here, we review the progress of the past several years in the understanding

of the HSC niche, emphasizing the cellular composition of the BM HSC niche and the molecular mechanisms and signals that underlie HSC–niche communication during homeostasis, ageing and malignancy. Finally, we discuss some unanswered questions in the field and their implications for regenerative medicine and the treatment of haematological and other cancers.

Bone marrow architecture

Understanding how HSCs and niche regulators interact requires knowledge of the microanatomical organization and properties of adult BM (FIG. 1). The BM is an intricate organ that encompasses several haematopoietic and non-haematopoietic cell types that are interconnected by a vascular and innervated network within the cavities of long bones and axial bones³⁵. Most current knowledge about the HSC niche comes from studies of the long bones in mice (FIG. 1a). However, whereas all bones support haematopoiesis in mice, in humans the axial skeleton (which includes the cranium, sternum (FIG. 1b–d), ribs, vertebrae and ilium) is the major haematopoietic site. The haematopoietic (red) marrow of human long bones is progressively replaced in adolescence by ‘fatty’ yellow marrow that has negligible haematopoietic activity, except for in the proximal regions of the long bones³⁶.

Despite the differences in morphology, trabecular content and the ratio of endosteal to central marrow of various bones (such as the femur, pelvis and sternum), the HSCs isolated from them have similar gene expression profiles and long-term multilineage reconstitution abilities³⁷. Analyses of the distribution patterns of *ex vivo*-purified and transplanted HSCs revealed that they tend to home to near the endosteal bone surfaces after bone marrow ablation by irradiation^{38,39} as well as in non-ablated recipients⁴⁰. Haematopoietic stem and progenitor cells (HSPCs) also home preferentially to the trabecular-rich metaphysis of femurs in non-ablated mice, a process that depends in part on hyaluronic acid⁴¹. Other imaging studies have reported that endogenous HSCs localize eccentrically toward the bone surface rather than in the central medullary regions^{12,30,42–44}. HSCs isolated from the endosteal region show greater *in vivo* homing, lodgement and reconstitution potential than their central marrow counterparts^{45,46}. However, a recent study using optically cleared BM suggested that HSCs identified by α -catulin and KIT expression (TABLE 1) are uniformly distributed in the marrow space⁴⁷ (see BOX 1).

The periosteum is a highly vascularized and innervated tissue, allowing the entry and exit of blood vessels⁴⁸ and nerve fibres to the bone and marrow cavity⁴⁹. Of the autonomic nerves that reach the bone, the sympathetic nerves have been shown to penetrate the BM space^{50,51}. Conversely, parasympathetic fibres may innervate the distal femoral metaphysis⁵². In the adult mouse BM, arterioles are wrapped by sympathetic nerves and perivascular stromal cells that express both the pericyte marker neural–glial antigen 2 (NG2) and the type VI intermediate filament protein nestin (NES), forming structural networks termed the neuro-reticular complex^{30,53,54}.

A unique dense vascular network enables the efficient removal of waste products and the optimal delivery of nutrients, oxygen, hormones, neurotransmitters and growth factors to various locations in the bone and BM. Independently of the bone type, the oxygenated arterial blood connects with an extended network of sinusoids and exits through collecting

veins⁵⁵. Typical long bones, such as the femur, contain a central artery that enters the cortical bone through a nutrient canal and branches to form ascending and descending smaller arteries and thin-walled arterioles that mostly run parallel to the long axis of the marrow cavity, close to the endosteum^{30,56}. The connection between arterioles and the sinusoidal network preferentially occurs adjacent to endosteal and trabecular bone, mostly at the metaphysis but also in the diaphysis, and is mediated by type H vessels (also called transitional vessels). The endothelium of type H vessels expresses both arteriolar and sinusoidal markers. The number of type H vessels declines when bones have reached their adult size and is markedly reduced in old mice^{57–59}. Sinusoidal endothelial cells represent the majority of BM endothelium and seem to be less affected by ageing than transitional vessel endothelial cells. Sinusoidal vessels connect to the central venous sinus, which is found at the centre of the marrow cavity in cross-section and runs the entire length of the diaphysis and metaphysis^{31,57,60}. In turn, the central venous sinus empties into nutrient veins that pass through the nutrient canal along with the central artery, carrying deoxygenated blood and nutrient waste out of the marrow into the general circulation⁶¹. The vasculature of the skeletal system has been extensively studied (reviewed elsewhere^{55,62}).

Blood vessels are crucial for haematopoiesis. Definitive HSCs emerge from endothelial cells of the largest artery and HSCs continue to be closely associated with blood vessels in embryonic and extra-embryonic sites⁶³. In the adult mouse BM, early studies suggested the existence of a vascular niche that regulates haematopoiesis⁶⁴. However, the idea of a vascular HSC niche was supported by direct imaging of HSC distribution in their native microenvironment close to blood vessels^{54,65}. As discussed below, the niche has grown in complexity with several stromal and haematopoietic cells contributing to its activity⁶⁶.

Constituents of the HSC niche

Transgenic mouse models have been developed that enable the ablation of various endogenous cell types or depletion of niche factors or the tracing of lineages. Coupled with the use of advanced microscopy techniques (BOX 2), these models have led to the identification of several non-haematopoietic and haematopoietic cell types, as well as niche factors and their receptors as indispensable regulators of HSC activity; these cell types and factors (FIG. 2 and FIG. 3) are discussed below.

Regulation by non-haematopoietic cells

Various bone marrow stromal cells and other cell types have been implicated in the regulation of HSC activity, including mesenchymal stem cells (MSCs), adipocytes and glial cells.

Osteolineage cells.—Osteolineage cells were the first population of BM cells to be linked to the regulation of HSPCs. Early studies preceding Schofield's niche concept suggested that HSPCs are enriched in the endosteal region of the bone^{67,68}. In addition, studies in which HSC-enriched cell populations were transplanted into non-myeloablated recipients suggested that HSPCs are preferentially located near the bone surface⁴⁰. The presence of osteoblasts in the endosteal region, together with their ability to support expansion of haematopoietic progenitor cells *in vitro*⁶⁹ led to the hypothesis that osteoblasts

are a candidate niche cell. Later, *in vivo* studies using early cell-surface markers (which define Lineage[−]SCA1⁺KIT⁺ (LSK) cells) showed a correlation between the numbers of osteoblasts and HSPCs^{70–72}. Conditional ablation of osteoblasts, by ganciclovir treatment of mice expressing a truncated form of the herpes virus thymidine kinase (TK) gene under the control of a 2.3 kb fragment of the rat $\alpha 1$ type I collagen promoter, induces acute alterations in haematopoiesis, including reduced numbers of lymphoid, erythroid, and myeloid progenitors⁷². Enforced signalling through parathyroid hormone, a potent regulator of bone turnover, increases the number of osteoblastic cells and leads to HSC expansion mediated, at least in part, by the Notch ligand Jagged 1 (REF⁷⁰). Similarly, the conditional inactivation of bone morphogenetic protein receptor type IA (BMPRIA) using Cre recombinase (Cre) expressed from the *Mx1* promoter (*Mx1*-Cre) increases the number of N-cadherin⁺ osteoblasts, which also leads to HSC expansion⁷¹. This effect was thought to be the result of interactions between HSCs and osteoblasts mediated by the adhesion molecule N-cadherin, although functional evidence implicating N-cadherin in this interaction is still lacking⁷¹. Indeed, subsequent studies using new markers that are highly specific for HSCs dispute these findings^{73–75}.

Two of the best studied cytokines that are known to be non-cell-autonomously required for HSC maintenance — CXC-chemokine ligand 12 (CXCL12, also known as SDF1)^{76–79} and stem cell factor (SCF, also known as KIT ligand)^{80,81}—bind to CXC-chemokine receptor 4 (CXCR4) and KIT on HSCs, respectively^{82,83}. Deletion of *Cxcl12* or *Scf* in mature osteoblasts or osteoblastic progenitors using *Col.2.3*-Cre, *Bglap*-Cre (*Bglap* encodes osteocalcin) or *Osx*-Cre (*Osx* encodes osterix) mice has little effect on HSCs, suggesting that these osteolineage cells do not directly maintain HSCs, at least not via CXCL12 or SCF^{84–86}. Osteoblasts can also produce other molecules that are implicated in the maintenance of HSCs, including osteopontin (OPN), a negative regulator of HSC pool size^{87,88}, and thrombopoietin (THPO)^{89,90} and angiopoietin 1 (ANGPT1)¹², which bind to their receptors myeloproliferative leukaemia protein (MPL) and tyrosine kinase with Ig and EGF homology domains-2 (TIE2), respectively, and regulate HSC quiescence. However, the physiological contribution of osteoblast-derived THPO and ANGPT1 in HSC regulation has been questioned^{91,92}. Hepatocytes, not local marrow niche cells, seem to be the major functional source of THPO required for HSC maintenance⁹². HSPCs, megakaryocytes and leptin receptor-positive (LEPR⁺) stromal cells seem to be the main sources of ANGPT1 in the mouse BM⁹¹. This study also suggested that ANGPT1 does not directly affect HSC function but rather regulates niche regeneration after irradiation.

Although osteoblasts were the first cell population in the BM to be linked to HSC regulation, 3D imaging studies have shown that endogenous HSCs are not significantly associated with osteoblasts^{30,43}. Taken together, these studies suggest that mature osteoblasts do not have a direct role in regulating HSC activity. However, osteolineage cells seem to support the maintenance of more committed haematopoietic progenitors, in particular, the lymphoid lineage^{72,85,86,93,94}. The regulation of lineage-committed progenitors by BM niches has been reviewed elsewhere⁶⁶.

Perivascular cells.—The discovery that adult mouse BM HSCs and haematopoietic progenitor cells differentially express receptors of the signalling lymphocytic activation

molecule (SLAM) family (which include CD150, CD48 and CD244) has greatly facilitated imaging of highly purified HSCs in their native microenvironment. Bone marrow CD150⁺CD48⁺CD41⁺Lineage⁻ HSCs are localized in relatively close proximity to sinusoids rather than being confined to the endosteum, suggesting the existence of a vascular niche⁶⁵. Subsequent studies revealed that perivascular cells in human and mouse BM are highly enriched in niche factor expression and mesenchymal stem cell (MSC) activity^{54,95}.

Bone marrow-derived MSCs are rare haematopoiesis-supporting stromal cells that have self-renewal potential and the capacity to differentiate into bone, fat and cartilage. They wrap tightly around arterioles and more loosely around sinusoidal blood vessels. MSCs form colony-forming unit-fibroblasts (CFU-Fs) or mesospheres (non-adherent mesenchymal spheres) *in vitro* and, when transplanted, they contribute to ectopic formation of an organized haematopoietic niche in which BM stromal cells and active haematopoiesis can be detected⁹⁶. Furthermore, human BM CD146⁺ mesenchymal stem and progenitor cells (MSPCs) subcutaneously transplanted into mice can generate a heterotopic haematopoietic niche with host-derived HSCs and donor-derived perivascular CD146⁺ MSPCs⁹⁵. CD105⁺CD51⁺ MSPCs isolated from fetal mouse bones and transplanted into the renal capsule of adult mice are capable of inducing ectopic haematopoietic marrow⁹⁷. These studies indicate that MSPCs may organize the HSC niche.

HSCs are released into the circulation in a circadian manner in response to adrenergic signals from the sympathetic nervous system (SNS) that regulate the synthesis of CXCL12^{98–100}. Tracking the stromal cells that are targeted by SNS nerves has led to their identification as perivascular cells that are marked by GFP expression from regulatory elements of the *Nes* promoter. *Nes*-GFP⁺ cells associate tightly with nerves and HSCs and are enriched for MSC activity, suggesting that the BM niche may consist of the two stem cell populations, MSCs and HSCs⁵⁴. *Nes*-GFP⁺ perivascular cells express high levels of key factors involved in HSC maintenance, such as CXCL12 and SCF, as well as other regulatory factors, such as ANGPT1, OPN, interleukin-7 (IL-7) and vascular cell adhesion molecule 1 (VCAM1). A similar cell population can also be isolated from the BM of non-transgenic mice and from human fetal BM using the surface markers platelet-derived growth factor receptor- α (PDGFR α) and CD51 (REF. ¹⁰¹). Knock-in of a GFP reporter at the *Cxcl12* locus has revealed the existence of CXCL12-abundant reticular (CAR) stromal cells, which are adipo-osteogenic progenitors that are mainly distributed around sinusoids^{79,102}. CAR cells are also major producers of SCF¹⁰², IL-7, which is required for the maintenance of lymphoid progenitors and mature B cells¹⁰³, and the transcription factors forkhead box C1 (FOXC1) and early B cell factor 3 (EBF3), which are both required for CAR cell self-maintenance^{104,105}.

Subsequent studies in mice with a GFP knock-in at the *Scf* locus showed GFP expression by endothelial cells and perivascular cells marked by the adipo-osteogenic regulator *Lepr*^{84,106}. Approximately 90% of *Lepr*-Cre⁺ cells overlap with CAR cells¹⁰⁷ and represent a large subset (~80%) of *Nes*-GFP⁺ cells^{30,108}. Deletion of *Scf* or pleiotrophin (*Ptn*) using *Lepr*-Cre results in depletion of HSCs in the BM^{84,109,110}. However, conditional deletion of *Cxcl12* using *Lepr*-Cre mobilizes HSCs from the BM to the circulation and the spleen but has no effect on their abundance or localization in the marrow^{85,109}. In *Nes*-Cre^{ER}

transgenic mice, which express a tamoxifen-inducible Cre–oestrogen receptor (ER) fusion protein, deletion of *Cxcl12* or *Scf* does not markedly alter HSC numbers^{84,85}, even though *Nes*–GFP⁺ cells overlap substantially with LEPR⁺ cells in the BM^{30,101,108}. As *Nes* transgenic reporters were generated and screened to label neural stem cells, their expression in the BM is variable and depends on the transgenic strain. Accordingly, *Nes*–Cre^{ER} recombination efficiency in *Nes*–GFP⁺ cells is very low and a *Nes* transgene may not necessarily reflect endogenous *Nes* expression, possibly owing to the incomplete *Nes* promoter regulatory elements in the *Nes*–GFP construct and the slow turnover of GFP. *Nes*–Cre^{ER} may be more appropriate for early development studies, as the deletion of *Cxcl12*, when tamoxifen is administered within the first 3 weeks after birth, depletes HSCs in the early postnatal BM¹¹¹.

Whole-mount 3D imaging of the BM has uncovered heterogeneity among perivascular cells — rare *Nes*–GFP^{high} cells that express the pericyte marker NG2 (REF³⁰) or the smooth-muscle marker MYH11 (REF¹⁰⁹) are associated with arterioles and a subset of quiescent HSCs, whereas *Nes*–GFP^{low} stromal cells overlap with LEPR⁺ cells and CAR cells and are ubiquitously distributed near sinusoids³⁰. In contrast to *Lepr*–Cre, *Cxcl12* deletion using *Ng2*–Cre, which has high Cre-mediated recombination efficiency in almost all *Nes*–GFP⁺ cells and in >80% in LEPR⁺ cells and CAR cells, leads to a severe reduction in the number of HSCs in the BM, their exit from quiescence and redistribution of a subset away from arterioles¹⁰⁹. The conditional deletion of *Cxcl12* using paired-related homeobox 1 (*Prx1*)–Cre also depletes HSCs from the BM, likely because PRX1 is expressed in limb bud mesoderm during development and broadly by adult BM mesenchymal progenitor cells^{85,86}.

Using *Nes*–GFP and inducible *Ng2*–Cre^{ER} together with a reporter line, ~30% of *Nes*–GFP^{high} cells are labelled with the reporter but no labelling of *Nes*–GFP^{low} cells is detected. Although the recombination efficiency of *Ng2*–Cre^{ER} inducible diphtheria toxin receptor (iDTR) is sufficient to reduce *Nes*–GFP^{bright} cells and the marrow HSC population after tamoxifen and diphtheria toxin administration³⁰, conditional deletion of *Cxcl12* or *Scf* using *Ng2*–Cre^{ER} has no effect in BM HSCs using this tamoxifen administration protocol is used⁴⁷. However, with an extended tamoxifen administration period, starting at 2–3 weeks of age to maximize the efficiency of depletion, the selective deletion of *Cxcl12*, but not of *Scf*, in *Ng2*–Cre^{ER} cells reduced HSC numbers in the BM and altered HSC localization¹⁰⁹. These studies suggest that distinct inputs of cytokines required for HSC maintenance are derived from distinct perivascular niches (LEPR⁺ cells versus NG2⁺ cells), although it is still unclear if this differential contribution is a simple reflection of a difference in niche cell numbers (to explain the lack of an effect of *Scf* deletion) or a reflection of the crucial role of cytokines in HSC localization (to explain strong effect of *Cxcl12* deletion). Because bone marrow arterial endothelial cells secrete SCF¹¹², it is also possible that the difference in phenotype may be due to the contributions of other stromal cell types. Recent studies further support the notion of niche heterogeneity — the quiescence and localization of von Willebrand factor (*Vwf*)–GFP[–] HSCs are regulated by NG2⁺ perivascular cells, whereas those of myeloid-biased *Vwf*–GFP⁺ HSCs are selectively regulated by megakaryocytes³² (see below).

It is now clear that there is substantial overlap between the adult BM MSC populations that have been identified using *Nes*-GFP, *Ng2*-Cre, CAR, *Scf*-GFP, *Lepr*-Cre and *Prx1*-Cre^{101,106,108,113,114}. In addition, two populations of bone MSCs have been identified based on the expression of PDGFR α and SCA1(REF¹¹⁵) or *Cd73*-GFP(REF¹¹⁶), and two populations of skeletal stem cells isolated from whole bones have been identified based on the expression of CD51 and CD200 (REF¹¹⁷) or gremlin 1 (REF¹¹⁸). The contributions of these populations of cells to steady-state regulation of HSCs, as well as their precise lineage relationship with the previously described MSC populations, have yet to be resolved.

When dissecting the relationship between different MSC populations identified using cell surface markers or genetic reporters or when comparing studies, the tissue processing technique utilized for cell isolation is an important consideration. Different enzymatic treatments or bone-processing methods, such as crushing or marrow flushing, can elicit phenotypically and functionally different cellular subsets, preventing direct comparison of studies. For example, SCA1 and GREMLIN1 seem to be expressed mostly by bone-associated MSCs (for example, in the periosteum), which are not in direct contact with haematopoietic cells. Cells isolated from the bone exhibit distinct gene expression profiles and greater clonogenicity, growth and differentiation capacity compared to those in the BM^{96,119,120}. Bulk-cell analysis shows that niche activity measured by the expression of HSC niche factors correlates with MSC activity in the mouse and human fetal BM¹⁰¹. However, only a fraction of *Nes*-GFP⁺ cells, CAR cells, LEPR⁺ cells and *Prx1*-Cre cells contain CFU-F and multilineage differentiation capacity, suggesting that heterogeneity exists in the stromal cell population. However, the homogeneity of the stromal cell population can be substantially improved by the routine inclusion of CD51 as a marker in the analysis¹²¹. Interestingly, the majority of BM CD45⁻Ter119⁻CD31⁻ stromal cells are HSC-derived and contain previously unappreciated populations of CD45⁻ erythroid and B lymphoid progenitors¹²¹. Although the hierarchical organization of bona fide stromal cell types may be as complex as the haematopoietic lineage tree, there is currently no evidence supporting this idea; the on-going characterization of stromal cell types using single-cell techniques will undoubtedly provide further insight.

Adipocytes.—In humans, ageing accelerates BM adiposity, which is especially apparent in long bones where haematopoietic sites are progressively replaced by ‘fatty’ yellow marrow with reduced haematopoietic activity³⁶. Adiponectin, an adipocyte-secreted protein, impairs the proliferation of haematopoietic progenitors in vitro¹²². Furthermore, adipocyte-rich marrow (such as in the vertebrae of the mouse tail) contains fewer HSPCs than adipocyte-poor marrow (such as in the thoracic vertebrae). In the A-ZIP/F1 ‘fatless’ mouse, BM engraftment by HSCs is accelerated¹²³. In addition, the BM recovers more quickly after transplantation or chemotherapy when mice are treated with a peroxisome proliferator-activated receptor γ (PPAR γ) antagonist, which inhibits adipogenesis^{123,124}. These results suggest that adipocytes are negative regulators of HSC function, although alterations in MSCs may also indirectly contribute to this result. A novel MSC population (CD45⁻CD31⁻SCA1⁺CD24⁺) that is regulated by diet and ageing and can give rise to osteochondrogenic and adipogenic precursors, has been identified¹²⁵. Consistent with the negative influence of adipocytes, co-transplantation of adipogenic precursors in an intratibial injection model

inhibits HSPC engraftment and bone fracture healing, in a manner mediated by the negative regulator dipeptidyl peptidase 4¹²⁵. By contrast, adipocyte progenitors derived from *Adipoq*-Cre^{ER}-labelled cells transiently support the regeneration of HSCs by producing SCF¹²⁶. Further studies are needed to clarify the mechanisms of adipolineage cell regulation of the HSC niche, particularly at steady state.

Neural regulation.—Innervation in the skeletal system has been suggested to regulate haematopoiesis in the bone and BM^{49,98,127}. Whereas parasympathetic nerves only innervate the bone and do not fully penetrate the marrow^{52,128}, sympathetic nerves^{50,51} and sensory nerves^{51,129} innervate both the bone and BM. Sympathetic fibres release the neurotransmitter noradrenaline, which signals through adrenergic receptors. HSPC mobilization out of the marrow to peripheral organs depends on an intact gradient of CXCL12. Granulocyte colony-stimulating factor (G-CSF) decreases the steepness of the gradient by selectively reducing BM CXCL12 levels, leading to rapid HSPC egress from the BM^{77,130}. The SNS has a role in HSC mobilization in response to G-CSF treatment⁹⁸, as pharmacological or genetic ablation of adrenergic neurotransmission suppresses the HSC migratory response to G-CSF stimulation, which indicates that adrenergic signalling controls G-CSF-induced osteoblast suppression and CXCL12 downregulation⁹⁸. Furthermore, an intact osteocyte network, which is regulated by SNS fibres, is also required for G-CSF-induced HSPC mobilization¹³¹.

Bone marrow *Nes*-GFP⁺ MSCs are targeted by the SNS, as at steady state, adrenergic signals transduced by the $\beta 3$ adrenergic receptor (ADR $\beta 3$), which is expressed by *Nes*-GFP⁺ stromal cells mediate circadian oscillations of CXCL12 expression and HSC egress in mice^{54,99} and probably in humans¹⁰⁰. Importantly, G-CSF-induced mobilization is associated with suppression of MSC and osteolineage cell function^{98,99,131–133}.

SNS signals are also important for haematopoietic regeneration following genotoxic stress^{134,135}. Chemotherapy and/or irradiation-mediated toxicity can promote sympathetic neuropathy in the BM and excessive proliferation of MSCs and endothelial cells, which leads to a reduction in their niche support capacity. The administration of neuroprotective¹³⁵ or neuroregenerative¹³⁴ drugs rescues sympathetic nerve fibres and promotes the survival of niche cells and haematopoietic recovery after genotoxic insults, highlighting the potential benefit of using these drugs to protect haematopoietic niches from therapeutic injury.

Nonmyelinating Schwann cells, which are glial cells that express the marker glial fibrillary acidic protein (GFAP), insulate sympathetic and sensory nerves along arteries and promote HSC quiescence through activation of transforming growth factor β (TGF β) and SMAD signalling¹³⁶. Surgical sympathectomy by transection of the postganglionic sympathetic nerve results in the loss of BM GFAP⁺ cells and HSCs by increased proliferation. However, as less invasive methods of surgical sympathectomy do not result in reduced HSC numbers^{98,99,134}, it is unclear how SNS nerves signal to Schwann cells and to what extent marrow denervation contributes to the effects on HSCs.

Whether nerves can directly influence HSC function remains poorly understood. Adrenergic signals can act directly on human haematopoietic progenitors that express the $\beta 2$ -adrenergic

receptor, promoting their migration and engraftment¹³⁷. Furthermore, the BM is also innervated by sensory nerves^{51,129}, although very few studies have implicated them in haematopoiesis^{138,139}. Similarly, very little is known about the role of parasympathetic signalling in regulating HSC activity or their microenvironment. However, the type 1 muscarinic receptor (CHRM1), which is one of the receptors for the parasympathetic neurotransmitter acetylcholine in the hypothalamus, has been shown to regulate G-CSF-induced HSC mobilization from the BM via glucocorticoid hormone release¹⁴⁰.

Endothelial cells.—The BM is a well-vascularized tissue, enabling several blood cell types, including HSCs, to enter or leave the bloodstream. Bone marrow endothelial cells lining the interior of all blood vessels produce various factors, such as Notch ligands, CXCL12, SCF and pleiotrophin, which regulate HSCs and progenitors' activity^{58,84,86,110,141–143}. Endothelial cells also play a crucial role after myelosuppressive injury, by producing factors that promote haematopoietic regeneration^{110,141,142,144–147}. *In vivo* studies have shown that deletion of *Gp130*^{REF148}, *Scf*⁸⁴, *Cxcl12*^{85,86} or *Jag1*^{REF145} using endothelial cell-specific Cre strains (*Tie2*-Cre or *Cdh5*-Cre (*Cdh5* encodes VE-cadherin)), impairs HSC maintenance at steady state, and confirms the role of endothelial cells as niche regulators. Arteriolar endothelial cells (AECs) and sinusoid endothelial cells (SECs) can be distinguished by their differential expression of SCA1 and podoplanin (PDPN); AECs are CD45⁺Ter119⁺SCA1^{high}PDPN⁺, whereas SECs are uniformly CD45⁺Ter119⁺SCA1⁺PDPN⁺. Interestingly, AECs secrete nearly all detectable endothelial-derived SCF in the BM, as genetic deletion of *Scf* in AECs, but not in SECs, substantially reduced the number of functional HSCs¹¹². Intriguingly, although the overall abundance of BM endothelial cells is comparable to that of MSPCs¹⁴⁹, the levels of CXCL12 and SCF in BM endothelial cells are much lower than in MSPCs¹⁰⁹. Thus, it remains possible that extra-medullary *Tie2*-Cre⁺ cells may influence the BM niche. Additionally, as the structural integrity and function of endothelial cells and perivascular cells are intimately connected, it is conceivable that deletion of a specific niche or loss of specific factors could have deleterious effects on other niches. In fact, depletion of LEPR⁺ cells using an inducible diphtheria toxin system produced alterations in VEGFR3⁺ sinusoids, which became more tortuous, dilated and leaky¹⁵⁰. Similarly, the conditional deletion of *Angpt1* in perivascular cells and haematopoietic cells resulted in increased vascular leakiness⁹¹.

Although hypoxia has been suggested to be an inherent feature of HSPCs that is independent of their spatial localization in the BM architecture⁴³, a recent study indicated that the permeability of different vessels (arterioles versus sinusoids) to blood plasma affects the levels of reactive oxygen species (ROS) in neighbouring HSCs and consequently their localization in the marrow. HSPCs in the vicinity of the less permeable AECs contain low levels of ROS and are thus quiescent, whereas those close to the leakier SECs have increased ROS levels, leading to their activation and augmenting their differentiation and migration³¹. It is unclear whether these differences arise from their specific metabolic profiles. Future studies should focus on imaging more refined HSC populations, as the higher ROS tolerance of contaminating progenitors may confound the analysis. The large number of studies that investigated the metabolic requirements for HSC activity have been reviewed elsewhere¹⁵¹.

In-depth analyses of the BM vasculature in mice of different ages identified distinct subtypes of blood vessels that have unique molecular identifiers^{57,59,152} and are associated with distinct types of perivascular cells^{31,58,60}. Notch signalling in BM endothelial cells promotes osteogenesis and the expansion of type-H and arterial vessels as well as their coverage by perivascular PDGFR β ⁺ mesenchymal cells^{58,152}, which is accompanied by increased SCF levels and HSC abundance in the BM⁵⁸. Together, these studies further emphasize the complexity of BM niches by revealing the existence of molecular pathways that couple the behaviour of endothelial cells and perivascular mesenchymal cells.

Regulation by HSC descendants

In addition to the stromal niche components, HSCs' own progeny can also regulate HSC activity in a feedback loop.

Megakaryocytes.—Emerging models of haematopoiesis propose that there is a close relationship between the megakaryocyte lineage and HSCs. Megakaryocyte lineage may even bypass differentiation from multipotent progenitors and instead differentiate directly from HSCs^{16,18,19,29,153–155}. In fact, a subpopulation of HSCs exists that is biased toward megakaryocyte potential and expresses platelet markers, such as CD41 and von Willebrand factor (vWF)^{19,29}, and megakaryocytes directly regulate HSC quiescence^{156–159}. Whole-mount 3D imaging studies of HSC localization combined with computational randomization analyses showed that a subset of HSCs specifically associate with megakaryocytes and that megakaryocyte depletion leads to HSC proliferation¹⁵⁶. Megakaryocytes might regulate HSC quiescence by several mechanisms, including secretion of the chemokine CXCL4 (also known as PF4)¹⁵⁶, TGF β (signalling in HSCs requires SHIP1 (also known as PTPN6))^{157,160} and THPO (mediated by C-type lectin-like receptor 2)^{158,159}. However, selective deletion of *Thpo* in haematopoietic cells (including megakaryocytes) using *Vav1*–Cre mice does not alter HSC abundance in the BM⁹². In contrast to steady-state conditions, megakaryocytes promote HSC niche remodelling following a lethal dose of radiation, through osteolineage cell expansion^{161,162} and the secretion of fibroblast growth factor 1 (FGF1)¹⁵⁷. During emergency myelopoiesis, the production of TGF β 1 and CXCL4 by megakaryocytes surrounding clusters of granulocyte/macrophage progenitor cells (cGMPs) may be an important mechanism to re-establish HSC quiescence and limit the duration of the regenerative response¹⁶³. Thus, these studies suggest that separate HSC niches exist that promote quiescence — megakaryocytes and arterioles with NG2⁺ cells and GFAP⁺ nerves^{30,136}. *Vwf*–GFP⁺ HSCs, which are biased towards both platelet formation and the myeloid lineage, closely associate with and are regulated by megakaryocytes, indicating that HSCs are regulated in a feedback loop by their progeny³². Furthermore, megakaryocyte depletion selectively expands *Vwf*–GFP⁺ HSCs, whereas depletion of NG2⁺ arteriolar niche cells selectively depletes *Vwf*–GFP[–] HSCs. Thus, these studies demonstrate the existence of two spatially and functionally distinct niches for HSC subpopulations that have distinct developmental potential.

Phagocytic cells.—Investigation of the mechanism of G-CSF-induced HSC mobilization revealed that phagocytic cells in the BM regulate HSC migration. BM macrophages promote HSC retention by regulating osteolineage cells and MSCs, thus antagonising the SNS-

mediated inhibition of HSC retention in the BM^{133,164,165}. Splenic red pulp macrophages have an important role in HSC retention in the splenic niche by providing adhesion via VCAM1¹⁶⁶. *In vivo* depletion of macrophages using genetic models or clodronate-loaded liposomes promotes the mobilization of HSPCs into the blood¹⁶⁴. Furthermore, BM CD169⁺ macrophages promote the retention of HSCs in the BM, by inducing the expression of CXCL12 and other HSC retention factors by *Nes*-GFP⁺ MSCs¹⁶⁵. Additionally, G-CSF-receptor signalling in BM monocytic cells has an important role in eliciting HSPC mobilization¹³³. The crosstalk between macrophages and *Nes*-GFP⁺ MSCs may be mediated by macrophage-derived oncostatin M¹⁶⁷. Macrophages may also directly regulate HSCs^{168,169}. A rare population of α -smooth muscle actin⁺ macrophages localized adjacent to HSCs in the BM may protect HSPCs from exhaustion by limiting the production of ROS under stress¹⁶⁸. A subset of macrophages expressing duffy antigen receptor for chemokines (DARC) regulates HSC quiescence via TGF β -SMAD3 signalling¹⁶⁹. However, whether the macrophages that directly influence HSCs and those that exert their effects indirectly via the niche are the same, is unknown.

The homeostatic clearance of aged CD62L^{low}CXCR4^{high} neutrophils from the circulation also regulates the HSC niche¹⁷⁰. The daily release and clearance of neutrophils is regulated in a circadian manner, altering CAR cell function and promoting the circadian egress of HSPCs into the circulation. Modulation of the niche is ultimately mediated by BM phagocytic macrophages — which are responsible for neutrophil clearance — through liver X receptor signalling¹⁷⁰. While studying the mechanism of fever in models of mobilization, another group showed that G-CSF-induced sympathetic tone stimulates the production of prostaglandin E2 by neutrophils, which can target osteolineage cells to promote HSPC retention¹⁷¹. Tumour necrosis factor (TNF) produced by neutrophils improves vascular recovery following irradiation via TNF receptors on host endothelial cells, which results in enhanced haematopoietic engraftment¹⁷². This study highlights a potential novel approach to improve BM transplantation by enhancing the recovery of the niche compartment. Together, these studies provide a formal demonstration that macrophages and neutrophils directly and indirectly regulate HSC activity.

Regulatory T cells.—Initial studies suggested that subsets of T lymphocytes may be beneficial in allogeneic HSC (allo-HSC) engraftment after BM transplantation^{173,174}. Improvements in intravital microscopy techniques enabled researchers to show that allo-HSCs co-localized with FOXP3⁺ regulatory T (T_{reg}) cells in the endosteal area after transplantation¹⁷⁵. T_{reg} cells promote the survival of donor allo-HSCs by secreting the immunoregulatory cytokine IL-10. Moreover, transplanted donor-derived HSCs can persist in non-irradiated recipient mice for 30 days without immunosuppression. However, depletion of FOXP3⁺ T_{reg} cells leads to rapid loss of allo-HSCs, establishing that T_{reg} cells are thus capable of endowing the HSC niche with immune privilege. Bone marrow CD150^{high} T_{reg} cells seem to localize close to HSCs and promote their quiescence at steady state (potentially by the CD39-catalysed production of adenosine), thereby facilitating allo-HSC engraftment¹⁷⁶. The direct and indirect roles of T_{reg} cells in the HSC niche may establish an immune-privileged ‘sanctuary’ from immune attack, not only for healthy

HSPCs but also for malignant cells; thus, further investigation of these cells in cancer therapy is warranted.

The ageing niche—Mammalian ageing is characterized by progressive tissue attrition, which is associated with degenerative diseases and an increased incidence of many types of cancers. In the haematopoietic system, age-associated alterations in immune responses that have been attributed to a decline in HSC function contribute to increased susceptibility to infections, autoimmunity, anaemia and myeloproliferative diseases¹⁷⁷. Although the absolute number of phenotypically identified HSCs increases with age in mice, the regenerative potential of aged HSCs is reduced^{178–181}. However, these changes in HSC number are not observed in all mouse strains; in fact, the abundance of functional HSCs in BALB/c mice decreases with age¹⁸². Furthermore, aged HSCs show myeloid-biased differentiation upon transplantation^{180,181,183–185}, enhanced mobilization into the circulation and reduced homing back to the BM^{178,186,187}. Aged HSCs also localize to sites further away from the endosteum in competitive short-term homing assays in conditioned mice¹⁸⁸ and are redistributed away from arteriolar and megakaryocytic niches at steady state¹⁸⁹ compared with young HSCs, suggesting that altered HSC distribution is an additional hallmark of ageing.

The functional impairment of aged HSCs compared with their young counterparts when transplanted into young recipients^{178,180,181} suggests that HSC ageing is a consequence of cell-intrinsic alterations. Indeed, many cell-intrinsic mechanisms involved in HSC ageing have been described, including defects in cell polarity, altered transcriptional and epigenetic profiles, and altered metabolism and DNA damage (reviewed elsewhere^{190,191}). However, transplanted young HSCs engraft at a lower efficiency in aged recipients than in young mice^{180,192}, indicating a role for cell-extrinsic mechanisms in HSC ageing (FIG. 4a). Interestingly, a subpopulation of aged, myeloid-restricted HSCs behaved as ‘latent’ multipotent HSCs, as they had a myeloid-restricted potential in primary transplantation but not in secondary transplantation¹⁹³. This study suggests that the rejuvenation of HSCs and restoration of their multilineage potential are complex but attainable and highlights a possible role of the HSC microenvironment in this process¹⁹⁴ (TABLE 2). For example, increased secretion of the pro-inflammatory chemokine CC-chemokine ligand 5 (CCL5; also known as RANTES) may contribute to the myeloid-biased differentiation of aged HSCs¹⁹². *Ex vivo* treatment of HSCs with CCL5 results in fewer T cells and deletion of *Ccl5* in mice rescues the myeloid-biased differentiation. OPN expression by BM stromal cells decreases with age and the injection of thrombin-cleaved OPN attenuates the functional deficits of aged HSCs after transplantation¹⁹⁵. MSCs also show skewed differentiation during ageing, which leads to decreased bone formation and increased adipogenesis^{196,197}. The accumulation of adipocytes in the BM may contribute to haematopoietic alterations in aged mice, owing to their negative effects on haematopoiesis^{123,125} and the concomitant expansion of the myeloid lineage^{198,199}. Age-related bone loss in mice has also been associated with changes in the BM vasculature^{58,189,200}. The number of transitional vessels, arterioles and Osterix⁺ cells is substantially reduced in aged mice and can be restored by the activation of endothelial Notch signalling, although HSC function is not rejuvenated⁵⁸. Furthermore, the BM vasculature exhibits increased leakiness, elevated ROS levels and

decreased expression of CXCL12, SCF and Jagged 1 with age²⁰⁰. Co-culture of young HSPCs with aged endothelial cells, as well as injection of endothelial cells into mice following myelosuppressive injury, demonstrated that aged endothelial cells impair the function and increase the myeloid differentiation bias of young HSCs. Conversely, young endothelial cells can restore the repopulation capacity of aged HSCs but are unable to reverse their myeloid differentiation bias²⁰⁰.

Ageing also leads to marked remodelling of the BM vasculature, including shortening of arteriolar segments and loss of innervation of arterioles¹⁸⁹. Surgical denervation of the bone in young mice results in all the major features of HSC ageing, such as the expansion of myeloid-biased HSCs that have reduced repopulating activity, the acquisition of cell polarity defects, and an increased number of γ H2AX foci (a marker of DNA damage) in HSCs. Denervation also induced remodelling of the BM microenvironment that is reminiscent of that in aged mice, including shortening of arteriolar vessels and expansion of MSCs that have reduced clonogenic capacity and expression of HSC niche factors, such as CXCL12 and SCF. Importantly, administration of an ADR β 3-selective agonist in old mice substantially rejuvenated the *in vivo* function of aged HSCs by acting on BM stromal cells¹⁸⁹. Future studies to investigate the interplay between HSC-intrinsic and HSC-extrinsic mechanisms that regulate HSC ageing should offer therapeutic opportunities to mitigate the ageing-related decline in the haematopoietic system.

The haematopoietic stem cell niche in cancer

Consistent with the regulation of haematopoiesis by the niche (FIG. 3), emerging evidence suggests that niche constituents can also drive neoplasia (FIG. 4b) or be remodelled to support malignant cells (FIG. 4c).

Niche-driven malignancies

One of the first indications that an altered BM niche may contribute to malignant transformation comes from two studies^{201,202} that found that retinoic acid receptor- γ (RAR γ) deficiency triggers a myeloproliferative neoplasm (MPN)-like phenotype. This phenotype is observed even when wild-type cells are transplanted into RAR γ knockout mice²⁰¹, showing that RAR γ expression in the stromal cells rather than in haematopoietic cells causes neoplasia. In addition, deletion of the gene encoding retinoblastoma-associated protein (*Rb1*) in both the stromal compartment and myeloid cells results in MPN-like disease²⁰². Similarly, deletion of mind bomb-1 (*Mib1*), an essential component for Notch ligand endocytosis, in non-haematopoietic cells causes MPN-like disease that can be reverted by activation of Notch in the microenvironment²⁰³. These studies show that signals from the microenvironment can influence malignant transformation.

Mesenchymal-derived stromal cells have also been implicated in driving malignancies. Deletion of the gene encoding the RNA-processing enzyme Dicer 1 in MSPCs using *Osx*-Cre causes myelodysplastic syndrome (MDS) that can sporadically transform to acute myeloid leukaemia (AML). However, deletion of *Dicer1* in mature osteoblasts using *Bglap*-Cre does not result in malignancy²⁰⁴. Loss of Dicer 1 in MSPCs also leads to the reduced expression of *Sbds*, the gene mutated in human Shwachman–Bodian–Diamond syndrome,

which is associated with BM failure and leukaemic predisposition. *Sbds* deletion in MSPCs stimulates p53 signalling and secretion of the inflammatory molecules S100A8 and S100A9, which impairs haematopoiesis and favours leukaemogenesis by inducing genotoxic stress in HSPCs²⁰⁵. Conditional expression of a mutant protein tyrosine phosphatase non-receptor type 11 (*Ptpn11*), a positive regulator of RAS signalling, in MSCs and osteoprogenitors can also drive MPN development and progression²⁰⁶. Additional support for the role of osteolineage cells in leukaemogenesis comes from studies of mice with osteoblast-specific expression of constitutively active β -catenin, which results in MDS and from increased Notch signalling in HSCs²⁰⁷. Interestingly, activation of the parathyroid hormone receptor in mouse osteoblasts augments MLL–AF9 oncogene-driven AML, whereas it reduces BCR–ABL chronic myelogenous leukaemia (CML)-like disease, suggesting a disease-specific role for osteolineage cells in leukaemogenesis²⁰⁸. Other cells of the microenvironment may also influence malignant transformation, as genetic disruption of Notch signalling in endothelial cells alters miR-155 expression, activates NF- κ B signalling, and increases proinflammatory cytokine production, which can trigger MPN-like disease²⁰⁹. Overall, these studies provide evidence that the microenvironment can cause and/or support haematological malignancy in mice (FIG. 4b). However, it is unclear whether similar alterations in human in the niche only can cause human malignancies, although there are anecdotal reports of donor cell-derived neoplasias after allogeneic stem cell transplantation from a healthy donor²¹⁰. Whether this results from undetected malignant clones in the donor sample or from an altered microenvironment in the recipient has not been resolved.

Niche remodelling by malignancies

Just as healthy HSPCs can modulate the niche⁹¹, so too can leukaemia stem cells (LSCs) in order to compete for the BM niche²¹¹ and further remodel it to create a cancer-supportive environment. Leukaemic cells upregulate the expression of several molecules, including CXCR4^{REF212–215}, VLA4^{REF216} (also known as integrin $\alpha 4\beta 1$) and the glycoprotein CD44^{REF217,218}; these molecules mediate leukaemia cell adhesion and survival that confer chemoresistance and are thus being explored as therapeutic targets. BCR–ABL CML cells can induce the differentiation of MSCs into altered osteolineage progenitors through direct cell–cell contact and the secretion of THPO and CCL3, which may impair their capacity to support healthy HSCs and instead promote the growth of leukaemia cells²¹⁹. Similar observations were reported for various mouse models of acute leukaemia, such as an MLL–AF9-driven model of AML^{220,221}, a BCR–ABL-driven model of blast-crisis CML²²² and a model of T cell acute lymphoblastic leukaemia (T-ALL)^{223,224}. Furthermore, studies of primary AML samples from patients also showed that leukaemia cells suppressed BM adipogenesis in favour of AML-supportive osteolineage differentiation from MSCs^{225,226}. Other HSC niche regulators, such as vascular endothelial cells, are also altered in leukaemia. In mouse and human AML, an overall increase in the BM vasculature occurs^{220,227}, although a selective reduction in the number of endosteal blood vessels is observed by intravital microscopy²²¹. In the latter study, vascular remodelling results from the accumulation of local proinflammatory and anti-angiogenic cytokines produced by endosteal AML cells. Interestingly, chemical or genetic rescue of endosteal vessel defects prevented the loss of healthy HSCs and increased the efficacy of chemotherapy²²¹. Similarly, inhibition of endothelial-derived nitric oxide normalizes the BM vascular leakiness observed

in AML xenografts and improves the response to the chemotherapeutic agent cytarabine²²⁸, suggesting that vascular regulation by malignant cells is a key component of chemoresistance.

Emerging data suggest that nerves — which are predominantly associated with blood vessels in tissues — can regulate solid cancers^{229–231} and haematologic malignancies^{220,232}. In the MLL–AF9 mouse model of AML, infiltration of leukaemia cells into the BM leads to the degeneration of sympathetic nerve fibres around arterioles, which further promotes AML²²⁰. Consistent with this observation, treatment of these mice with the neurotoxin 6-hydroxydopamine, which specifically damages catecholaminergic neurons^{98,99}, increases the number of phenotypic LSCs. Neuropathy is accompanied by the proliferation of NES⁺ MSPCs that are primed for osteoblastic differentiation and a reduction in the abundance of HSC-maintaining NG2⁺ periaarteriolar niche cells. Blockade of the β 2-adrenergic receptor enhances AML infiltration in the BM, whereas a β 2-adrenergic agonist delays disease progression²²⁰. In a mouse model of MPN induced by the expression of a mutant form of Janus kinase 2 (JAK2^{V617F}), the number of sympathetic fibres and Schwann cells is also reduced, which is triggered by the production of the proinflammatory cytokine IL-1 β by mutant HSPCs²³². In contrast to its effect in the MLL–AF9 mouse model of AML, neuropathy in this MPN model induces apoptosis of NES⁺ MSCs. MPN progression is attenuated by the administration of a β 3-adrenergic agonist, which restores the sympathetic regulation of MSCs and indirectly reduces the number of LSCs²³². Other studies of MPN myelofibrosis found that abnormal megakaryocytes secrete proinflammatory paracrine signals, such as TGF β ²³³, whereas the aberrant differentiation of LEPR⁺ MSPCs²³⁴ and/or Gli1⁺ MSPCs²³⁵ gives rise to excess myofibroblasts that promote BM fibrosis. Collectively, these studies indicate that although different haematological malignancies share common oncogenesis pathways, it is likely that their interaction with the BM microenvironment may be specific to the leukaemia subtype. Solid tumours of non-haematopoietic origin, such as prostate and breast cancer, can metastasize to the BM and modulate the HSC niche to support tumour growth²³⁶. Human metastatic prostate cancer cells can bind to niche-derived CXCL12 and compete with donor HSCs to occupy the niche^{237,238}. Manipulating the neoplastic niche in combination with chemotherapy may thus be a beneficial therapeutic strategy, particularly in malignancies for which conventional therapies have been unsuccessful.

Conclusions and future perspectives

The past 10 years have seen extraordinary growth in the understanding of BM niches and the specific cellular and molecular components that regulate HSC activity. A range of genetically modified mouse strains, combined with reporters and sophisticated imaging techniques associated with computational analyses have enabled the study of the dynamics of endogenous HSCs in their native environment. These studies support the idea that the niches for HSCs and their progeny consist of complex arrangements of cellular constituents whose contributions remain to be fully elucidated. Accumulating data have identified a major role for vascular and perivascular cells in HSC niche regulation. However, the most purified candidate niche cells (for example, CAR cells, *Nes*–GFP⁺ cells or LEPR⁺ cells) far outnumber HSCs, which suggests that these mesenchymal-derived populations require

further fractionation or that establishment of a bona fide niche requires the participation of other cellular constituents⁶⁶. Ongoing studies using single-cell approaches should provide new insights about the heterogeneity of stromal cells in the HSC niche. Although conditional gene deletions or cellular depletion are powerful tools to investigate the factors and cellular participants that are involved in establishment and/or regulation of the HSC niche, the specificity of promoter and Cre recombinase excision activity is an important consideration for the interpretation of experiments in these models. Studies in which single factors are deleted in niche cells must be cautiously interpreted, as there may be compensatory production of the factor by other cells or long-range signals from outside the bone marrow (BOX 2).

The fact that ‘space’ must be made in the BM niche, using preparative myeloablative regimens, to enable HSCs to engraft has led to the notion that niches are saturated at steady state. However, a recent study suggested that the BM may contain numerous unoccupied niches at steady state²³⁹, which raises a number of important questions, such as how vacant niches can be unlocked, whether they form reservoirs for haematopoietic emergencies and whether leukaemia cells take advantage of these reservoirs to promote disease progression. These important questions also raise the basic yet important issue of how HSC numbers are regulated in the BM. As current imaging approaches to localize endogenous HSCs in fixed tissues may underestimate niche availability, improvements in intravital microscopy methods and genetic reporters to image HSC–niche interactions in real-time may bypass this limitation.

Studies suggest that haematopoietic progenitors have a major role in the regulation of steady-state haematopoiesis; therefore, further studies are needed to carefully evaluate the structure of putative niches established by haematopoietic progenitors and to identify novel factors that regulate stemness and the maintenance of specific types of progenitors. RNA sequencing and gene-targeting approaches may help with the discovery of these factors and their specific role in HSC and progenitor function, but proteomics-based and lipidomics-based methods ultimately be necessary to investigate more thoroughly the various secreted factors that are presumably required to sustain haematopoiesis. These advances would enable the development of improved chemically defined culture systems and the design of ex vivo HSC expansion protocols to improve gene editing and transplantation efficiency. The development of novel mouse models with a humanized microenvironment that is more permissive for human HSC niche studies would be beneficial, as currently it is difficult to recapitulate certain haematological malignancies in mice.

The underlying changes in the BM niches that promote the development of leukaemia suggest that different leukaemia subtypes can induce specific niche abnormalities and may differ in their niche dependence. Other important questions about niche involvement in malignancy include whether LSCs reside in specific niches and if so, whether LSCs of different classes (for example, myeloid versus lymphoid) localize in different niches; and what the role of these niches is in transformation from pre-leukaemia to acute leukaemia. As the incidence of haematological malignancies increases with age, understanding the mechanisms of HSC and niche ageing and their contribution to malignant transformation

may help in the design of therapeutic approaches to rejuvenate an aged haematopoietic system or prevent or treat haematological malignancies.

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Glossary terms

Transplantation

An *in vivo* procedure to replace the haematopoietic system of a recipient by delivering a sufficient number of donor total bone marrow cells or enriched populations of haematopoietic stem and progenitor cells.

Self-renewal

The capacity of a cell to divide and give rise to identical cells with equivalent developmental potential, resulting from either an asymmetric cell division that yields a daughter cell and a cell committed to differentiation, or from a symmetric cell division that yields two identical daughter cells.

Multipotent

The capacity of a single cell to give rise to progeny of multiple lineages.

Quiescence

The state of being inactive or dormant in the G₀ phase of the cell cycle, which is important for long-term function.

Exhaustion

A state in which the numbers and/or function of HSPCs are compromised owing to high turnover as a consequence of high demand for reconstitution in stress or serial transplantation.

Homing

The return of haematopoietic stem cells to the bone marrow by trans-endothelial migration.

Lodgement

The process by which, following homing, haematopoietic stem cells anchor in a specific region.

Engraftment

The process by which donor haematopoietic stem cells persist in the host, generate mature progeny as well as re-populate the haematopoietic stem cell pool.

Trabecular bone

A type of bone tissue, also known as cancellous or spongy bone, which actively remodels and is mostly present in flat and irregular bones, as well as in the epiphysis and metaphysis of long bones.

Cortical bone

A type of bone tissue, also known as compact bone, which has a mechanical function, forming a hard shell for the long bone diaphysis and accounting for the majority of total bone mass in the human skeleton.

Endosteum

Inner surface of the bone cavities and the outer surface of the trabeculae bone spicules within the cavities covered by bone-forming osteoblasts and bone-resorbing osteoclasts.

Periosteum

Tissue that covers the outer surface of the bone and consists of two layers: an outer fibrous layer that contains fibroblasts and collagen fibres and an inner cambium layer that consists of osteoblasts and skeletal stem cells with bone regeneration capacity.

Sinusoids

Specialized thin-walled capillaries with a wide lumen. Sinusoids distribute evenly throughout the bone marrow and form a web of fenestrated vessels that facilitate trafficking of haematopoietic cells and factors in and out of the circulation.

Perivascular niche

A specialized microenvironment located adjacent to the bone marrow vasculature, which regulates the maintenance of haematopoietic stem cells and/or progenitor cells.

Bone marrow stromal cells

Cells of non-haematopoietic origin (classically defined as CD45⁻Ter119⁻) in the bone marrow. Stromal cells may or may not include endothelial cells, depending on whether cells that express CD31 or TIE2 are included in the definition.

Mesenchymal stem cell

(MSC). A self-renewing precursor cell that can differentiate into bone, fat, cartilage and forms stromal cells of the bone marrow.

Mesenchymal stem and progenitor cells

(MSPCs). A population of stromal cells that remains undefined but is expected to contain self-renewing mesenchymal stem cells and colony-forming mesenchymal progenitor cells.

Sympathetic nervous system

(SNS). A branch of the autonomic nervous system that emerges from the thoracolumbar spinal cord and prepares the body for situations requiring alertness by releasing noradrenaline, which binds to adrenergic receptors.

Genotoxic insults.

Radiation or chemical agents that induce damage to the genetic material in cells.

Allogeneic.

In the context of transplantation, cells or tissue that are from the same species but are genetically different from the recipient.

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Box 1 –**The localization of HSCs in the niche**

Assessment of the anatomical location of haematopoietic stem cells (HSCs) and their proximity to candidate niche cells are major criteria for defining *bona fide* niches²⁴⁰ and are major topics of debate in the field. Direct visualization of the bone marrow (BM) is therefore essential to fully understand how HSCs are maintained and regulated *in situ*. However, this is a major challenge owing to a lack of exclusive markers for the identification and tracking of endogenous HSCs and niche cells and to the calcified nature of bones. Initial studies traced the homing of exogenously labelled or GFP-marked haematopoietic stem and progenitor cells (HSPCs) transplanted into the BM of recipient mice using classical histological or intravital microscopy imaging techniques. These studies indicated that HSPCs have a close relationship with the endosteum^{38–40}. However, the extensive manipulation required to purify HSCs, the possible effect of anaesthesia or surgical stress during transplantation and the limited depth of imaging are all major drawbacks of this approach.

The discovery of signalling lymphocytic activation molecule (SLAM) family receptors (such as CD150, CD48 and CD244) as HSPC markers, studies using thinly sliced BM sections revealed that CD150⁺CD48⁺CD41[−]Lineage[−] HSCs are distributed broadly in close proximity to endothelial cells and *Nes*–GFP⁺ perivascular cells^{54,65}. In mice expressing GFP from the haematopoietic promoter of *Gata2*, GFP⁺SCA1⁺ cells enriched in HSC activity localize in close proximity to the endosteum⁴⁴. Endogenous HSCs have also been shown to localize in perivascular niches that are enriched near the endosteum^{43,241,242}. However, the use of histological sections is limited by the restricted z-depths. Further technological improvements enabling 3D visualization of endogenous HSCs *in situ* uncovered a substantial association between a subset of quiescent HSCs and small arterioles, which themselves show a higher density in the endosteum region³⁰. Upon activation and proliferation, this HSC subset distributes away from arterioles. 3D whole-mount imaging analyses revealed that a subset of quiescent HSCs is located adjacent to megakaryocytes in a niche that seems to be distinct from the arteriolar niche¹⁵⁶. Although the ‘non-arteriolar niche’ is often referred to as the ‘sinusoidal niche’, it is important to emphasize that sinusoids occur throughout the BM and that most, if not all, haematopoietic cells are located in close proximity to sinusoids. However, HSC distribution in perivascular niches may not be random and may be influenced by specific microenvironments.

Of note, other studies using different tissue processing to improve translucence and transgenic mice with selective expression of HSC reporters (TABLE 1) suggested that HSCs are uniformly distributed in perisinusoidal niches. Studies using KIT immunostaining of optically cleared BM from *Ctnn1l*–GFP (*Ctnn1l* encodes α-catulin) reporter mice found that GFP⁺ HSCs are not associated with arterial vessels but instead are broadly and randomly distributed in the BM (in a manner indistinguishable from randomly placed dots)⁴⁷. Furthermore, there was no enrichment of quiescent HSCs in arteriolar areas. Another study found that *Hoxb5*–mCherry⁺ HSCs (imaged after chemical clearing) are homogeneously associated with VE-cadherin⁺ endothelial cells²⁴³.

In the future, it will be interesting to evaluate in greater detail the localization of HOXB5⁺ HSCs in the context of vascular compartments. Although clearing techniques enable deep imaging of the marrow, they also require tissue decalcification or marrow plug extraction and additional processing steps, that may compromise the marrow architecture and the expression of surface markers²⁴⁴. By contrast, and in support of the existence of multiple niche structures, other studies detected arteriolar and/or megakaryocyte niches containing quiescent HSCs with low levels of reactive oxygen species (ROS)³¹, quiescent HSCs in close proximity to arteriolar-associated Schwann cells¹³⁶ and HSCs associated with transitional vessel endothelial cells connecting arterioles and sinusoids⁵⁸. *Tie2*-GFP⁺ HSCs that are highly enriched for HSC activity also show a strong association with arterioles²⁴⁵ as well as the CD82⁺ HSC subset that is enriched for long-term HSC potential¹⁶⁹. Imaging of myeloid-biased HSCs using *Vwf*-GFP shows separate BM niches for *Vwf*-GFP⁺ HSCs and *Vwf*-GFP⁻ HSCs, which are regulated by megakaryocyte and arteriole-associated niche cells, respectively³². Thus, these studies using different HSC markers have reported differing results about HSC localization in the BM; whether these discrepancies result from the use of different methodologies or reflect the existence of distinct HSC subpopulations will require further investigation.

Box 2.**The identification of HSC niche cells**

A “stem cell niche” refers to the local microenvironment within a tissue in which stem cells are maintained in an undifferentiated and self-renewable state and receive stimuli that determine their fate. Therefore, the location of stem cells in a tissue is not enough to define a niche, as the niche must have both anatomical and functional consequences. In the haematopoietic system, niche inputs are experimentally evaluated by determining their effects on haematopoietic stem cell (HSC) numbers, proliferation, localization, *in vivo* repopulating capacity and trafficking. Stem cell niches share several key characteristics, such as physical association between niche and stem cells or selective synthesis of stem cell maintenance factors, that make their *in vivo* identification possible using different methodologies.

Targeted cell ablation studies.

Genetic manipulation can be used to deplete candidate niche cells *in vivo* and evaluate its effect on HSCs and haematopoiesis. Ablation can be achieved by the expression of a suicide gene, such as thymidine kinase (TK)⁷² or the diphtheria toxin receptor (DTR), under the control of the promoter of a gene specifically expressed in the target cell type. Use of the DTR approach led to the identification of different perivascular constituents of the bone marrow niche^{30,54,102,156–158}. Conversely, genetic manipulations can also be used to expand or activate putative niche candidates; for example, a correlation between the number of osteoblasts and haematopoietic stem and progenitor cells (HSPCs) was demonstrated using this approach^{70,71}. However, these approaches rely on the specificity of the genetic manipulation in the candidate niche cell type. Additionally, *in vivo* genetic cell depletion cannot discriminate between direct niche-dependent and indirect niche-independent effects that result from the systemic depletion of the cell population. The experimental dissection of direct and indirect effects should always be an important consideration in studies manipulating candidate niche cells.

Identification of niche cells by the expression of niche factors.

An alternative approach to identify niche cells is determining which cells synthesize HSC regulators, which include CXC chemokine ligand 12 (CXCL12), stem cell factor (SCF), thrombopoietin (THPO), osteopontin (OPN), transforming growth factor- β (TGF β), CXCL4, vascular cell adhesion molecule 1 (VCAM1), GP130, Notch ligands, fibroblast growth factor 1 (FGF1) and pleiotrophin (FIG 2).

Identification of niche cells by conditional deletion of niche factors.

Putative niche cells can also be functionally identified by conditionally deleting a gene encoding a known HSC niche factor (such as *Cxcl12*^{85,86,109} or *Scf*^{84,109}) by Cre-mediated recombination in candidate niche cells. This approach minimizes the off-target effects associated with the disruption of the BM cellular architecture that occurs in cell ablation studies. Furthermore, it enables a much more detailed evaluation of the individual contributions of candidate cells to overall niche activity. The main drawbacks of this approach are that it also relies on the availability of genetic models and the final

readout will depend on the specificity of Cre expression and recombination efficiency. In addition, the niche capacity of some cells might also be overlooked owing to compensation by other cell types.

Other approaches.

The physical proximity of HSCs and candidate niche structures may offer further insight into the HSC niche. Imaging techniques can reliably identify HSCs in their native microenvironment, and the distance between HSCs and the nearest putative niche can be easily measured. To determine whether potential associations are functionally relevant, the distribution of HSCs relative to candidate structures can be statistically compared to computer-generated models of random spots distribution in the marrow space^{30,47,156,157,243}. The HSC niche capacity of stromal cells can also be tested using heterotopic transplantation assays. In these assays, candidate niche cells are embedded in a matrix or ossicle and transplanted into recipient mice to test their ability to generate haematopoietic niches with active haematopoiesis of host origin supported by a stroma of donor origin^{54,95,97,101}.

Figure 1. Microarchitecture of the adult mouse femur and sternum bone marrow.

Postnatally, the bone marrow (BM) is the primary site of haematopoietic stem cell (HSC) maintenance and haematopoiesis. **a** | Longitudinal view of a femur illustrating the arrangement of blood vessels and nerves (marked in yellow) that accompany the arteries within the BM cavity. The periosteal layer covers the outer surface of the bone and the endosteal layer is at the interface of bone and BM. Branching arteries (red) run parallel to the long axis of the marrow cavity, often close to the endosteum. These vessels feed into the sinusoidal network, which is distributed evenly throughout the marrow cavity and then coalesces to form the venous circulation (blue). **b** | Anterior view of the adult human sternum with attached ribs. Unlike the long bones, which mostly contain adipocytes in adult humans, the sternum has rich haematopoietic activity in both mice and humans, which makes it a suitable site to study haematopoiesis. The blood supply to the sternum originates from sternal/perforating branches located in the intersegmental spaces (between the ribs) from internal thoracic arteries that extend along the inside of the ribcage parallel to the sternum. **c** | Mid-sagittal section of the mouse sternum illustrating the six BM compartments (the human sternum comprises two bones, the manubrium and the body) with **a** representative image of a sternal segment showing the vasculature, which is labelled using intravenously injected antibodies against CD31/CD144 (red) and SCA1 (green). Arterioles can be distinguished from the sinusoidal network by their SCA1^{high} expression.

Figure 2. Cellular and molecular constituents of the HSC niche.

Various cell types have been implicated in regulating haematopoietic stem cell (HSC) activity, including perivascular mesenchymal stem cells (MSCs), endothelial cells, osteoblasts, adipolineage cells, sympathetic nervous system (SNS) nerves, nonmyelinating Schwann cells, macrophages, megakaryocytes and regulatory T (T_{reg}) cells. The target plot illustrates how bone marrow (BM) niche cells contribute to HSC regulation indirectly or directly by synthesizing niche factors in the form of cell-bound or secreted molecules. The colour of the radial spokes indicates the HSC activity that is affected. *Molecules involved in bone marrow regeneration after ablation. Bold type indicates those molecules for which there is functional data using cell-specific genetic evidence. SCF, stem cell factor; CXCL12, CXC-chemokine ligand 12; CXCL4, CXC chemokine ligand 4; FGF1, fibroblast growth factor 1; TGFβ, transforming growth factor-β; DARC, duffy antigen receptor for

chemokines; GP130, glycoprotein 130; VCAM1, vascular cell adhesion molecule 1; IL-10, interleukin-10.

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Figure 3. The adult bone marrow HSC niche in homeostasis.

Schematic representation of the adult bone marrow haematopoietic stem cell (HSC) niche in homeostasis, showing various cell types and niche factors that directly or indirectly regulate HSC activity. Emerging evidence highlights the vasculature and associated stromal cells, such as periarteriolar *Nes-GFP^{high}* cells, *NG2⁺* cells and *MYH11⁺* cells and perisinusoidal *Nes-GFP^{low}* cells, CAR cells and *LEPR⁺* cells, as key regulators of HSC maintenance. Sympathetic nervous system (SNS) nerves regulate HSC mobilization and non-myelinating Schwann cells may contribute to HSC quiescence. Osteoblasts have been implicated in HSC regulation but the precise molecular signals have not been clearly elucidated; however, they may have a role in regulating lymphoid progenitors. Adipocytes may negatively affect HSC maintenance. Haematopoietic cells, such as macrophages, neutrophils, regulatory T (*T_{reg}*) cells and megakaryocytes, are examples of HSC-derived progeny that can feedback and contribute to HSC maintenance or mobilization. Regional localization of HSC subsets has shown that platelet-biased or myeloid-biased *Vwf-GFP⁺* HSCs and *Vwf-GFP⁻* HSCs are located in, and regulated by, separate BM niches containing megakaryocytes and arterioles, respectively. CAR cells, CXCL12-abundant reticular cells; SCF, stem cell factor; CXCL4, C-X-C-chemokine ligand 4; CXCL12, CXC chemokine ligand 12; DARC, duffy antigen receptor for chemokines; IL-7, interleukin-7; LEPR, leptin receptor; MYH11, myosin heavy chain 11; NG2, neuron/glia antigen 2; OPN, osteopontin; OSM, oncostatin M; SCF, stem cell factor; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; vWF, von Willebrand factor.

Figure 4. The adult bone marrow HSC niche in ageing and malignancy.

a | Schematic representation of the aged haematopoietic stem cell (HSC) niche. Aged HSCs are highly proliferative and exhibit increased myeloid-biased differentiation and reduced regenerative capacity. Ageing-related alterations of the bone marrow (BM) niche that influence HSC ageing include alterations in the vasculature and mesenchymal stem and progenitor cells (MSCs), increased adipogenesis and reduced osteogenesis, altered secretion of niche factors and a reduced number of adrenergic nerves. **b** | HSC niche alterations that promote cancer. Epigenetic or genetic lesions (lightning bolt) in stromal niche regulators can lead to the loss of inhibitory signals that control the growth of premalignant clones and ultimately promote myeloid malignancies. These lesions include alterations in the expression of RBPJ in endothelial cells, β -catenin in osteoblasts and Dicer 1, SBDS and PTPN11 in MSCs; and deletion of *Rarg* (which encodes RAR γ), *Rb* or *Mib1* in undefined stromal cells. **c** | Cancer promotes niche remodelling. The majority of myeloid

malignancies are caused by epigenetic and/or genetic mutations in haematopoietic stem and progenitor cells (HSPCs), which lead to BM niche remodelling that supports cancer cell growth at the expense of normal haematopoiesis. Alterations produced by different malignancies can lead to a proinflammatory environment characterized by impaired MSPC differentiation, fibrosis, vascular remodelling, neuropathy and reduced production of HSC-niche factors by stromal cells. Leukemic stem cells (LSCs) can also upregulate the expression of CXCR4, VLA4 and CD44, to hijack the mechanisms of adhesion used by healthy HSPCs. ADR β 3, adrenergic β 3 receptor; AML, acute myeloid leukaemia; CCL3, CC chemokine ligand 3; CML, chronic myeloid leukaemia; CXCL12, CXC chemokine ligand 12; CXCR4, CXC chemokine receptor type 4; IL-1 β , interleukin-1 β ; MDS, myelodysplastic syndrome; MIB1, mind bomb 1; MPN, myeloproliferative neoplasm; NG2, neuron/glia antigen 2; OPN, osteopontin; PTPN11, protein tyrosine phosphatase, non-receptor type 11; RAR γ , retinoic acid receptor- γ ; RB, retinoblastoma protein; RBPJ, recombination signal binding protein for immunoglobulin- κ J region; ROS, reactive oxygen species; SBDS, Shwachman–Bodian–Diamond syndrome protein; SCF, stem cell factor; SNS, sympathetic nervous system; T-ALL, T cell acute lymphoblastic leukaemia; TGF β , transforming growth factor- β ; THPO, thrombopoietin; VLA-4, very late antigen 4.

TABLE 1:

Transgenic mice for labelling of adult bone marrow HSPCs.

Mouse strain	Genetic modification	Specificity within the adult haematopoietic compartment	Analysis	Refs
<i>Hoxb5-Tri-mCherry</i>	Knock in	Specific to long-term HSCs	<ul style="list-style-type: none"> • Modified CUBIC clearing and light-sheet microscopy • Flow cytometry • Transplantation 	243
<i>Ctnnal1-GFP</i>	Knock in	Restricted to HSCs and haematopoietic progenitors; requires KIT staining to enrich for HSCs	<ul style="list-style-type: none"> • Modified Murray's clearing, immunostaining and confocal and multi-photon microscopy • Flow cytometry • Transplantation 	47
<i>Fgd5-mCherry</i>	Knock in	Restricted to HSCs; low expression in haematopoietic progenitors	<ul style="list-style-type: none"> • Flow cytometry • Transplantation 	246
<i>Vwf-GFP</i>	Transgenic	Labels platelet/myeloid-biased HSCs, megakaryocyte progenitors, megakaryocytes and platelets	<ul style="list-style-type: none"> • Flow cytometry • Transplantation • Immunostaining and whole-mount confocal microscopy 	29,32
<i>Msi2-GFP</i>	Knock in	Also labels haematopoietic progenitors	<ul style="list-style-type: none"> • Confocal microscopy • Flow cytometry 	247
<i>Pdzk1ip1-GFP</i>	Transgenic	Enriches for highly purified HSCs but also labels a small subpopulation of haematopoietic progenitors and mature granulocytes	<ul style="list-style-type: none"> • Doxycycline chase • Transplantation • Flow cytometry 	16
<i>Evi1-GFP</i>	Knock in	Also labels haematopoietic progenitors	<ul style="list-style-type: none"> • Flow cytometry • Transplantation 	248
<i>Scl-tTA-H2B-GFP</i>	Transgenic	H2B-GFP ^{high} label-retaining cells are enriched in quiescent long-term HSCs; also labels haematopoietic progenitors	<ul style="list-style-type: none"> • Doxycycline chase • Transplantation • Flow cytometry • Immunostaining and confocal microscopy 	6,249
<i>Tie2-GFP</i>	Transgenic	Enriches for highly purified HSCs but also labels haematopoietic progenitors	<ul style="list-style-type: none"> • Flow cytometry • Transplantation • Immunostaining and whole-mount confocal and multi-photon microscopy 	245
<i>Gprc5c-GFP</i>	Transgenic	Enriches for dormant HSCs but also labels haematopoietic progenitors	<ul style="list-style-type: none"> • Flow cytometry • Transplantation 	250
<i>Hdc-GFP</i>	Transgenic	Enriches for myeloid-biased HSCs but also labels haematopoietic progenitors and mature myeloid cells	<ul style="list-style-type: none"> • Flow cytometry • Transplantation • Immunostaining and confocal microscopy 	251
<i>Krt7-GFP</i>	Knock in	Specific to HSCs	<ul style="list-style-type: none"> • Flow cytometry 	252
<i>Gata2-GFP</i>	Knock in	Enriches for HSCs and haematopoietic progenitors; SCA1 or Lineage staining are required for HSC selectivity	<ul style="list-style-type: none"> • Flow cytometry • Transplantation • Immunostaining and microscopy 	44
<i>Hoxb4-YFP</i>	Knock in	Also labels haematopoietic progenitors	<ul style="list-style-type: none"> • Flow cytometry • Transplantation 	253

Ctnnal1, α -catulin; CUBIC, clear, unobstructed brain imaging cocktails and computational analysis; *Evi1*, ecotropic virus integration site 1 homologue; *Fgd5*, FYVE, RHOGEF and PH domain-containing 5; *Gata2*, GATA-binding 2; *Gprc5c*, G-protein coupled receptor family C group 5 member C; H2B, histone H2B; *Hdc*, histidine decarboxylase; HSCs, haematopoietic stem cells; HSPCs, haematopoietic stem and progenitor cells; *Hoxb5*, homeobox b5; *Krt7*, keratin 7; *Msi2*, Musashi 2; *Pdzk1ip1*, PDZK1-interacting 1; *Scl*, stem cell leukaemia; *Tie2*, tyrosine kinase with immunoglobulin and EGF homology domains 2; tTA, tetracycline-controlled transactivator protein; *Vwf*, von Willebrand factor.

TABLE 2:

Niche alterations associated with ageing in the mouse bone marrow.

Cell type	Alterations associated with ageing
Endothelial cells	<ul style="list-style-type: none">• Overall increase in vascular density¹⁸⁹ but reduced number and length of arterioles^{58,189} and type H capillaries⁵⁸• Increased vascular leakiness and elevated intracellular ROS levels²⁰⁰• Decreased expression of CXCL12, Jagged 1²⁰⁰ and SCF^{58,200}
Bone marrow MSCs	<ul style="list-style-type: none">• Decreased osteogenesis and increased adipogenesis^{189,196,197}• Reduced number of osteoprogenitors⁵⁸• Reduced number of perivascular αSMA⁺ cells^{58,189}, PDGFRβ⁺ cells and NG2⁺ cells⁵⁸• Expansion of bone marrow MSCs with reduced CFU-F capacity and reduced expression of HSC niche factors¹⁸⁹
Compact bone MSCs	<ul style="list-style-type: none">• Reduced number and CFU-F capacity of bone-associated Nes-GFP⁺ cells¹⁸⁹
Osteolineage cells	<ul style="list-style-type: none">• Increased expression of CCL5¹⁹²• Decreased expression of OPN¹⁹⁵• Reduced number of CD45-Ter119-CD31-SCA1-CD51⁺ osteoblast-enriched cells¹⁹⁵
Adipolineage cells	<ul style="list-style-type: none">• Increased number of adipolineage cells¹²⁵
Nerves	<ul style="list-style-type: none">• Reduced number of TH⁺ adrenergic and β-III tubulin⁺ nerve fibres¹⁸⁹• Reduced number of synaptophysin⁺ synapses¹⁸⁹
Megakaryocytes	<ul style="list-style-type: none">• Increased number of megakaryocytes and megakaryocyte progenitors¹⁸⁹

α SMA, α -smooth muscle actin; CCL5, CC-chemokine ligand 5; CFU-F, colony-forming unit-fibroblast; CXCL12, CXC-chemokine ligand 12; HSC, haematopoietic stem cell; MSCs, mesenchymal stem cells; MSCs, mesenchymal stem and progenitor cells; Nes, nestin; NG2, neural/glial antigen 2; OPN, osteopontin; PDGFR β , platelet-derived growth factor receptor- β ; ROS, reactive oxygen species; SCA1, stem cell antigen 1; SCF, stem cell factor; TH, tyrosine 3-hydroxylase.