INTRODUCTION

Haematopoietic stem cells (HSCs) sustain multilineage haematopoiesis during the lifetime of an organism. Adult HSCs reside in dedicated bone marrow (BM) niches, which sustain their functions. Extensive efforts have been devoted into unveiling the HSC niche due to its medical relevance.\(^1\)\(^-\)\(^6\) The BM constitutes a highly vascularised organ. Anatomically two major BM-niche regions can be distinguished: the vascular niche (including arterioles and sinusoids that converge in the central vein) and the endosteal niche, more closely associated with the bone\(^2\) (Figure 1). Within the vascular niche, besides endothelial cells (ECs), which are an intrinsic part of the blood vessels, the stromal cells provide a tri-dimensional scaffold to the BM and include mesenchymal stem cells (MSCs), which are multipotent cells with the ability to differentiate into osteocytes, adipocytes and chondrocytes. MSCs comprise periairteriolar NG2\(^+\) cells, Nestin\(^{high}\) cells, MYH11\(^+\) cells, CXCL12-abundant reticular (CAR) cells, perisinusoidal LepR\(^+\) cells and Nestin\(^{low}\) cells, amongst others.\(^1\)\(^-\)\(^5\)\(^,\)\(^7\) In addition, sympathetic nerves and non-myelinating Schwann cells associate with arteries, while megakaryocytes (MKs) connect with sinusoids. In the endosteal niche, macrophages, osteoblasts and osteoclasts do not normally associate with the vasculature.\(^7\) Interestingly, HSCs locate to specific areas within the BM. Particularly, HSCs concentrate close to the vasculature during homeostasis and closer to the endosteum after transplantation.\(^8\) Different cell types have been...
implicated as components of the HSC niche, such as sinusoidal endothelium, perivascular stromal cells, macrophages, MKs, osteoblasts and sympathetic nerves (Figure 1). The cellular composition of the BM dramatically changes with age, upon drug treatments (e.g. chemotherapy) and during disease (e.g. leukaemia). Anatomical and histological analyses of bone preparations provided the first insights into the function and cellular components of the BM. Modern molecular biology and genetic models enabled more sophisticated approaches to study the BM niche involving: (i) depletion and expansion of candidate niche cells, (ii) genetic deletion of essential supportive factors from candidate cell types, (iii) stem cell transplantation and (iv) high-resolution imaging techniques (Table 1). Furthermore, recent broader implementation of single-cell sequencing techniques has exposed a tremendous cell diversity within the BM.

These approaches are limited by one or more of these factors: (i) infidelity of Cre-recombinase (CRE) expression (e.g., lack of specificity for subsets of mesenchymal cells and contradictory results on the role of osteoblasts), (ii) low-resolution, (iii) low-throughput, (iv) inability to label and visualise niche cells of unknown phenotype, and (v) insufficient sequencing depth to capture the small number of cells that are in cell–cell contact with the rare HSC population.

Thus, although a remarkable body of data has been produced, the exact composition of the niches that harbour and maintain HSCs and haematopoietic stem and progenitor cells (HSPCs) are still a matter of intense debate. In this review, we focus on the niches that support HSCs as the most extensively studied and on mouse as the most widespread mammalian model of haematopoiesis (Table 2). We provide a historical perspective on how our understanding of the structure and cellular composition of the BM has evolved over time based on the implementation of new approaches. We describe in detail these research strategies, their technical limitations and advantages, what was learnt from them and how new methods complement previous techniques to elucidate the cellular interactomes in the BM.

**STEM CELLS AND NICHE CELLS: MORE THAN NEIGHBOURS**

In 1896 Artur Pappenheim conceptualised the term *stem cell* describing a precursor cell capable of generating other mature...
| Strategy | Findings | Advantages | Disadvantages | References |
|----------|----------|------------|---------------|------------|
| Anatomical and histological studies | Identified a variety of cell types, suggested the existence of stem cells and the importance of the marrow in blood production. | Information on tissue architecture. | Limited functional and molecular information and difficulty to address the identity of specific cell types. | 27–33 |
| Transplantation of stromal components | Defined the presence of a haematopoietic supportive microenvironment able to recruit and support host haematopoietic activity. | Molecular and functional information. | Lack of the full plethora of steady-state bone marrow niche components. | 23,24,35–41,43 |
| Expansion and depletion of candidate niche cells by drug treatments | Provided insights into the role played by some niche components (e.g., osteoblasts) in the maintenance of HSCs. | Functional information. | Lack of spatial information. Broad and non-specific side-effects affecting multiple molecular and cellular targets that complicate the interpretation of the results. | 46,48–53 |
| Expansion and depletion of candidate niche cells via genetic manipulation | Explored the role of niche cells (e.g., CAR cells, megakaryocytes and macrophages) in a more specific fashion than drug treatments. | Molecular and functional information. | Lack of specificity of most promoters complicates the identification of defined candidate niche cells. The use of various promoters is advised to achieve solid conclusions. Global changes in the numbers of cells in the BM may indirectly lead to the activation of other mechanisms to regain homeostasis. | 7,21,22,41,51,54,56,58–66 |
| Genetic deletion of essential supportive factors in the niche | Identified cellular sources of key cytokines and unveiled HSC niche components and their role regulating HSC dynamics, quiescence and self-renewal. | Molecular and functional information. | Lack of spatial information. Promiscuous promoters target non-specific populations complicating conclusions. The use of inducible models should be favoured over constitutive models to discern among embryonic and adult effects. | 69–72 |
| 2D Imaging | The use of HSC and stromal cell markers combined with genetic tracing allowed a simpler and specific identification of populations of interest. | Spatial information. | Conclusions based on limited number of cell layers. Lack of functional information. Biased by the selection of the markers used to define cell types. | 8,9,41,47,77,79,81,82,84 |
| 3D Imaging | Statistical analysis on the distribution of candidate niche cells and HSCs. Provided a comprehensive quantitative picture on the abundance of niche components. | Quantitative spatial and molecular information. | Lack of functional information and limited by the number of antibodies that can be employed at one single time. | 61,83,85–88 |
| Intravital microscopy | Single-cell resolution imaging in live animals. | Spatial, cell behavioural and longitudinal information. | Reduced molecular information and limited by the number of used markers (e.g., via genetic fluorescent labelling). | 8,13,41,47,70,83,85,89–97 |
| Single-cell sequencing techniques | Uncovered the diversity and heterogeneity of the BM and allowed to characterise the identity of different BM cells. | Molecular and functional insights from specific and defined subsets of marrow populations. | Lack of spatial information. Insufficient sequencing depth to capture rare populations in bulk samples. | 2,3,6,15,103 |

(Continues)
types of blood cells. Early experiments during the atomic age demonstrated that lead-shielding the spleens of lethally irradiated mice prevented mortality.\(^6\) Stem cells were proved later to be the critical protective factor. Till and McCulloch\(^9\) reported that mice marrow cells injected in irradiated recipients could lead to the formation of colonies of proliferating cells with self-renewing abilities in their spleens (CFU-S). Noticing that CFU-S stem cells were less robust than the cells of the BM at reconstituting haematopoiesis in irradiated animals, Ray Schofield\(^{20}\) formulated the niche hypothesis in which a stem cell is associated with other cells that determine its behaviour and fate. The identification of heterologous cells influencing stem/progenitor cells in mammals provided experimental evidence for this hypothesis.\(^{21-24}\)

Overall, BM stem cell niches can be defined as highly specialised and dynamic microenvironments that support HSPCs. Moreover, these niches integrate a variety of cues to efficiently respond to a plethora of insults, including infection and bleeding, to maintain tissue homeostasis throughout life.\(^4,5,10\) This implies balancing stem cell differentiation and self-renewing decisions to generate the billions of blood cells required daily, while simultaneously preserving the stem cell population size and avoiding leukaemia development.\(^{20}\)

### UNVEILING THE BM NICHE: EVOLUTION IN RESEARCH APPROACHES

#### Anatomical and histological analyses: early studies

Numerous research methods have shaped our view on the role and composition of the HSC niches. Aristotle (384–322 BC) described the marrow as some ‘sort of bone waste byproduct’,\(^14\) while Hippocrates (460–375 BC) and Galen (130–200) considered it the source of nutrients for the bone.\(^{14,25}\) In the 18th century, Jacques-François-Marie Duverney and Charles Robin noticed that bone is formed before marrow during development and that not every adult bone harbours a marrow, leading them to consider the marrow as the vascular element of the bones.\(^{14,25,26}\) In the 19th century, Ernst Neumann, Giulio Bizzozero (disciples of Rudolph Virchow) and William Osler described nucleated red blood cells, white blood cells and giant marrow cells (i.e. MKs) in the BM of humans and rabbits,\(^{27-29}\) leading to the modern view of the BM as the site of adult haematopoiesis.

Anatomically, Xavier Bichat (1771–1802) had already described the presence of red marrow and yellow fatty marrow.\(^{30,31}\) Neumann noted that in most bones the marrow changes from red marrow at birth to a yellow adult marrow and claimed that blood production is confined to the red marrow in the central bones.\(^{32}\) Osler described the medulla of patients with leukaemia as similar to the ‘matter in the core of an abscess’ and the marrow in pernicious anaemia as comparable to the red marrow of a child.\(^{14,29}\) Paul Ehrlich, using acid and basic aniline coal tar dyes, identified

---

**TABLE 1**

| Strategy | Findings | Advantages | Disadvantages | References |
|----------|----------|------------|---------------|------------|
| Spatially resolved transcriptomics | Locate transcriptionally profiled cells to particular areas previously dissected via laser-capture microdissection. | Molecular and spatial information from specific and defined subsets of marrow populations. | Lack of information on proteomic profiles and cannot establish cell-cell interactions but only predicts them. | 6,105–107 |
| Fluorescent labelling of cells in spatial proximity | Allowed labelling of neighbouring cells and the study of the cellular environment (e.g., in leukaemia, AML). | Spatial information of the cells of interest and prospective isolation and characterisation of niche cells. | Lack of functional information. Unable to distinguish whether cells are in cell-cell contact or distant. | 108,109 |

Abbreviations: AML, acute myeloid leukaemia; BM, bone marrow; CAR, CXCL12-abundant reticular (cells); (2)(3)D, (two)(three)-dimensional; HSC, haematopoietic stem cell.
| Strategy | Mouse strain | Description | Major findings | References |
|----------|--------------|-------------|----------------|------------|
| ‘HSC-reporter’ mouse strains | Mds1-GFP | GFP-labelling of haematopoietic stem and progenitor cells (HSCs and MPPs). | LT-HSCs localise close to sinusoidal blood vessels and the endosteal surface. MPPs locate closer to transition zone vessels in intravital imaging studies in calvarium. | 95 |
| | Flt3-Cre; Mds1-GFP | GFP* LT-HSCs: CRE expression in differentiating HSCs ablates floxed GFP in MPPs limiting GFP expression to LT-HSCs. | Significant motility of tdTomato+ HSCs in the perivascular space. Intermittent contacts with SCF-expressing perivascular stromal cells. | 97 |
| | Pdeltl-CreERt; R26LoxStopLox-tdTom | Tamoxifen-inducible expression of tdTomato in HSCs. | MCherry+ HSCs associated with VE-cadherin+ endothelial cells. | 93 |
| | HoxB5-tri-mCherry | mCherry-labelling of long-term HSCs. | α-Catulin–GFP+ c-Kit+ HSCs preferentially locate to the central marrow in close contact with LepR+ and CXCL12+ niche cells. | 85,92 |
| | α-Catulin-GFP | GFP-labelling of HSCs. | Myeloid-biased and lymphoid-biased HSCs occupy distinct BM niche microenvironments that are differentially regulated. | 94 |
| | VWF-eGFP | GFP-labelling of myeloid-biased HSCs. | DOX-pulsing visualises dormant and non-dormant HSPCs which show similar localisation in the BM. | 85 |
| | SCL-tTA; H2BGFP | Doxycycline-inducible labelling of HSPCs with Histone H2B-GFP. | Abundance of empty HSC niches available for engraftment upon transplantation of non-conditioned recipients. | 98 |
| | Rosa26-M2-rtTA; TetOP-H2B-GFP | Doxycycline-inducible labelling of HSCs with Histone H2B-GFP. Most functional HSCs contained within H2B-GFP+ c-Kit+ cells | | |
| ‘Stroma-reporter’ mouse strains | Nestin-GFP | GFP-labelling of mesenchymal stem cells. | Studies of 3D-imaging describe two subtypes of Nestin-GFP cells in the BM: NG2+ Nestin-GFP+BRIGHT stromal pericyte cells in arterioles and LepR+ Nestin-GFPLOW in sinusoids. | 83 |
| | Scf-GFP | GFP-labelling of SCF expressing cells including CXCL12-expressing perivascular stromal and endothelial cells. | Scf-GFP+ cells express high levels of mesenchymal stem/stromal cell markers and localise close to HSCs. | 69 |
| | Cxcl12-DsRed and Cxcl12-GFP | Constitutive fluorescent labelling of CXCL12-expressing cells. | Brightest DsRed and GFP-expressing cells include perivascular stromal cells and endothelial cells. Distributed around sinusoids | 47,70 |
| | Col2.3-GFP; Cxcl12-DsRed | GFP+ osteoblasts. DsRed+ CXCL12-expressing cells. | Osteoblasts express CXCL12. | 70 |
| | Scf-GFP; Cxcl12-DsRed | GFP+ SCF-expressing cells. DsRed+ CXCL12-expressing cells. | In the perivascular stroma all Scf-GFP+ cells are Cxcl12-DsRed+ and vice versa. | 70 |
| | Lepr-Cre; loxP– EYFP | YFP+ LepR-expressing perivascular stromal cells. | LepR+ perivascular stromal cells do not express Nestin but express mesenchymal stem/stromal cell markers including CXCL12, PDGFRα and PDGFRβ. | 69,70 |
| | mCol2.3-PPR | Exogenous PPR expression in osteoblasts. | Increased numbers of osteoblasts leading to accumulation of HSPCs via activation of NOTCH1 receptor in HSCs. | 21 |

(Continues)
| Strategy                      | Mouse strain                  | Description                                                                 | Major findings                                                                                                                                                                                                 | References |
|-------------------------------|-------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| ‘Stroma-deleter’ mouse strains | Nestin-CreERT2; iDTR         | Tamoxifen/Diphtheria toxin-induced ablation of Nestin’ MSCs.                | Reduction in CD150+ CD48+ LSK cells in the BM. No effect on global BM and Lin+ CD48− cellularity.                                                                                                                | 41         |
|                               | Cxcl4-cre; iDTR               | Diphtheria toxin-induced ablation of megakaryocytes.                         | Increased HSC numbers with no effect in the number of osteolineage cells, Nestin<sup>dim</sup>, CD51<sup>+</sup> PDGFR-α<sup>+</sup> perivascular cells and Nestin<sup>high</sup> cells.         | 61         |
|                               | Pf4-Cre; iDTR                 | Diphtheria toxin-induced ablation of megakaryocytes. Potential promiscuous Cre expression in HSCs. | Reduction in the number of HSCs and repopulating units in the BM. Potential unspecific effects as expression of PF4 in HSCs cannot be ruled out.                                                                 | 62,63      |
|                               | Pf4-Cre; Mos-iCsp3            | AP20187-induced ablation of megakaryocytes. Promiscuous Cre expression in HSCs. | Global loss of bone marrow cellularity, including lymphoid, myeloid and erythroid progenitors and HSCs. Specificity problems due to ‘bystander killing’.                                                                 | 56,57      |
|                               | ratCol2.3-TK                  | Ganciclovir-inducible ablation of osteoblasts.                              | Loss of osteocalcin-positive osteoblasts and HSC mobilisation. Mafia transgene is also expressed in CD11b<sup>+</sup> and Ly-6G<sup>+</sup> myeloid cells.                                                                 | 51         |
|                               | Macrophage Fax-induced apoptosis (Mafia) | AP20187-inducible ablation of c-fms expressing cells (macrophages) through a suicide fusion protein. | Severe reduction in HSC numbers. Promiscuous expression of Mx1– Cre complicates interpretation of results.                                                                                          | 16,47      |
|                               | Cxcl12-DTR-GFP                | Diphtheria toxin-inducible ablation of CAR cells (suicide gene).            | Selective increase in the number of VWF<sup>a</sup> HSCs.                                                                                                                                                   | 94         |
|                               | Cxcl4-cre; iDTR; VWF-eGFP     | Diphtheria toxin-inducible ablation of megakaryocytes.                      | Selective increase in the number of VWF<sup>a</sup> HSCs.                                                                                                                                                   | 94         |
|                               | Dmp-1-DTR                     | Diphtheria toxin-inducible ablation of osteocytes.                          | Resistant to G-CSF-induced HSC mobilisation.                                                                                                                                                                | 66         |
| ‘Niche factors-deleter’ mouse strains | Mx1-Cre; Cxcr4<sup>fl/fl</sup> | pIpC-inducible ablation of Cxcr4 in BM MSCs, Nestin’ cells and perivascular cells. | Severe reduction in HSC numbers. Promiscuous expression of Mx1– Cre complicates interpretation of results.                                                                                  | 16,47      |
|                               | Vav-Cre; Scf<sup>fl/fl</sup>  | Scf ablation in haematopoietic cells.                                      | Scf ablation in haematopoietic cells.                                                                                                                                                                            | 69         |
|                               | mCol2.3-Cre; Scf<sup>fl/fl</sup> | Scf ablation in osteoblasts.                                                 | No effect on the numbers and function of HSCs in the BM.                                                                                                                                                     | 69         |
|                               | Nestin-Cre; Scf<sup>fl/fl</sup> | Scf ablation in mesenchymal stem cells.                                    | No effect on the numbers and function of HSCs in the BM.                                                                                                                                                     | 69         |
|                               | Nestin-CreERT; Scf<sup>fl/fl</sup> | Tamoxifen-inducible ablation of Scf in mesenchymal stem cells.            | Severe reduction in HSC numbers. Promiscuous expression of Mx1– Cre complicates interpretation of results.                                                                                  | 16,47      |
|                               | Lepr-Cre; Scf<sup>fl/fl</sup> | Constitutive Scf ablation in perivascular stromal cells.                   | Reduction in the number of HSCs.                                                                                                                                                                            | 69         |
|                               | Ubc-CreERT; Scf<sup>fl/fl</sup> | Tamoxifen-inducible ubiquitous Scf ablation.                               | Decreased cellularity in the bone marrow and spleen and depletion of HSCs.                                                                                                                                  | 69         |
|                               | Tie2-Cre; Scf<sup>fl/fl</sup> | Scf genetic ablation in endothelial cells.                                  | Reduction in the number of HSCs and haematopoietic defects during embryonic development. This highlights the importance of using TAM-inducible Cre-ERT/Cre-ERT2 models to distinguish among embryonic and adult BM specific defects. | 69         |
numerous haematopoietic cell types and classified leucocytes based on the staining properties of their granules. Remarkably, Neumann also proposed the presence of ‘great lymphozyt stem cell’ capable of both self-renewing and producing lymphocytes and erythroid cells in the BM. Thus, early anatomical and histological studies demonstrated not only the presence of different anatomical types of BM but also identified a variety of cell types. Moreover, these pioneer studies cemented the importance of the marrow niche as the source for blood production and suggested the presence of stem cells.

**Evaluation on the ability of niche cells to transfer haematopoietic niche activity in vivo**

In the 1960s, transplantation of stromal tissues provided the first experimental evidence on the presence of a

### TABLE 2 (Continued)

| Strategy                  | Mouse strain Description | Major findings                                                                 | References |
|---------------------------|--------------------------|--------------------------------------------------------------------------------|------------|
| *Vav-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 genetic ablation in haematopoietic cells. | No effect on the function and numbers of HSCs and HSPCs. | 70,71      |
| *Nestin-Cre; Cxcl12*<sup>f/f</sup> | Constitutive ablation of Cxcl12 in mesenchymal stem cells. |                                                                      |            |
| *Oc-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in mature mineralising osteoblasts. |                                                                      |            |
| *Tie2-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in endothelial cells. |                                                                      |            |
| *Prx1-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in osteoblasts, osteocytes, CAR cells and CD45<sup>−</sup>Lin<sup>−</sup>PDGFRα<sup>−</sup>B2<sup>−</sup>Cxcl12<sup>−</sup>Nestin<sup>−</sup>LepR<sup>−</sup>mesenchymal progenitors. | Additive phenotype among Col2.3-Cre; Cxcl12<sup>f/f</sup> and Lepr-Cre; Cxcl12<sup>f/f</sup>mice as a consequence of Prx1-Cre expression in both perivascular stromal cells and osteoblasts. | 70,71      |
| *Osx-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in mature osteoblasts, osteocytes and CAR cells. | HSPC mobilisation and loss of B-lymphoid progenitors but normal HSC function. | 71         |
| *Lepr-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in sinusoidal perivascular niches. | Mobilisation of HSCs into the circulation and effect on HSCs location. | 70–72      |
| *mCol2.3-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in endosteal osteoblasts. | Reduction in the number of CLPs and LMPPs. No effect in the number and engraftment potential of HSCs. | 70         |
| *NG-2-Cre; Cxcl12*<sup>f/f</sup> | Cxcl12 ablation in perivascular niches. | NG2<sup>+</sup> Nestin<sup>+</sup>arteriolar and Lepr<sup>+</sup>Nestin<sup>+</sup>sinusoidal niches maintain HSCs in the BM. Depletion of Cxcl12 from Lepr<sup>−</sup>sinusoidal cells affect HSC location. | 72         |
| *NG-2-Cre<sup>ERT</sup>; Cxcl12*<sup>f/f</sup> | Tamoxifen-inducible ablation of Cxcl12 in arteriolar perivascular niches. |                                                                      |            |
| *Myh11-CreERT2; Cxcl12*<sup>f/f</sup> | Tamoxifen-inducible ablation of Cxcl12 in arteriolar perivascular niches. |                                                                      |            |
| *Ng2-Cre; Scf*<sup>f/f</sup> | Scf genetic ablation in perivascular niches. |                                                                      |            |
| *Ng2-Cre<sup>ERT</sup>; Scf*<sup>f/f</sup> | Tamoxifen-inducible ablation of Scf in arteriolar perivascular niches. |                                                                      |            |
| *Lepr-Cre; Scf*<sup>f/f</sup> | Scf ablation in sinusoidal perivascular niches. |                                                                      |            |
| *Mx1-Cre; Bmprr1a*<sup>f/f</sup> | pIpc-inducible Bmprr1a ablation in BM MSCs, Nestin<sup>−</sup>cells, perivascular cells, and cells in other tissues. | Increased number of osteoblasts and BrdU<sup>−</sup>LSK cells. | 22         |

Abbreviations: BM, bone marrow; Bmpr1a, bone morphogenetic protein receptor type 1a; CAR, CXCL12-abundant reticular cells; CLP, common lymphoid progenitor; Col2.3, collagen 2.3 promoter; Cxcl12, CXC-chemokine ligand 12; Dmp-1, dentin matrix protein-1; DT, diphtheria toxin; DTR, DT receptor; GFP, green fluorescent protein; Hoxb5, homeobox B5; HSC, haematopoietic stem cell; HSPCs, haematopoietic stem and progenitor cells; Lepr, leptin receptor; LMPPs, lymphoid-primed multipotent progenitors; Mds1, myelodysplastic syndrome 1; Myh11, myosin heavy chain 11; Ng2, neural-glial antigen 2; Oc, osteocalcin; Ox, osteonectin; PDGFR, plateled-derived growth factor receptor; Pf4, platelet factor 4; PPR, PTH/PTH-related protein receptor; Prx-1, paired-related homeobox protein 1; PTH, parathyroid hormone; Scf, stem cell factor; TK, thymidine kinase, VWF, von Willebrand factor.
haematopoietic microenvironment and on the role of niche factors. Particularly, subcutaneous marrow implantation revealed that stromal fibroblast-like precursors are able to support and reconstitute a haematopoietic microenvironment upon autologous and heterotopic transplantation.23,24,35,36

Notably, co-transplantation of murine osteoblasts, but not dendritic cells, enhances multilineage engraftment of transplanted Lineage− haematopoietic progenitor cells in lethally irradiated mice.37 Moreover, subcutaneous transplantation of human CD146+ multipotent subendothelial reticular BM cells into immunocompromised mice resulted in human-derived bone tissue, which was colonised by murine haematopoietic progenitor cells.38 Similarly, foetal transplantation of human CD146+ multipotent subendothelial cell factor (SCF, also known as c-Kit ligand [KitL] and steel factor [SL]) levels are severely reduced and exhibit low HSPC numbers.42,43 Transplantation of wild-type (wt) spleen stroma into spleens of non-irradiated S1/S1d mice locally triggered host erythropoiesis and provided some of the first evidence on the role of SCF as a niche factor.43

Interestingly, in the Steel-Dickie S1/S1d mutant mice stem cell factor (SCF, also known as c-Kit ligand [KitL] and steel factor [SL]) levels are severely reduced and exhibit low HSPC numbers.42,43 Transplantation of wild-type (wt) spleen stroma into spleens of non-irradiated S1/S1d mice locally triggered host erythropoiesis and provided some of the first evidence on the role of SCF as a niche factor.43

Selective expansion and depletion of candidate niche cells

Expansion and ablation of candidate niche cells via drug treatments and genetic manipulation followed by prospective evaluation on the numbers of HSC/HSPCs offered new means to functionally evaluate the role of these cells in the HSC niche.21,22

Drug treatments

Expansion of candidate niche cells by drug treatments

Parathyroid hormone (PTH) administration in wt mice expands the number of osteoblasts, Nestin+ MSCs and Lin−Scal1+c-Kit+ (LSK) HSPCs in the BM and improves survival in BM transplantation.21,41 A primary role for the osteoblasts increasing LSK numbers was suggested; however, the multicellular effects of PTH makes it difficult to disentangle the role of those perturbed cells. Unfortunately, PTH administration after umbilical cord blood transplantation in human patients has shown no effect on blood count recovery in phase II clinical trials.44

Conversely, in vivo administration of strontium, a bone anabolic agent, increases osteoblast number (although not N-cadherin+ osteoblasts), bone volume, and trabecular thickness, but does not affect HSPC numbers.45

Depletion of candidate niche cells by drug treatments

Granulocyte colony-stimulating factor (G-CSF) administration mobilises HSPCs to the blood circulation through complex mechanisms involving various BM cell types. Importantly, G-CSF reduces chemokine ligand 12 (CXCL12) (i.e. stromal cell-derived factor-1 [SDF-1]) levels,46 whose receptor, CXCR4, is highly expressed in HSCs.47 The CXCL12–CXCR4 axis plays a critical role in HSC trafficking and niche retention, as illustrated by a severe reduction in BM-HSC numbers following Cxcr4 ablation in Mx1Cre-Cxcr4lox/null mice.47 Genetic and pharmacological disruption of the sympathetic system inhibits G-CSF-induced HSC mobilisation,48 suggesting a regulatory role for this system on the HSC niche. Specifically, G-CSF increases the duration of sympathetic noradrenaline signals on β2- and β3-adrenergic receptors. In BM stromal cells, activation of these receptors downregulates CXCL12 expression promoting HSC release.49,49,50

Additionally, G-CSF simultaneously depletes a population of endosteal macrophages (osteomacs, which normally support osteoblast) and induces G-CSF receptor-expressing BM leucocytes to suppress osteoblasts,51,52 obscuring their specific role.

Administration of liposome-embedded clodronate, which specifically kills phagocytic cells as the only cells capable of engulfing liposomes, phenocopies G-CSF effects, including HSC mobilisation and loss of osteoblasts, supporting a role for macrophages in the HSC niche.51

Treatments with anti-vascular endothelial (VE)-cadherin and vascular endothelial growth factor receptor 2 (VEGFR2) blocking monoclonal antibodies downregulate angiogenic NOTCH ligand expression in ECs and impair HSC engraftment in vivo, indicating a role for sinusoidal ECs in this context.53 In contrast, zoledronate treatment, which severely decreases the number of endosteal osteoclasts, does not affect HSC mobilisation or numbers.51

Overall, drug treatments often exhibit multiple cellular and molecular targets. Thus, it is not immediately possible to discern among direct versus indirect effects and their relevance in the BM niche. Genetic models aimed to specifically target cell types of interest are helping to elucidate the roles of particular BM-niche components.

Genetic-based models

Expansion of candidate niche cells by genetic manipulation

As with PTH treatments, expression of a constitutive active version of the PTH/PTH-related protein receptor (PPR) in
osteoblasts (mCol2.3-PPR mice) increases the number of osteoblasts, which produce higher levels of NOTCH-ligand JAGGED-1 and trigger LSK expansion via NOTCH1 receptor activation, supporting a role for osteoblasts in the HSC niche.21

Bone morphogenetic protein receptor, type IA (BMPRIA) expression inhibits osteoblastic lineage differentiation from mesenchymal progenitors.24 Accordingly, blocking BMP signalling via Bmpr1a depletion in Mx1–Cre^+/T;Bmpr1a^/Pf4- Cre;iDTR mice results in increased numbers of osteoblasts and LSKs.22 Additionally, transplantation of wt LSKs into Bmpr1a-depleted recipient mice induces the expansion of wt transplanted LSKs, supporting a non-cell autonomous HSPC effect.22 However, promiscuous expression of Mx1–Cre (e.g., in BM MSCs, Nestin+ cells and perivascular cells)16 obscures the specific cell type responsible for LSK expansion in this context.

Depletion of candidate niche cells by genetic manipulation (‘suicide genes’)22

Other studies have investigated the role of candidate niche cells by genetically depleting osteoblasts, Nestin+ MSC, CAR cells and MKs from the BM through linear expression and activation of ‘suicide genes’ including the herpesvirus thymidine kinase (TK), the diphtheria toxin (DT) receptor (DTR) or genetically modified dimersable Caspase genes (e.g., FK506-Fas, Mos-iCsp3) in the cells of interest (Table 2).

TK expression confers sensitivity to the initially non-toxic pro-drug ganciclovir (GCV). GCV phosphorylation by TK and subsequent phosphorylation yield triphosphate-GCV (the active metabolite), which incorporates into the DNA causing single-strand breaks and apoptosis.55 GCV treatment of Col2.3-TK transgenic mice (TK under a osteoblast promoter [Rat-Col2.3]) results in conditional ablation of osteoblasts and a global loss of BM cellularity, including HSCs/HSPCs.56 Importantly, not only cells expressing TK but neighbouring cells can undergo cell death by so-called ‘bystander killing’, making this approach less specific than desired.

Macrophage Fas-induced apoptosis (Mafia) transgenic mice express FK506-FAS (a suicide fusion protein) under the c-fms ‘macrophage-specific’ promoter. Administration of AP20187 ligand induces dimerisation and activation of the suicide protein triggering FAS-mediated apoptosis in c-fms-expressing cells, leading to loss of osteocalcin (Oc) osteoblasts and HSC mobilisation.51 c-fms-Mafia is not expressed in osteoblasts, MSCs and ECs; however, CD11b+ and Ly-6G+ myeloid cells express it. Thus, a broader role of myeloid cells in HSC regulation cannot be formally excluded in this model.51

DTR expression provides an analogous strategy for selective cell lineage depletion. DTR-expressing cells are sensitive to DT while wt murine cells are insensitive.28 Different mouse lines have been genetically engineered to express DTR.

HSCs locate close to reticular CAR cells (which express high CXCL12 levels)57 and to assess their role, CARs were ablated in Cxcl12-DTR-GFP mice (DTR knocked in the Cxcl12 locus) through DT treatment.59 This abolished adipogenic and osteogenic differentiation potential of marrow cells and SCF and CXCL12 production in the BM and led to a marked reduction in HSC number59 implicating adipogenic CAR cells as part of the HSC niche.

In Cre-inducible DTR transgenic mouse strain (iDTR),60 CRE mediated excision of a floxed transcriptional STOP cassette yields DTR expression. In iDTR; Cre-ERT2 double transgenic mice, combined administration of tamoxifen (Tam) and DT ablates CRE-ERT2-expressing cells. Nestin+ MSCs depletion in Nes-CreERT2/iDTR mice halves the numbers of CD150+CD48+ LSK LT-HSCs in the BM. Global BM and Lin+CD48+ cellularity were not affected in these mice, supporting a cell-specific effect of Nestin+ cells on HSCs.41

Ablation of MKs has led to conflicting results. MK ablation in Cxcl4-cre;iDTR mice yielded a substantial increase of CD105+CD150+ HSCs and re-populating units,61 with no effect in the BM cellularity suggesting a direct effect of MKs on HSC quiescence by CXCL4 secretion.61 Nevertheless, MK depletion in Pf4-Cre;DTR and Pf4-Cre;Mos-iCsp3 mice produced a significant reduction on HSCs and re-populating units in the BM.62,63 In Pf4-Cre;Mos-iCsp3 mice, administration of AP20187 triggers Caspase-induced apoptosis via homodimerisation of iCsp3.64 Importantly, platelet factor 4 (PF4) is reportedly expressed in HSCs, which could lead to undesired apoptosis of HSCs in Pf4-Cre;Mos-iCsp3 and Pf4-Cre;iDTR mice.65 However, Pf4-Cre–lineage traced BM cells failed to reconstitute irradiated mice63 questioning if HSCs express PF4.

Osteocyte depletion in dentin matrix protein-1 (DMP-1)-DTR transgenic mice yields a strain resistant to G-CSF-induced HSC mobilisation.66 Likewise, klotho hypomorphic (kl/kl) mice, which display osteoporosis and a disrupted osteocyte network, exhibit a lack of HSC mobilisation in G-CSF treatments, supporting a role for osteocytes in regulating HSPC egress from the BM. The role of the osteocytes in the HSC niche may work indirectly through effects on osteoblasts and macrophages.66

Overall, the lack of specificity of most promoters obscures the identification of defined candidate niche cells and advises the use of various promoters to infer consistent conclusions. Additionally, ablation of large numbers of cells in the BM may indirectly activate HSPCs to regain homeostasis.7 Even in the event of cell-type-specific ablation, discerning if the effect on HSC numbers arises from direct or indirect perturbations on other niche components requires a detailed characterisation.7

Inducible ablation of genes encoding niche factors

Conditional ablation of genes encoding critical niche factors such as Cxcl12 or Scf by CRE-mediated recombination
in candidate niche cells followed by the evaluation of HSC numbers has been employed to unveil HSC niche components and their role in HSC regulation.

The c-Kit receptor (c-Kit)–SCF axis plays a critical role regulating quiescence and self-renewal in HSCs, which express high c-Kit levels. 56,68

Conditional floxed Scf alleles (Scf<sup>fl/+</sup>) allow Scf genetic deletion via Cre activity. 69 Scf depletion from haematopoietic cells (in Vav-Cre; Scf<sup>fl/−</sup> mice), osteoblasts (Col2.3-Cre; Scf<sup>fl/−</sup> mice) or MSCs (Nestin-Cre; Scf<sup>fl/−</sup> and Nestin-CRE-ERT; Scf<sup>fl/−</sup> mice) does not affect the numbers and function of LT-HSCs (CD150<sup>+</sup>CD48<sup>−</sup> LSK cells) in the BM. 69 However, HSC numbers decrease following Scf deletion in ECs (Tie2-Cre;Scf<sup>fl/−</sup> mice) and perivascular stromal cells (Leptin receptor, Lepr-Cre;Scf<sup>fl/−</sup> mice), supporting the role of endothelial and Leptin<sup>+</sup> stromal cells as HSC-niche components. 69 Constitutive CRE activity can lead to haematopoietic defects during embryonic development (e.g., in Tie2-Cre; Scf<sup>fl/−</sup> embryos) precluding a proper interpretation of defects observed during adulthood. 69 Thus, the use of inducible TAM-regulated CRE activity (i.e., via Cre-ERT or Cre-ERT2) is advisable whenever possible.

As aforementioned, the CXCR4–CXCL12 axis is critical in HSC regulation. Cxcl12 depletion from haematopoietic cells (in Vav-Cre; Cxcl12<sup>−/−</sup> mice), osteoblasts (Col2.3-Cre; Cxcl12<sup>−/−</sup> mice) and mature mineralising osteoblasts (Oc-Cre; Cxcl12<sup>−/−</sup> mice) does not affect HSCS/HSPCs. 70,71 Conversely, Cxcl12 depletion in ECs (Tie2-Cre; Cxcl12<sup>−/−</sup> mice) modestly reduces the numbers of transplantable HSCs in the BM without mobilisation, 70,71 while Cxcl12 ablaiton in perivascular stromal cells (Lepr-Cre;Cxcl12<sup>−/−</sup> mice) results in the mobilisation of HSCs into the circulation. Prx1-Cre; Cxcl12<sup>−/−</sup> mice show an additive phenotype among Col2.3-Cre;Cxcl12<sup>−/−</sup> and Lepr-Cre;Cxcl12<sup>−/−</sup> mice, 70,71 reflecting Prx1-Cre wider expression. Deletion of Cxcl12 from osterix (Osx)–expressing stromal cells (Osx-Cre;Cxcl12<sup>−/−</sup>) results in constitutive HSPC mobilisation and loss of B-lymphoid progenitors, but normal HSC function. 71 Both Osx–Cre and Prxl–Cre transgenes drive CRE expression in mature osteoblasts, osteocytes and CARs but Prx1–Cre also targets CD45<sup>−</sup>Lin<sup>−</sup>PDGFRα<sup>−</sup>SCA1<sup>−</sup>Nestin<sup>−</sup>Lepr<sup>−</sup> MSCs, which seem required for HSC and common lymphoid progenitors (CLP) maintenance. 71 Overall, Cxcl12 deletion in BM candidate niche cells suggests perivascular endothelial, Lepr<sup>−</sup> stromal and Nestin<sup>−</sup>Lepr<sup>−</sup> MSCs as HSC-niche components and that CLPs occupy an endosteal osteoblastic niche. 70,71 Accordingly, Cxcl12 deletion from osteoblasts (Col2.3-Cre; Cxcl12<sup>−/−</sup> mice) diminishes CLP numbers and lymphoid reconstitution potential, albeit no effect on HSCs. 70

Additionally, ablation of Scf or Cxcl12 from sinusoidal (via Lepr-Cre mice), arteriolar (Ng2-Cre-ERT and Myh11-Cre-ERT2) or both (Ng2-Cre) perivascular niches show that Ng2<sup>−</sup>Nestin<sup>−</sup> arteriolar and Lepr<sup>−</sup>Nestin<sup>low</sup> sinusoidal niches have a role in maintaining HSCs in the BM, and that depletion of Cxcl12 from Lepr<sup>−</sup> sinusoidal cells also affects HSC location. 72

Moreover, genetic deletion of Ebf3 and Foxcl transcription factors from all mesenchymal cells (via Prxl-Cre) or more specifically in CAR cells (Lepr-Cre mice) has revealed that these factors are essential to maintain the BM niches for HSCs. 73,74 Ebf3 and Foxcl are expressed preferentially in CAR cells. Particularly, Ebf3 and Foxcl (via Runxl expression) prevent the differentiation of CAR cells into osteoblasts and adipocytes, respectively. 73,74 This supports CAR cells as specialised professional niche cells, whose specific features and identity are actively regulated. 76

### Imaging techniques

Unveiling the cellular structure of the BM niche via imaging studies relies on the use of highly-specific HSC markers and of markers specific to the candidate niche cells so that location can be effectively assessed.

### Haematopoietic stem cell markers

Initial studies showed that transplanted carboxyfluorescein succinimidyl ester (CFSE)-labelled Lineage<sup>−</sup> BM cells (a broad population of HSPCs) locate closer to the endostele, while CFSE<sup>−</sup>Lineage<sup>−</sup> cells preferentially distribute around the central marrow. 77 Staining for LSK phenotype labels a mixed population of HSCs and HSPCs. 78 In vivo bromodeoxyuridine (BrdU) pulsing identifies more quiescent HSCs (i.e., CD45<sup>−</sup>LSK BrdU<sup>−</sup>), 22 which locate around N-cadherin<sup>−</sup>CD45<sup>−</sup> osteoblastic cells in the BM endosteum. 22 The remarkable discovery of signalling lymphocyte attractant molecule (SLAM) markers with the ability to identify bona fide transplantable murine HSCs as about one in three CD150<sup>−</sup>CD48<sup>−</sup> LSK cells 79,80 enabled a simple antibody combination to precisely distinguish HSCs. 79 Two-dimensional (2D) microscopy, and other imaging approaches discussed below, support the presence of vascular niches where most Lin<sup>−</sup>CD48<sup>−</sup>CD41<sup>−</sup>CD150<sup>−</sup> LT-HSCs locate to extraluminal perisinusoidal spaces and associate with sinusoidal endothelium and mesenchymal CAR cells. 81,82 A minor portion localise around arteria and arterioles vessels. 83,84

### Three-dimensional (3D) whole-mount imaging

Initial studies analysed HSC localisation taking into account single BM populations and lacked resolution at tissue level. 3D whole-mount imaging of optically cleared BM preparations coupled with simulations of randomly assigned positions has allowed for testing of the significance of the distribution of candidate niche cells to HSCs and compare them with a null distribution. 61,85-87 3D microscopy showed that Lineage<sup>−</sup>CD41<sup>−</sup>CD48<sup>−</sup> c-Kit<sup>−</sup> Sca1<sup>−</sup> HSPCs preferentially localise in the endosteum interacting with sinusoidal and non-sinusoidal BM microvessels. 88 Importantly, imaged-based quantitative spatial analysis of BM tissues revealed...
that sinusoidal ECs (SECs) and CAR cells are ~30-fold more abundant than previously assumed by flow cytometry analyses. This suggests that enzymatic and mechanical methods employed for tissue dissociation prior to flow cytometry analyses are not efficient in extracting every cell type, which can lead to confounding conclusions. Moreover, high abundance of SEC and CAR cells makes them widely available in the BM for cell interactions. Other 3D-microscopy studies have shown that CD41+ MKs are not randomly distributed to Lin−CD48+CD41+CD150−HSCs in the BM sinusoids and that quiescent HSCs associate with small endosteal arterioles ensheathed by NG2+ pericytes. As the criteria employed to define a random distribution of dots and the methods used to test the statistical significance of cell location largely diverge among studies, this can be a source of variability and can lead to conflicting conclusions on cell interactions.

Intravital microscopy (IVM)

Intravital microscopy combines high-resolution confocal microscopy and two-photon video imaging. It allows longitudinal in vivo studies of cellular dynamics including cell migration, division, death and cell–cell interactions. IVM has exposed that LT-HSCs preferentially locate in the endosteal surface while MPPs concentrate to transitory zone vessels. However, another group reported the endosteal surface while MPPs concentrate to transitory zone vessels.95 Interestingly, α-catulin-GFP+ HSCs located close to sinusoidal blood vessels and arteriolar niches, highlighting the presence of two functionally distinct niches.94

Haematopoietic stem cell genetic reporters

In α-catulin-GFP knock-in mice only 0.02% of BM cells are α-catulin–GFP+, all HSCs are α-catulin–GFP+ and 30% of α-catulin–GFP+ c-Kit+ cells are LT-HSCs. Thus α-catulin–GFP+ very specifically identifies LT-HSCs. Dividing and non-dividing α-catulin–GFP+ c-Kit+ cells locate around LepR+CXCL12+ niche cells in perisinusoidal BM locations.92

Likewise, mCherry expression from the Hoxb5 locus in Hoxb5-tri-mCherry mice is mostly limited to LT-HSCs. Only HOXB5+ cells harbour LT-engraftment potential and the majority of mCherry+ cells associate with VE-cadherin+ perivascular ECs.93

Von Willebrand factor (VWF)-eGFP expression in VWF-eGFP knock-in mice segregates platelet/myeloid-biased HSCs (VWF-eGFP+) from lymphoid-biased HSCs (VWF-eGFP−). Intriguingly, VWF-eGFP+ HSCs associate with MK niches, while VWF-eGFP− HSCs mostly occupy NG2+ arteriolar niches, highlighting the presence of two functionally distinct niches.85

In Mds1-GFP;Flt3-Cre mice, a floxed Mds1-GFP allele is expressed in HSCs and Flt3-Cre expression in differentiating HSCs restricts GFP to LT-HSC. IVM shows LT-HSCs located closer to sinusoidal blood vessels and the endosteal surface while MPPs concentrate to transition zone vessels.95 However, another group reported (Lin−CD41+CD48−cKIT+CD150−FLT3−) MPPs residing in HSC niches.96 Interestingly, Mds1-GFP;Flt3-Cre LT-HSCs exhibit limited motility during steady-state haematopoiesis in IVM.95 Paradoxically, in vivo imaging of tdTomato+ labelled HSCs in Pdzklip-Cre;loxSTOPlox-tdTomato mice (Pdzklip-Cre drives HSC-specific-CRE expression) showed significant motility for tdTomato+ HSCs in the perivascular space with occasional interactions with SCF+ perivascular cells.97 Differences in the expression pattern among genetic reporters and analysed bones (calvaria vs. long bones) could explain these discrepancies.

Recently, quantitative 3D-microscopy studies have analysed the localisation of HSCs (labelled with different strategies) in relation to four simultaneous BM components in different bones.85 In this report, α-catulin-GFP+ HSCs and Mds1GFP+/Flt3Cre HSCs located close to sinusoidal CXCL12+ stromal cells and MK but not to bone, adipocyte or Schwann cells.85 Additionally, dormant (non-dividing) HSCs, labelled as GFP-retaining c-Kit+ cells in doxycycline (DOX)-chased Scl-1Tα;H2B GFP mice, showed similar location to α-catulin-GFP+ HSCs.85 Importantly, HSC cells in Col2.3GFP+ mice) also express CXCL12 as shown in Col2.3Gfp+;Cxc12DsRed+ mice, albeit at lower levels.70

Remarkably, in Sclgfp+/Cxc12DsRed+ mice all Scf-GFP+ cells are Cxc12-DsRed+ and vice versa, suggesting that perivascular cells are a major source of both factors. LepR+ perivascular cells are exceptionally high CXCL12 producers (EYFP+ cells from Lepr−Cre;loxSTOPlox–EYFP mice express ~15000-fold the mRNA levels in the whole BM).78

Genetic reporters for niche components

Fluorescent protein expression in niche cells has greatly facilitated their identification. Green fluorescent protein (GFP) labelling of Nestin+ MSC cells in Nestin-GFP knock-in mice showed that CD150+/CD48−Lin−HSCs localise around Nestin-GFP+MSCs and tyrosine hydroxylase+ catecholaminergic fibres.41 Nestin-GFP+ cells encompass NG2+ Nestin-GFP+ cells (in ‘Haematopoietic stem cell genetic reporters’).
locations reflected the abundance of the analysed BM niche cell types rather than the presence of specific microenvironments within the analysed populations. Notably, these niche cell types are much more frequent in the BM than previously assumed. Remarkably, the transplantation of very large numbers of HSCs into non-myeloablated recipients strikingly demonstrated that donor HSCs are able to engraft and occupy niches distant from host HSCs without replacing host HSCs as visualised by DOX-chased Rosa26-M2-rTA; TetOP-H2B-GFP labelled HSCs. This further highlights the abundance of empty HSC niches available for engraftment.

**Single-cell profiling of BM cells**

To date, imaging techniques are still biased by preselection of antibodies and limited by how many BM niches can be simultaneously analysed. Mass cytometry, or cytometry by time of flight (CyTOF) recently unveiled 28 subsets of non-haematopoietic cells in the BM during homeostasis. This single-cell technique allows measurements of ~50 targets per cell and enables a detailed taxonomy of the BM niche; nevertheless, it is still restricted by the number and preselection of antibodies.

Broader implementation of single-cell RNA-sequencing (scRNAseq) procedures recently yielded the first transcriptional profiles of the BM at single-cell level. scRNAseq provides an unbiased means with which to characterise BM cells with extraordinary precision. A total of 17 cellular subtypes were identified among non-haematopoietic unfractonated cells (7AAD−Calcein total of 17 cellular subtypes were identified among non-haematopoietic unfractionated cells (7AAD−Calcein−) comprising MSCs, osteolineages, chondrocytes, fibroblasts, ECs and pericytes. Similarly, scRNAseq of VE-Cadherin+ endothelial, LepR+ cells and COL2.3+ osteoblasts fractionated from Tom+CD45lowTer119low stro- plus clustering of the BM niche components.

Progressive depletion of abundant cell types in the BM (i.e., major immune populations and erythroid progenitors) followed by scRNAseq served to capture rare niche cellular components and exposed 32 cell clusters. They encompass Schwann cells, smooth muscle cells, myofibroblasts, EC clusters (Scal arterial and Emcn sinusoidal ECs) and nine Pdgfra+ mesenchymal populations (chondrocytes, osteoblasts, fibroblast-like populations, Ng2 Nestin+ MSCs, and two CAR clusters). These two CAR populations, namely Adipo-CAR (similar to Lepr-Cre+ cells) and Osteo-CAR cells, showed the highest cyto- kine levels among all BM cells.6

The scRNAseq data lack spatial distribution information. Circumventing this, laser-capture microdissection coupled with sequencing (LCM-seq or spatial transcriptomics) of BM fixed sections allows assignment of cells to particular spatial locations. Particularly, Adipo-CARs preferentially locate to perisinusoidal endothelial areas, while Osteo-CARs to non-vascular regions and arteriolar endothelium. An emerging challenge from scRNAseq databases is how to readily compare cell clusters identified by different laboratories. Even more important is to establish the functional relevance of any of these novel cellular populations, which will need to rely on genetic-based approaches (e.g., genetic ablation of candidate cells).

To predict the likelihood of interaction among cells, various algorithms and databases (e.g., RNA magnet, CellPhone DB, NicheNet) have emerged based on the expression pattern of cell-surface receptors and their known surface-expressed ligands. Thus, scRNAseq data can be interrogated to expose cell–cell interactions and potential niche components.

**Strategies to fluorescently label cells in cell proximity**

Although progressive depletion of abundant populations enriches samples for less frequent cell populations, rare populations may be missed. Particularly, rare HSCs (~20000 total HSCs in an adult mouse) may interact with a very reduced number of bona fide niche cells, especially if HSCs show low motility. Additionally, current spatial transcriptomics lack the ability to directly capture cell–cell interactions. Tackling this, a soluble lipid-permeable mCherry (sLP-mCherry) protein secreted by transduced cells and which can be absorbed by neighbouring cells, allows spatial location of the producer cells and prospective isolation and characterisation of niche cells (mCherry+) within the bulk tissue. This strategy was recently used to analyse the early niche in contact with LP-mCherry-expressing human acute myeloid leukaemia (AML) leukaemic cells xenografted in immunocompromised mice by the isolation and transcriptional profiling of mCherry+ cells. In this regard, Table 3 provides a summary of various changes in cellular composition in the BM niche in different malignant and non-malignant diseases and conditions.
| Disease/condition          | Experimental findings (BM niche)                                                                                                                                                                                                 | Highlights                                                                                                                                                                                                 | Experimental model-species | References   |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|-------------|
| Ageing                    | Telomere shortening in stromal cells limits HSC function and engraftment. MSCs lose osteogenic potential and switch into an adipogenic state. Increase in Adipo-CAR numbers. Increased myelopoiesis, anaemia, thrombopoiesis. Loss of EMCN^+CD31^high vessels and β3-adrenergic innervation. Reduced number of sinusoidal ECs and increased vessel diameter. | Age-related BM niche defects impact HSC functions.                                                                                                                                                     | Human and mouse.          | 117,118     |
| Chronic viral infections  | Persistent viral infections induce cytotoxic damage, interfere with normal signalling pathways and cellular trafficking in the BM. Viral DNA is often found in the human BM (e.g., herpesviruses, hepatitis B virus, Merkel cell polyomavirus and human papillomavirus). In mice chronic infection with lymphocytic choriomeningitis virus causes long-lasting destruction of the CAR cell network due to accumulation of activated CD8^+ T-cells in the BM via interferon-dependent mechanisms decreasing HSC functionality. | Chronic viral infections perturb the BM niche leading to a decreased competitive fitness in HSCs.                                                                                                       | Human and mouse.          | 119–122     |
| Aplastic anaemia (AA)     | Hypoplastic, fatty BM, severe reduction in HSPCs and HSCs. Acquired AA triggered by autoimmune dysregulated CD8^+ T-cells. Overproduction of pro-inflammatory cytokines including interferon γ and TNFα. In AA patients: increased numbers of TNFα producing macrophages, fewer endosteal cells, vascular and perivascular cells. MSCs from AA patients display decreased clonogenic potential and proliferation and are biased to differentiate into adipogenic lineages. Allogeneic transplantation from unmanipulated BM is preferred over PB-derived transplantation as BM niche elements recover more efficiently, suggesting MSCs in the BM contribute to a better engraftment. | It is unclear if the alterations in the BM in AA patients contribute to AA or if they are a consequence of AA, especially in acquired AA.                                                                 | Human and mouse.          | 123–126     |
| β-thalassaemia            | Altered bone metabolism. Osteoporosis. Osteopenia. Expansion and premature apoptosis of immature erythroid precursors in the BM. Reduced quiescence of HSCs. Compromised HSC activity. Reduced PTH levels. Transplantation of thalassaemic HSCs into wt mice rescues HSC activity. | HSC self-renewal deficiency in β-thalassaemia may be caused by an altered BM niche.                                                                                                                   | Human and mouse.          | 127–129     |
| Myelodysplastic syndrome (MDS) | Human MDS cells reprogram MSCs into a transplantable niche disease. Altered inflammatory signalling in niche cells could facilitate somatic mutations, clonal selection and expansion. MDS cells preclude osteolineage differentiation of MSCs thru extracellular vesicles (EVs) resulting in defective haematopoiesis. | BM failure in MDS patients results at least partially from the differentiation block of MSCs.                                                                                                              | Human and mouse.          | 130–132     |
| Acute myeloid leukaemia (AML) | Drastic remodelling of endosteal vasculature in AML. Endosteal AML cells produce pro-inflammatory and anti-angiogenic factors. Loss of osteoblasts, HSCs and HSC niches. Sympathetic neuropathy blocks the differentiation of Nestin^+ MSCs into NG2^+ cells. Increased vascular permeability allowing HSC egress from the BM. Overall loss of BM stroma in AML mouse models. Reduced numbers and activity of osteoblasts in AML patients. | AML cells severely modify and highjack the BM niche to thrive.                                                                                                                                              | Human and mouse.          | 11–13,133–135 |
| Multiple myeloma (MM)     | MM cells impair osteoblast differentiation via secretion of Dickkopf-1 and IL3; activate osteoclasts by VEGF secretion; and induce angiogenesis by secreting VEGF, HGF and other cytokines. MM cells inhibit T-cells by TGFβ and IL10 creating an immunosuppressive environment. The modified BM niche supports MM growth, disease progression and chemoresistance via a combination of secreted cytokines, chemokines and an altered extracellular matrix. | MM cells alter the cytokine milieu in the BM. The BM niche facilitates survival signals and disease progression.                                                                                          | Human and mouse.          | 136–138     |
Of note, sLP-mCherry producing cells label cells in proximity but cannot distinguish between distant and direct physical interactions, and transient and stable contacts.\textsuperscript{108,109} Unveiling the type of interactions among HSCs and niche components is likely vital to define bona fide cellular and molecular cues that regulate HSCs.

\section*{CONCLUSIONS AND FUTURE PERSPECTIVE}

The development and implementation of new research techniques has dramatically changed our view of the BM from a source of nutrients for the bones to a highly specialised and complex tissue responsible for maintaining haematopoietic homeostasis.

Initial studies suggested osteoblasts as a major HSC-niche component.\textsuperscript{21,22,37} However, more recent studies based on: (I) genetic ablation of critical molecular niche factors (mostly Scf and Cxcl12) in candidate niche cells, (II) the use of more stringent HSC markers (i.e., SLAM markers, genetic HSC reporters) and (III) sophisticated imaging techniques\textsuperscript{8,47,49,79}, support two major HSC niches in the BM: (i) sinusoidal niches containing ECs, MKs and CAR cells and (ii) arteriolar niches encompassing ECs, NG2\textsuperscript{+} pericytes, CAR cells, sympathetic nerves and non-myelinating Schwann cells.\textsuperscript{1,4,6,85} Surprisingly, simultaneous imaging of HSCs and multiple BM components indicate that HSCs randomly localise within sinusoids, Cxcl12\textsuperscript{+} stroma, and MKs.\textsuperscript{85} Furthermore, 3D-quantitative microscopy indicates that these HSC niches are ~30-times more frequent than previously assumed.\textsuperscript{1,86} Future research will determine the level of heterogeneity within the HSC niches and if some particular sub-compartments constitute specialised subniches.

In this regard, scRNAseq technologies are exposing an unappreciated cellular diversity in the BM; nevertheless, the functional relevance of most of these populations is still to be investigated. Next steps characterising the BM niche should provide a ‘proteomic’ perspective. Recently, proteogenomic techniques based on cellular indexing of transcriptomes and epigenomes by sequencing (CITE-seq) coupled with scRNAseq have allowed mRNA and protein expression analyses at the single-cell level.\textsuperscript{110,111} Additionally, multiplexed imaging techniques (e.g. CODEX\textsuperscript{112} and IBEX CITEX\textsuperscript{113}) render multi-parameter high-resolution images in tissue sections and can help to phenotypically dissect the cellular complexity in the niche.

Unveiling the type of interactions among HSCs and niche components is critical to determine the cellular and molecular signals that regulate HSCs. Novel algorithms predicting the likelihood of cellular interactions are based on known ligand receptors but ‘ignorant’ for any unknown molecular interactors. Soluble lipid-permeable fluorescent proteins allow for identification of cells in cell proximity and enrichment for scarce cells that may not be captured by current scRNAseq techniques.\textsuperscript{95,97,108,109} However, they do not provide information on the type of cellular interactions.
At this stage, only the implementation of novel unbiased methods (ideally modular and genetic) with the ability to identify and differentiate among frequent/stable cell–cell interactions versus transient and distant interactions in vivo will directly untangle the BM-niche components. These technologies will be extremely useful in revealing the microenvironments that support any other cell of interest in any tissue (including tumour chemotherapy-resistant cells). Neurobiology, with a long-standing interest in uncovering synaptic partners, has employed rabies viruses, optogenetics and split forms of GFP and CFP to investigate synaptic interactions. In summary, four decades of intense technical development and biological studies have allowed remarkable advances in our understanding of the BM niche. The foreseeable implementation of novel approaches will identify critical factors required for HSC maintenance, self-renewal and differentiation.

**AUTHOR CONTRIBUTIONS**

Raúl Sánchez-Lanzas, Foteini Kalampalika and Miguel Ganuza wrote the manuscript.

**ACKNOWLEDGEMENTS**

We thank the Centre for Haemato-Oncology at Barts Cancer Institute for critical discussions and reading of the manuscript. We are grateful to Victoria Godwin for English revision of the manuscript. This work was supported by the American Society of Haematology (Global Research Award) (Miguel Ganuza), Barts Charity (Rising Stars Programme) (Miguel Ganuza), Cancer Research UK (PhD-fellowship - Foteini Kalampalika), Leukaemia UK (John Goldman Fellowship, 2020/JGF/001) (Miguel Ganuza) and Medical Research Council (MRC Career Development Award, MR/V009222/1) (Miguel Ganuza).

**CONFLICT OF INTEREST**

Authors declare no conflicts of interest.

**DATA AVAILABILITY STATEMENT**

All the data reported here was gathered from published literature.

**ORCID**

Miguel Ganuza © https://orcid.org/0000-0002-1070-7961

**REFERENCES**

1. Pinho S, Frenette PS. Haematopoietic stem cell activity and interactions with the niche. Nat Rev Mol Cell Biol. 2019;20(5):303–20.

2. Tikhonova AN, Dolgalev I, Hu H, Sivaraj KK, Hoxha E, Cuesta-Dominguez A, et al. The bone marrow microenvironment at single-cell resolution. Nature. 2019;569(7755):222–8.

3. Baryawno N, Przybylski D, Kowalczyk MS, Kfoury Y, Severe N, Gustafsson K, et al. A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. Cell. 2019;177(7):1915–32 e16.

4. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014;505(7483):327–34.

5. Mendelson A, Frenette PS. Haematopoietic stem cell niche maintenance during homeostasis and regeneration. Nat Med. 2014;20(8):833–46.

6. Baccin C, Al-Sabah J, Velten L, Helbling PM, Grunschläger F, Hernandez-Malmierca P, et al. Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization. Nat Cell Biol. 2020;22(1):38–48.

7. Lucas D. The bone marrow microenvironment for hematopoietic stem cells. Adv Exp Med Biol. 2017;1041:5–18.

8. Lo Celso C, Fleming HE, Wu JW, Zhao CX, Mika-Lye S, Fujisaki J, et al. Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. Nature. 2009;457(7225):92–6.

9. Lee GY, Jeong SY, Lee HR, Oh JH. Age-related differences in the bone marrow stem cell niche generate specialized microenvironments for the distinct regulation of normal hematopoietic and leukemia stem cells. Sci Rep. 2019;9(1):1007.

10. Ganuza M, McKinney-Freeman S. Hematopoietic stem cells under pressure. Curr Opin Hematol. 2017;24(4):314–21.

11. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood. 2009;114(6):1150–7.

12. Schepers K, Campbell TB, Passegue E. Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell Stem Cell. 2015;16(3):254–67.

13. Duarte D, Hawkins ED, Akinduro O, Ang H, De Filippo K, Kong IY, et al. Inhibition of endostate vascular niche remodeling rescues hematopoietic stem cell loss in AML. Cell Stem Cell. 2018;22(1):64–77 e6.

14. Cooper B. The origins of bone marrow as the seedbed of our blood: from antiquity to the time of Osler. Proc (Baylor Univ Med Cent). 2011;24(2):115–8.

15. Wolock SL, Krishnan I, Tenen DE, Matkins V, Camacho V, Patel S, et al. Mapping distinct bone marrow niche populations and their differentiation paths. Cell Rep. 2019;28(2):302–11 e5.

16. Joseph C, Quach JM, Walkley CR, Lane SW, Lo Celso C, Purton LE. Deciphering hematopoietic stem cell niches in their niches: a critical appraisal of genetic models, lineage tracing, and imaging strategies. Cell Stem Cell. 2013;13(5):520–33.

17. Pappenheim A. Ueber entwickelung und ausbildung der erythroblasten. Virchows Arch. 1896;145:587–643.

18. Jacobson LO, Marks EK, Robson MJ, Gaston EO, Zirkle RE. Effect of spleen protection on mortality following x-irradiation. J Lab Clin Med. 1949;34:1538–43.

19. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res. 1961;14:213–22.

20. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;4(1–2):7–25.

21. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the hematopoietic stem cell niche. Nature. 2003;425(6960):841–6.

22. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003;425(6960):836–41.

23. Friedenstein AJ, Chailakhyan RK, Latsnik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation. 1974;17(4):331–40.

24. Friedenstein AJ, Petrakova KV, Kurolesova AI, Preobrazhenko VV, Vlasov SA, Timofeev EA, et al. Marrow niche cells: a microenvironment for hematopoiesis. Transplantation. 1968;6(2):230–47.

25. Robin C. Dictionnaire encyclopédique des sciences médicales. In: DeChambre A, editor. Paris; 1875.

26. Duverney M. De la structure et du sentiment de la moelle. Histoire de l’Academie Royale des Sciences. Amsterdam: Chez Pierre Mortier; 1700.
DIVERSITY IN THE BONE MARROW NICHE: CLASSIC AND NOVEL STRATEGIES
TO UNCOVER NICHE COMPOSITION

27. Du NE. Rôle de la meilleure des os dans les formations du sang. C R Acad Sci. (Paris). 1869;68:1112–3.

28. Bizzozero G. Sulla funzione emato poeticà del midollo del ossa. Zentralbl Med Wissensc. 1868;6:885.

29. Oser W. The principles and practice of medicine. 1st ed. New York: D. Appleton; 1892.

30. Bichat X. Traité d’anatomie descriptive. 1st ed. Paris, France: Chez Brosson, Libraire. Gabon, Libraire; 1812 An 10 (1801- an 12 (1803).

31. Tratwal J, Rojas-Sutterlin S, Bataclan C, Bluam S, Naveirs OA. Bone marrow adiposity and the hematopoietic niche: a historical perspective of reciprocity, heterogeneity, and lineage commitment. Best Pract Res Clin Endocrinol Metab. 2021;35(4):101564.

32. Neumann E. The law of distribution of yellow and red marrow in the bones of the extremities. Cent J Med Sci. 1882;20:321–3.

33. Ehrlich P. Beitrage zur Kenntnis der granulirten Bindegewebszellen und der eosinophilen Leukocythen. Arch Anat Physiol (Leipzig). 1879;3:166–9.

34. Neumann E. Hamatologische Student II. Die Variabilitat der Leukamie. Virchows Arch. 1912;12:379–412.

35. Tavassoli M, Crosby WH. Transplantation of marrow to extramedullary sites. Science. 1968;161(3836):54–6.

36. Maniatis A, Tavassoli M, Crosby WH. Origin of osteogenic precursor cells in extramedullary marrow implants. Blood. 1971;38(5):569–75.

37. El-Badri NS, Wang BY, Cherry GRA. Osteoblasts promote engraftment of allogeneic hematopoietic stem cells. Exp Hematol. 1998;26(2):110–6.

38. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2007;131(2):324–36.

39. Chan CK, Chen CC, Luppen CA, Kim JB, DeBoer AT, Wei K, et al. Endochondral ossification is required for hematopoietic stem-cell niche formation. Nature. 2009;457(7228):490–4.

40. Pinho S, Lacombe J, Hanoun M, Mizoguchi T, Bruns I, Kunisaki Y, et al. PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. J Exp Med. 2013;210(7):1351–67.

41. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and hematopoietic stem cells form a unique bone marrow niche. Nature. 2010;466(7308):829–34.

42. Boland GN, Svendsen BJ, Katoji K, Arai K, Ferraro F, Lira SA. Mesenchymal stem cells can control hematopoietic stem cell repopulation. Nat Med. 2013;19(7):792–800.

43. Wolf NS. Dissecting the hematopoietic microenvironment. III. Evidence for a positive short range stimulus for cellular proliferation. Cell Tissue Kinet. 1978;11(4):335–45.

44. Ballen K, Mendizabal AM, Cutler C, Politikos I, Jamieson K, Shpall EJ, et al. Phase II trial of parathyroid hormone after double umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2012;18(12):1851–8.

45. Lymeri S, Horwood N, Marley S, Gordon MY, Cope AP, Dazzi F. Strontium can increase some osteoblasts without increasing hematopoietic progenitor cell expansion. J Exp Med. 2013;210(7):1351–67.

46. Greenbaum AM, Link DC. Mechanisms of G-CSF-mediated hematopoietic stem and progenitor mobilization. Leukemia. 2011;25(2):211–7.

47. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006;25(6):977–88.

48. Katayama Y, Battista M, Kao WM, Hidalgo A, Peirad AJ, Thomas SA, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006;124(2):407–21.

49. Mendez-Ferrer S, Battista M, Frenette PS. Cooperation of beta(2)- and beta(3)-adrenergic receptors in hematopoietic progenitor cell mobilization. Ann N Y Acad Sci. 2010;1192:139–44.

50. Lucas D, Bruns I, Battista M, Mendez-Ferrer S, Magnon C, Kunisaki Y, et al. Norepinephrine reuptake inhibition promotes mobilization in mice: potential impact to rescue stem cell yields. Blood. 2012;119(17):3962–5.

51. Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, et al. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood. 2010;116(23):4815–28.

52. Christopher MJ, Link DC. Granulocyte colony-stimulating factor induces osteoblast apoptosis and inhibits osteoblast differentiation. J Bone Miner Res. 2008;23(11):1765–74.

53. Butler JM, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, et al. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. Cell Stem Cell. 2010;6(3):251–64.

54. Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, et al. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. J Cell Biol. 1998;142(1):295–305.

55. Moolten FL. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. Cancer Res. 1986;46(10):5276–81.

56. Visnjic D, Kalajzic Z, Rowe DW, Katavic V, Lorenzo J, Aguila HL. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. Blood. 2004;103(9):3258–64.

57. Kaneko Y, Tsukamoto A. Gene therapy of hepatica: bystander effects and non-apoptotic cell death induced by thymidine kinase and ganciclovir. Cancer Lett. 1995;96(1):105–10.

58. Saito M, Iwakami T, Taya C, Yonekawa H, Noda M, Inui Y, et al. Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. Nat Biotechnol. 2001;19(8):746–50.

59. Omatsu Y, Sugiyama T, Kohara H, Kondoh G, Fuji N, Kohno K, et al. The essential functions of adipose-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity. 2010;33(3):387–99.

60. Buch T, Heppner FL, Tertilt C, Heinzen TI, Kremer M, Wunderlich FT, et al. A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. Nat Methods. 2005;2(6):419–26.

61. Bruns I, Lucas D, Pinho S, Ahmed J, Lambert MP, Kunisaki Y, et al. Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. Nat Med. 2014;20(11):1315–20.

62. Nakamura-Ishizu A, Takubo K, Fujikoa M, Suda T. Megakaryocytes are essential for HSC quiescence through the production of thrombopoietin. Biochem Biophys Res Commun. 2014;454(2):353–7.

63. Zhao M, Perry JM, Marshall H, Venkatraman A, Qian P, He XC, et al. Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. Nat Med. 2014;20(11):1321–6.

64. Fujikoa M, Tokano H, Fujikoa KS, Okano H, Edge AS. Generating mouse models of degenerative diseases using Cre/lox-mediated in vivo mosaic cell ablation. J Clin Invest. 2011;121(6):2462–9.

65. Calaminus SD, Guitart AV, Sinclair A, Schachtmr H, Watson SP, Holyoake TL, et al. Lineage tracing of Pf4-Cre marks hematopoietic stem cells and their progeny. PLoS One. 2012;7(12):e51361.

66. Asada N, Katayama Y, Sato M, Minagawa K, Wakahashi K, Kawano H, et al. Matrix-embedded osteocytes regulate mobilization of hematopoietic stem/progenitor cells. Cell Stem Cell. 2013;12(6):737–47.

67. Thoren LA, Liuba K, Bryder D, Nygren JM, Jensen CT, Qian H, et al. Kit regulates maintenance of quiescent hematopoietic stem cells. J Immunol. 2008;180(4):2045–53.

68. Miller CL, Rebel VI, Helgason CD, Landsorp DM, Eaves CJ. Impaired steel factor responsiveness differentially affects the detection and long-term maintenance of fetal liver hematopoietic stem cells in vivo. Blood. 1997;89(4):1214–23.

69. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain hematopoietic stem cells. Nature. 2012;481(7382):457–62.
70. Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. Nature. 2013;495(7440):231–5.

71. Greenbaum A, Hsu YM, Day RB, Schuettpelz LG, Christopher MJ, Bortz, et al. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. Nature. 2013;495(7440):227–30.

72. Asada N, Kuniaki Y, Pierce H, Wang Z, Fernandez NF, Birbair A, et al. Differential cytokine contributions of perivascular haematopoietic stem cell niches. Nat Cell Biol. 2017;19(3):214–23.

73. Omatsu Y, Seike M, Sugiyama T, Kume T, Nagasawa T. Foxc1 is a critical regulator of haematopoietic stem/progenitor cell niche formation. Nature. 2014;508(7507):536–40.

74. Seike M, Omatsu Y, Watanabe H, Kondo G, Nagasawa T. Stem cell niche-specific Ebf3 maintains the bone marrow cavity. Genes Dev. 2018;32(5–6):359–72.

75. Omatsu Y, Aiba S, Maeta T, Higaki K, Aoki K, Watanabe H, et al. Runx1 and Runx2 inhibit fibrovascular conversion of cellular niches for hematopoietic stem cells. Nat Commun. 2022;13(1):2654.

76. Omatsu Y, Nagasawa T. Identification of microenvironmental niches for hematopoietic stem cells and lymphoid progenitor-bone marrow fibroblastic reticular cells with salient features. Int Immunol. 2021;33(12):821–6.

77. Nilsson SK, Johnston HM, Coverdale JA. Spatial localization of transplanted hematopoietic stem cells: inferences for the localization of stem cell niches. Blood. 2001;97(8):2293–9.

78. Okada S, Nakaochi H, Nagayoshi K, Nishikawa S, Miura Y, Suda T. In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells. Blood. 1992;80(12):3044–50.

79. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005;121(7):1109–21.

80. Oguro H, Ding L, Morrison SJ. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell. 2013;13(1):102–16.

81. Yilmaz OH, Kiel MJ, Morrison SJ. SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. Blood. 2006;107(3):924–30.

82. Kiel MJ, Radice GL, Morrison SJ. Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. Cell Stem Cell. 2007;1(2):204–17.

83. Kunisaki Y, Brunis I, Scheiermann C, Ahmed J, Pinho S, Zhang D, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. Nature. 2013;502(7473):637–43.

84. Itkin T, Gur-Cohen S, Spencer JA, Scha这边ovitz A, Ramasamy SK, Kusumbe AP, et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. Nature. 2014;532(7599):323–8.

85. Kokkaliaris KD, Kunz L, Cesaba-Recski S, Stathopoulou C, Renders S, Camargo F, et al. Adult blood stem cell localization reflects the abundance of reported bone marrow niche cell types and their combinations. Blood. 2020;136(20):2296–307.

86. Gomariz A, Helbling PM, Isringhausen S, Suessbier U, Becker A, Boss A, et al. Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy. Nat Commun. 2018;9(1):2532.

87. Zhang J, Wu Q, Johnson CB, Pham G, Kinder JM, Olsson A, et al. In situ mapping identifies distinct vascular niches for myelopoiesis. Nature. 2021;590(7846):457–62.

88. Nombela-Arrieta C, Pivarnik G, Winkel B, Canty KJ, Harley B, Mahoney JE, et al. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. Nat Cell Biol. 2013;15(5):533–43.

89. Xie Y, Yin T, Wiegraebe W, He XC, Miller D, Stark D, et al. Detection of functional haematopoietic stem cell niche using real-time imaging. Nature. 2009;457(7225):97–101.

90. Kohler A, Schmitzort V, Filippi MD, Ryan MA, Daria D, Gunzer M, et al. Altered cellular dynamics and endostial location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. Blood. 2009;114(2):290–8.

91. Winkler JG, Barbier V, Wadley R, Zannettino AC, Williams S, Levesque JP. Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. Blood. 2010;116(3):375–85.

92. Acar M, Kocherlakota KS, Murphy MM, Peyer JG, Oguro H, Inra CN, et al. Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. Nature. 2015;526(7571):126–30.

93. Chen JY, Miyashita S, Wang SK, Yamazaki S, Shinra R, Kao KS, et al. Hoxb5 marks long-term haematopoietic stem cells and reveals a homogenous perivascular niche. Nature. 2016;530(7599):223–7.

94. Pinho S, Marchand T, Yang E, Wei Q, Nerlov C, Frenette PS. Lineage-biased hematopoietic stem cells are regulated by distinct niches. Dev Cell. 2018;44(4):634–41 e4.

95. Christodoulou C, Spencer JA, Yeh SA, Turcotte R, Kokkaliaris KD, Panero R, et al. Live-animal imaging of native hematopoietic stem and progenitor cells. Nature. 2020;578(7794):278–83.

96. Cordeiro Gomes A, Harra T, Lim VY, Horned-Brandstetter D, Nevius E, Sugiyama T, et al. Hematopoietic stem cell niches produce lineage-instructive signals to control multipotent progenitor differentiation. Immunity. 2016;45(6):1219–31.

97. Upadhyaya S, Krichesky O, Akhmetzyanova I, Sawai CM, Fookstran DR, Reizis B. Intraval imaging reveals motility of adult hematopoietic stem cells in the bone marrow niche. Cell Stem Cell. 2020;27(2):336–45 e4.

98. Shimoto M, Sugiyama T, Nagasawa T. Numerous niches for hematopoietic stem cells remain empty during homeostasis. Blood. 2017;129(15):2124–31.

99. Foudi A, Hochdellinger K, Van Buren D, Schindler JW, Jaenisch R, Carey V, et al. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat Biotechnol. 2009;27(1):84–90.

100. Severe N, Karabacak NM, Gustafsson K, Baryawno N, Courjies G, Foudi A, et al. Stress-induced changes in bone marrow stromal cell populations revealed through single-cell protein expression mapping. Cell Stem Cell. 2019;25(4):570–83 e7.

101. Al-Sabah J, Baccin C, Haas S. Single-cell and spatial transcriptionomics approaches of the bone marrow microenvironment. Curr Opin Oncol. 2020;32(2):146–53.

102. Dalgalev I, Tikhonova AN. Connecting the dots: resolving the bone marrow niche heterogeneity. Front Cell Dev Biol. 2021;9:622519.

103. Zhong L, Yao L, Tower RJ, Wei Y, Miao Z, Park J, et al. Single cell transcriptomics identifies a unique adipose lineage cell population that regulates bone marrow environment. elife. 2020;9:e54695.

104. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell. 2014;15(2):154–68.

105. Silverstein I, Gonzales KA, Kharchenko PV, Turcotte R, Kfouri Y, Mercier F, et al. Proximity-based differential single-cell analysis of the niche to identify stem/progenitor cell regulators. Cell Stem Cell. 2016;19(4):530–43.

106. Efremova M, Vento-Tormo M, Teichmann SA, Vento-Tormo R. CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes. Nat Protoc. 2020;15(4):1484–506.

107. Browaeys R, Saels W, Saeks Y. nicheNet: modeling intercellular communication by linking ligands to target genes. Nat Methods. 2020;17(2):159–62.

108. Ombrato L, Nolan E, Kurelac I, Mavousian A, Bridgeman VL, Heizne I, et al. Metastatic-niche labelling reveals parenchymal cells with stem features. Nature. 2019;572(7771):603–8.

109. Passaro D, Garcia-Albornoz M, Diana G, Chakravarty P, Arizaga-McNaughton L, Batsivari A, et al. Integrated OMICs unveil the bone-marrow microenvironment in human leukemia. Cell Rep. 2021;35(6):109119.
110. Stocekius M, Hafemeister C, Stephenson W, Houck-Loomis B, Chattopadhyay PK, Swerdlow H, et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods. 2017;14(9):865–8.

111. Guilliams M, Bonnardel J, Haest B, Vanderborght B, Wagner C, Remmerie A, et al. Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches. Cell. 2022;185(2):379–96 e38.

112. Goltsvev Y, Samusik N, Kennedy-Darling J, Bhate S, Hale M, Vazquez G, et al. Deep profiling of mouse splenic architecture with CODEX multiplexed imaging. Cell. 2018;174(4):968–81 e15.

113. Radtke AJ, Kandov E, Lowekamp B, Speranza E, Chu CJ, Gola A, et al. The hematopoietic bone marrow microenvironment: Classic and novel strategies to uncover niche composition. How to cite this article: Sánchez-Lanzas R, Kalampalika F, Ganauz M. Diversity in the bone marrow niche: Classic and novel strategies to uncover niche composition. Br J Haematol. 2022;199:647–664. https://doi.org/10.1111/bjh.18355