Invited review
Best Practice & Research : Clinical Endocrinology & Metabolism

Title:
Bone Marrow Adiposity and the Hematopoietic Niche: A Historical Perspective of Reciprocity, Heterogeneity, and Lineage Commitment

Authors:

Josefine Tratwal, PhD, Postdoctoral Fellow[1]
Shanti Rojas-Sutterlin, PhD, Postdoctoral Fellow[1¶]
Charles Bataclan, PhD student[1]
Sabine Blum, MD, Hematology Consultant [2]
Olaia Naveiras, MD/PhD, Associate Professor [1,2,*]

Affiliations:

[1] Laboratory of Regenerative Hematopoiesis, Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL) & Department of Biomedical Sciences, University of Lausanne (UNIL), Lausanne, Switzerland

[2] Hematology Service, Departments of Oncology and Laboratory Medicine, Lausanne University Hospital (CHUV), University of Lausanne (UNIL), Lausanne, Switzerland

[¶] Current address: Department of Pharmacology & Biochemistry, Faculty of Medicine, Université de Montréal, Canada.

[*] Corresponding author: Department of Biomedical Sciences, University of Lausanne, Rue du Bugnon 27, 1011 Lausanne, Switzerland
E-mail address: olaia.naveiras@unil.ch (O. Naveiras)

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Abstract:

Purpose

Here we review the current knowledge on bone marrow adipocytes (BMAds) as active contributors to the regulation of the hematopoietic niche, and as potentially pivotal players in the progression of hematological malignancies. We highlight the hierarchical and functional heterogeneity of the adipocyte lineage within the bone marrow, and how potentially different contexts dictate their interactions with hematopoietic populations.

Recent Findings

Growing evidence associates the adipocyte lineage with important functions in hematopoietic regulation within the BM niche. Initially proposed to serve as negative regulators of the hematopoietic microenvironment, studies have also demonstrated that BMAds positively influence the survival and maintenance of hematopoietic stem cells (HSCs). These seemingly incongruous findings may at least be partially explained by stage-specificity across the adipocytic differentiation axis and by BMAds subtypes, suggesting that the heterogeneity of these populations allows for differential context-based interactions. One such distinction relies on the location of adipocytes. Constitutive bone marrow adipose tissue (cBMAT) historically associates to the "yellow" marrow containing so-called "stable" BMAs larger in size, less responsive to stimuli, and linked to HSC quiescence. On the other hand, regulated bone marrow adipose tissue (rBMAT)-associated adipocytes, also referred to as "labile" are smaller, more responsive to hematopoietic demand and strategically situated in hematopoietically active regions of the skeleton. Here we propose a model where the effect of distinct BM stromal cell populations (BMSC) in hematopoiesis is structured along the BMSC-BMAd differentiation axis, and where the effects on HSC maintenance versus hematopoietic proliferation are segregated. In doing so, it is possible to explain how recently identified, adipocyte-primed leptin receptor-expressing, CXCL12-high adventitial reticular cells (AdipoCARs) and marrow adipose lineage precursor cells (MALPs) best support active hematopoietic cell proliferation, while adipose progenitor cells (APCs) and maturing BMAd gradually lose the capacity to support active hematopoiesis, favoring HSC quiescence. Implicated soluble mediators include MCP-1, PAI-1, NRP1, possibly DPP4 and limiting availability of CXCL12 and SCF. How remodeling occurs within the BMSC-BMAd differentiation axis is yet to be elucidated and will likely unravel a three-way regulation of the hematopoietic, bone, and adipocytic compartments orchestrated by vascular elements. The interaction of malignant hematopoietic cells with BMAds is precisely contributing to unravel specific mechanisms of remodeling.

Summary

BMAds are important operative components of the hematopoietic microenvironment. Their heterogeneity directs their ability to exert a range of regulatory capacities in a manner dependent on their hierarchical, spatial, and biological context. This complexity highlights the importance of (i) developing experimental tools and nomenclature adapted to address stage-specificity and heterogeneity across the BMSC-BMAd differentiation axis when reporting effects in hematopoiesis, (ii) interpreting gene reporter studies within this framework, and (iii) quantifying changes in all three compartments (hematopoiesis, adiposity and bone) when addressing interdependency.

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Practice points:

- Reciprocity of bone marrow adiposity and hematopoietic cellularity has been long described and constitutes a diagnostic feature in numerous hematological diseases, likely reflecting a quiescent (cBMAT-associated, historically yellow marrow) versus a proliferative (rBMAT-associated, historically red marrow) hematopoietic stem and progenitor niche.
- The biological mechanisms for this reciprocity are only starting to be elucidated, and will account for regional specificity of heterogenous stromal subpopulations and their associated vascular structures.
- In homeostasis, the predictable distribution of red and yellow marrow areas offers a controlled access point to study the role of BMA (e.g., caudal vs. thoracic vertebrae in mouse models; in humans MRI-based imaging and femur or iliac crest vs sternal BM comparisons).
- In emergency hematopoiesis, stromal cells committed to the adipocyte lineage either correlate with or have been shown necessary for efficient hematopoietic recovery. Contrarily, mature BMAd have been associated with hematopoietic stem cell quiescence.

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Introduction: Origins of the marrow as the seedbed of our blood

From the perspective of hematopoiesis, bone marrow adipocytes (BMAds) are the most abundant non-hematopoietic component of the adult human bone marrow (BM). Many questions remain regarding the reciprocal relationship between hematopoiesis and adiposity via the control of the hematopoietic stem cell (HSC) niche.

What's in a niche?

> "In stem cell biology at large, a stem cell niche has come to be seen as the specific tissue site in which stem cells receive instructive cues that determine their behavior, in particular, their self-renewal throughout life. Stated in a simple way, a niche is the tissue site where stem cells remain, or even become, stem cells…However, self-renewal of stem cells may have additional, intrinsic, as well as population-based, determinants, all of which need to be integrated with the microenvironmental cues conceptualized in the niche." [1].

Two types of marrow and the stem cell theory

It was believed since at least the time of Hippocrates that marrow is the nutrient source of bone. However, the French anatomist Pierre Duverney observed in 1700 that some bones have no marrow (like those of the middle ear) and it would therefore unlikely be essential for nutrition of bone [2]–[4]. Ernst Neumann and Giulio Bizzozero who studied with Rudolf Virchow in Berlin, first described the presence of nucleated blood cells in the marrow in 1868, one and a half centuries later [5]–[7]. The following year, Neumann would ascertain that blood arises in the BM. He described that "it operates continually in the *de novo* formation of red blood cells" [8], accepting their finite lifespan and the necessity for continuous replenishment. He observed that "Also in the marrow rich in fat, the same cells are present but in lower quantity and their number decreases parallel to the decrease in the number of marrow cells and the increase in the number of fat cells." [7].

This was followed by his identification of leukemia as a disease of the marrow [9]. Charles Robin would a few years later notice that in the course of development, marrow is formed after bone [3]. Then in 1882, Neumann established the rule governing the development of yellow marrow and its reciprocity with hematopoiesis, what is now referred to as *Neumann's law,* which states that: at birth, all bones that contain marrow contain red marrow, and with age, the blood-producing capacity contracts toward the center of the skeleton, leaving the more peripheral bones with only fatty marrow [4], [10].

However, it was actually Xavier Bichat who at the end of the 18th century first recognized the two types of marrow, coining them *red marrow* as seen in the fetus and *fatty marrow* of the adult. These notions would be echoed centuries later [11] long after the presence of red marrow in the adult had been acknowledged, and the gelatinous transformation of the marrow had been recognized [4], [12], [13]. Notably, Bichat already observed that fatty marrow was distinct from other types of fat, which Dunlop and Nerking confirmed in the beginning of the 1900s through analysis of its chemical composition [13]–[15].

These seminal findings set the stage for eventually defining the HSC. As early as 1896, Arthur Pappenheim used the term *stem cell* to describe a precursor cell capable of giving rise to red and white blood cells (Figure 1) [16], [17]. Neumann followed, first designating the origin of all hematopoietic cells the "great lymphocyte" and later also adopting the term "stem cell" for the common precursor of the blood system [7], [18].

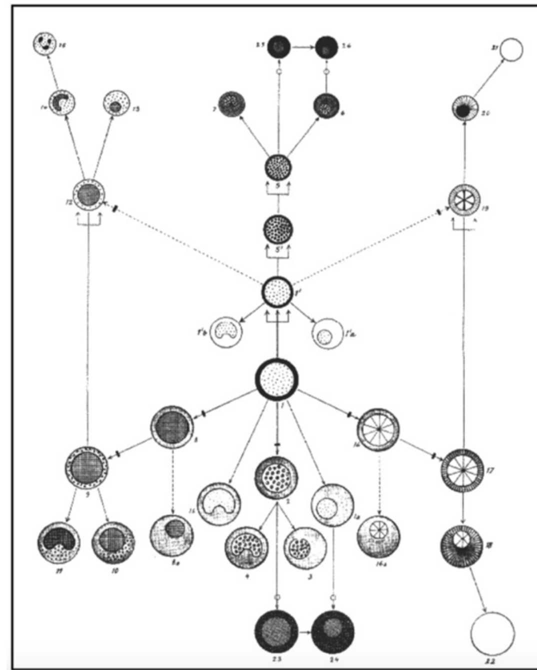

Figure 1|Stem cell origins. The recognition of a common progenitor as the source of hematopoietic cells by Ernst Neumann (1868), and the application of early staining techniques differentiating white blood cell lineages by Paul Ehrlich (1879), led to a discussion on the existence of a bona fide stem cell in the field. Arthur Pappenheim's illustration in 1905 of a precursor stem cell at the center of the hematopoietic system still holds true to some extent today. Reprinted with permission from [19].

Due to the limitations of experimental methods at the time, it wasn't until the 1960s however, that James Tim, Ernest McCulloch, and others provided definitive evidence of the existence of the first stem cell ever described, the common HSC, through the discovery of spleen colonies following irradiation and BM transplantation [20]–[22]. This lay the beautiful groundwork for clonal lineage tracing of cells *in vivo*, the isolation of stem cells, and the establishment of HSC transplantation as a treatment for malignancies of the blood (the first and nowadays still landmark stem cell therapy) through serial reconstitution. Today, beautifully elaborate roadmaps are available to infer from single cell transcriptomics the hematopoietic fate choices that take place within the marrow, for which the bone, the marrow stroma and their associated vasculature provide an essential environment [23]–[25].

Of the bone and its marrow

As experimental medicine was beginning to thrive, the relation of bone and marrow would finally be addressed beyond the ongoing speculative debates. Some of the first marrow grafts to extramedullary sites (such as the abdomen) were performed in the later 19th century [26]. Goujon and Bailkow noted that the grafted marrow would transform into bone, and as such made the first observations of the bone-forming potential of marrow cells [27]. However, it wasn't until a century later (following the same time course as the discovery of the HSC), that Mehdi Tavassoli and William Crosby, simultaneously with Friedenstein et al., determined that heterotopic transplants of marrow fragments could form not only bone but also marrow anew (Figure 2a) in the form of BM organoids or ossicles [28], [29], demonstrating that bone fragments retained "histological memory" such that they would reconstitute hematopoietic marrow and then undergo adipocytic transition if originating from regions of constitutively yellow marrow. The exploration of the BM stroma had begun. Tavassoli would go on to define the stable and labile fatty components of the marrow with differential lipid composition, and Friedenstein showed that indeed the bone and marrow derived from an osteogenic progenitor cell through establishing the colony

forming unit-fibroblast (CFU-F) assay. Seeding of BM cell suspensions at clonal density resulted in discrete colonies from single cells, with a linear dependence of colony formation on initial seeding [30]. Similarly, the colony forming unit (CFU) assay had been developed to quantify hematopoietic potential of HSCs and downstream progenitors plated in semi-solid medium [31], [32].

At the same time as the identification of the multipotent bone marrow stromal cell (BMSC), John J. Trentin first presented the concept of the hematopoietic microenvironment followed by the conceptualization of the stem cell niche hypothesis by Ray Schofield [33]–[36]. This notion that HSCs are regulated by their association with a discrete microenvironment of the BM, was substantiated with the advent of Dexter cultures [37]–[39]. And so, the HSC or "seed" is supported by a base of (hematopoietic stromal microenvironment) "soil" while some BMSC simultaneously function as a stem cell and provide a microenvironment for HSCs, thereby embodying properties of both the "seed" and the "soil" [40]–[43].

The search for the hematopoietic and stromal progenitor

Schofield proposed that HSCs age, or lose part of their regenerative potential, in situations of stress when challenged to sustain hematopoietic reconstitution. He maintained that HSCs are stem cells in their niche, where they remain quiescent or divide conservatively, but lose part of their potential when they are challenged and proliferate or differentiate as they move out of their niche [1]. Since HSCs were first observed to localize to endosteal surfaces, Schofield named the endosteum as the HSC niche, which other studies have since supported [44]–[50]. Genetic mouse models allowed for the determination of the osteoblast as a regulatory component of the HSC niche [51]–[53]. Notably, endosteal regions are also enriched in intra- and trans-cortical microvessels (the former lined by an adventitial layer of alkaline phosphatase positive (ALP^+) cells. These capillaries connect the marrow circulation, bone, and periosteal circulation, thereby contributing to the unique endosteal microenvironment [1], [47], [54]. The trans-cortical vessels, are either arterial or venous in nature connecting the periosteum with the BM, and make up the greatest contribution of blood flow in and out of the bone [54]. At the interface between the metaphysis and diaphysis, the blood flows from type H capillaries ($CD31^{hi}Endomucin^{low}$) into the branched sinusoidal network that constitutes the type L capillaries ($CD31^{low}Endomucin^{low}$) [55]–[57]. Interestingly, in aging animals and in ovarietomized mice, type H vessels and thereby blood flow, decline, whereas type L vessels do not decline with age [57]. Type H vessels are surrounded by $PDGFR\beta^+$ cells also expressing neural/glial antigen 2 ($NG2^+$). They secrete osteogenic factors maintaining $Osterix^+$ (Osx^+) progenitors that associate with these vessels [58], [59]. Meanwhile, type L vessels, are covered by two types of perivascular cells, namely leptin receptor ($LepR^+$) platelet-derived growth factor alpha ($PDGFR\alpha^+$) cells and CXCL12-expressing adventitial reticular (CAR) cells [60], the best studied cellular component of the HSC niche.

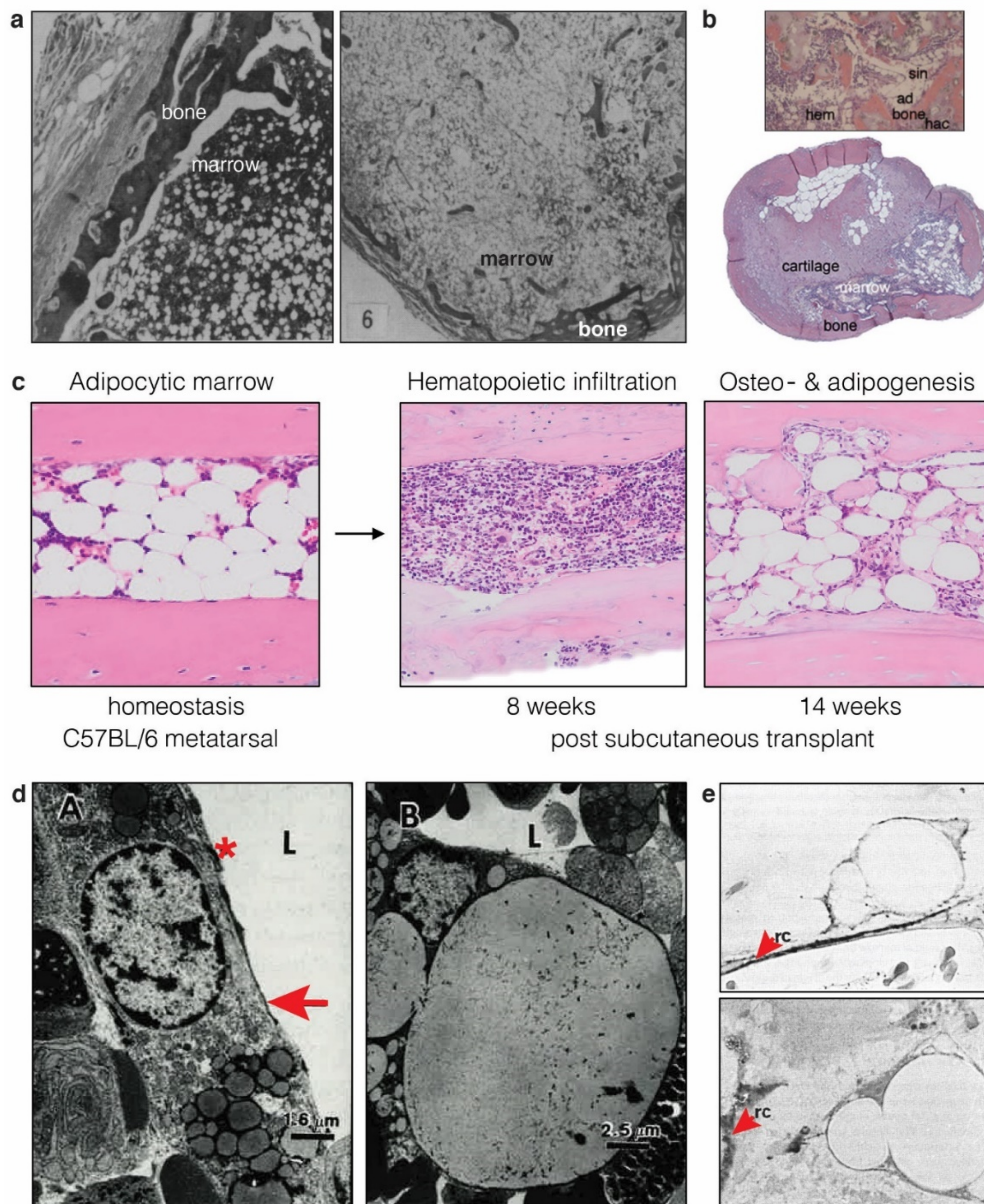

Figure 2 | The hematopoietic microenvironment. **a** Heterotopic ossicle formation recapitulates native bone marrow ontogeny from sites of red (left) and fatty (right) marrow [61]. **b** Ossicle formation *in vivo* occurs from CD146⁺ clonogenic BMSCs with a scaffold carrier (top, note adipocytes lining the sinusoid) or from a BMSC cartilage pellet (bottom)[62], [63]. **c** Hematoxylin and eosin stains of C57BL/6 female 8-week-old metatarsals containing adipocytic marrow in homeostasis and its regeneration post subcutaneous implantation into C57BL/6 female 8-week-old recipients, tibial-adjacent. After 8 weeks *in vivo*, hematopoietic infiltration is observed followed by renewed osteo- and adipogenesis after 14 weeks *in vivo*. Produced by Dr. Josefine Tratwal and Dr. Olaia Naveiras. **d** Transmission electron microscopy image of a marrow sinus segment from a rat femur. Adventitial reticular cell cytoplasm beneath the endothelium (asterisk) with multilocular lipid droplets as early signs of lipogenesis (left image) and the nucleus of a reticular cell compressed by two large lipid droplets in advanced lipogenesis (right image) Reprinted with permission ([40, p. 100]. **e** Multivacuolar maturing adipocyte in contact with abluminal reticular cell with protrusion extensions (top image). Alkaline-phosphatase positivity in reticular cells (arrows) and adipocyte membrane (bottom image). Reprinted with permission [64]. Ad: adipocyte, bm: bone marrow; hac: hydroxyapatite carrier; hem: hematopoiesis; L: lumen; rc: reticular cell; sin: sinusoid.

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Specifically, CAR cells reside on the subendothelial abluminal surface of BM sinusoids [62], [65]–[68]. Its skeletal stem cell (SSC) properties were delineated by stringent *in vivo* transplantation assays showing the *BM organizer* capacity of human, clonal CD146+ BMSCs (Figure 2b-c).Thus, the bone and its marrow truly is "a tale of two stem cells" that share a common niche, which may in fact house multiple different microenvironments for quiescent HSC versus the rapidly proliferating hematopoietic progenitors [1], [69], [70].

Ultrastructural studies hint that BM adipocytes (BMAds) seem to arise postnatally from anatomically-defined CAR-like cells [71]–[73]. In chemotherapy-induced hematopoietic ablation, rapid lipid accumulation converts these cells to adipocytes. Due to the location of the CAR cells on the abluminal part of the sinus wall, this conversion causes a constriction of the sinusoid [64]. Through the loss of lipids by lipolysis, the BMAds again release space so the sinusoids can dilate and resume blood flow to the microscopic anatomical region, coinciding with conversion of the marrow from yellow to red on the macroscopic scale and pointing to a vasculature-driven reciprocity of the yellow and red compartments [1], [74].

Adipose conversion of the BM may be thus explained as a physiological change of the niche affecting HSCs in defined regions of the skeleton [1]. Indeed, adipocyte-rich marrow correlates with lower HSC content and imposes reversible HSC quiescence in sites of yellow marrow [75], [76]. BMAds originate from one or several stromal progenitors, which are likely ALP+ and likely located at a pericyte position, as detailed below (Figure 2d). Para-trabecular and para-cortical adipocytes, long recognized in the context of diagnostic hematology [77], [78], may constitute a distinct BMAd population originating from bone lining cells under specific stress conditions. The lineage trajectories of the BMSC-to-BMAd differentiation axis are rapidly being elucidated through single cell transcriptomics, and currently available for murine BMSCs [79]–[81]. BMAds also contribute to the HSC niche responsible for instructive cues in the form of bound or secreted molecules for HSC quiescence, self-renewal, proliferation, and differentiation [64]. In concert with the extracellular matrix (ECM) stromal cells within the BMSC-BMAd differentiation axis also contribute regulatory signals through physical cues including contractile forces, shear stress, temperature, and oxygen tension [82], [83].

Perivascular stromal cells

Importantly, the C-X-C motif chemokine ligand receptor 12 (CXCL12, so-called stromal cell derived factor 1 (SDF1) or pre-B cell growth stimulating factor) and its receptor CXCR4 regulate the homing of HSC and their downstream progenitors (HPCs) and are critical for BM colonization and engraftment [84]–[86]. Multiple stromal cells of the niche are characterized by the expression of CXCL12 and may be the precursors to some or all BMAds [87], [88]. Stem cell factor (SCF, so-called steel factor (SF) or c-Kit Ligand, KitL) is also produced by perivascular stromal cells for the maintenance of HSCs which express the cKit receptor [89]. Differential roles have been uncovered for CXCL12 and SCF in the NG2+ periarteriolar subset of stromal cells as compared to perisinusoidal *LepR*-Cre targeted CAR cells [90]. Deletion of *Foxc1* in CAR cells during embryogenesis results in reduced hematopoietic stem and progenitor cells (HPCs), normal appearance of osteoblasts, and increased BM adiposity (BMA). *Foxc1* deletion in adulthood, was also shown to deplete HPCs by reducing CXCL12 and SCF in CAR cells without a conversion to yellow marrow [91]. *Foxc1* may thus be critical for CAR cell development, including CXCL12 and SCF expression, while inhibiting the adipogenic potential of these cells.

A wave of seminal studies carried out using transgenic mouse models show that most CXCL12 in the BM is derived from perivascular stromal cells marked by partially-overlapping stromal populations, as revealed by *Prx1*-Cre, *Nestin*-GFP, or *LepR*-Cre, and *Osterix*-Cre reporters [92]–[97]. These markers are expressed to varying degrees in the marrow, both at the level of the stromal population expressing the transgene and, for Cre-dependent models, also in the totality of their progeny. Results from these important studies thus have to be interpreted with some caution due to both limitations of GFP turnover or rate and efficiency of Cre recombination [98]. For example, the distribution of *Nestin*-GFP cells is specific to the skeletal location, and transgene dosage reveals differential functions. *Nestin*-GFP^{bright} cells are localized to arterioles (preferentially found in endosteal BM) while *Nestin*-GFP^{dim} cells are reticular in shape and associated with sinusoids (Figure 3) [99]. To better unravel the close ties of the BM-to-BMAds differentiation axis and the hematopoietic system, we must first do due diligence on understanding their specific roles in a stage-specific manner.

Filling the gaps: Distinct adipocyte subtypes

The SSC, CAR cell, and pre-adipocyte populations have been identified in the BM by expression of markers present in white adipose tissue (WAT) and pericytes [62], [65]–[68], [100]–[102]. Whereas in homeostasis, the bone marrow adipose tissue (BMAT) shares characteristics mostly with white adipose tissue (WAT), brown adipose tissue (BAT) marker uncoupling protein 1 (*Ucp1)* was found to be upregulated in whole tibia in response to exercise alone or in combination with rosiglitazone while reducing BMAT volume as well as BMAd size and number [103], [104]. In fact, *Ucp1* expression can be found in homeostasis at distal sites, as shown in Figure 3d for metatarsals, suggesting skeletal site-specific heterogeneity. This suggests that BMAT is plastic and can behave with properties of WAT or inducible white (termed beige, or, brite: brown-in-white), the term assigned to adipocytes of a mixed morphology and an overlapping but distinct signature compared to BAT [105]. To put such plasticity into perspective, it is interesting to note that Adiponectin (*Adipoq)*-expressing adipocytes make up the majority of the mammary gland. They dedifferentiate to PDGFRα^{+} preadipocytes and are replaced by alveolar structures during lactation to then reappear upon weaning [106], [107]. The cyclical de- and re-differentiation of the so-called "pink" adipocytes is indeed remarkable, and may present an inspiration for studying BMAT. This becomes intriguing in deciphering the changes in BMAT on stress hematopoiesis when the BM undergoes drastic changes in morphology and cell types. Indeed, it was suggested to call BMAds "yellow adipocytes" due to the gross anatomical color they impart [108]. However, this could lead to a misconception if applied to the entirety of BMAT due to differences incurred by its location.

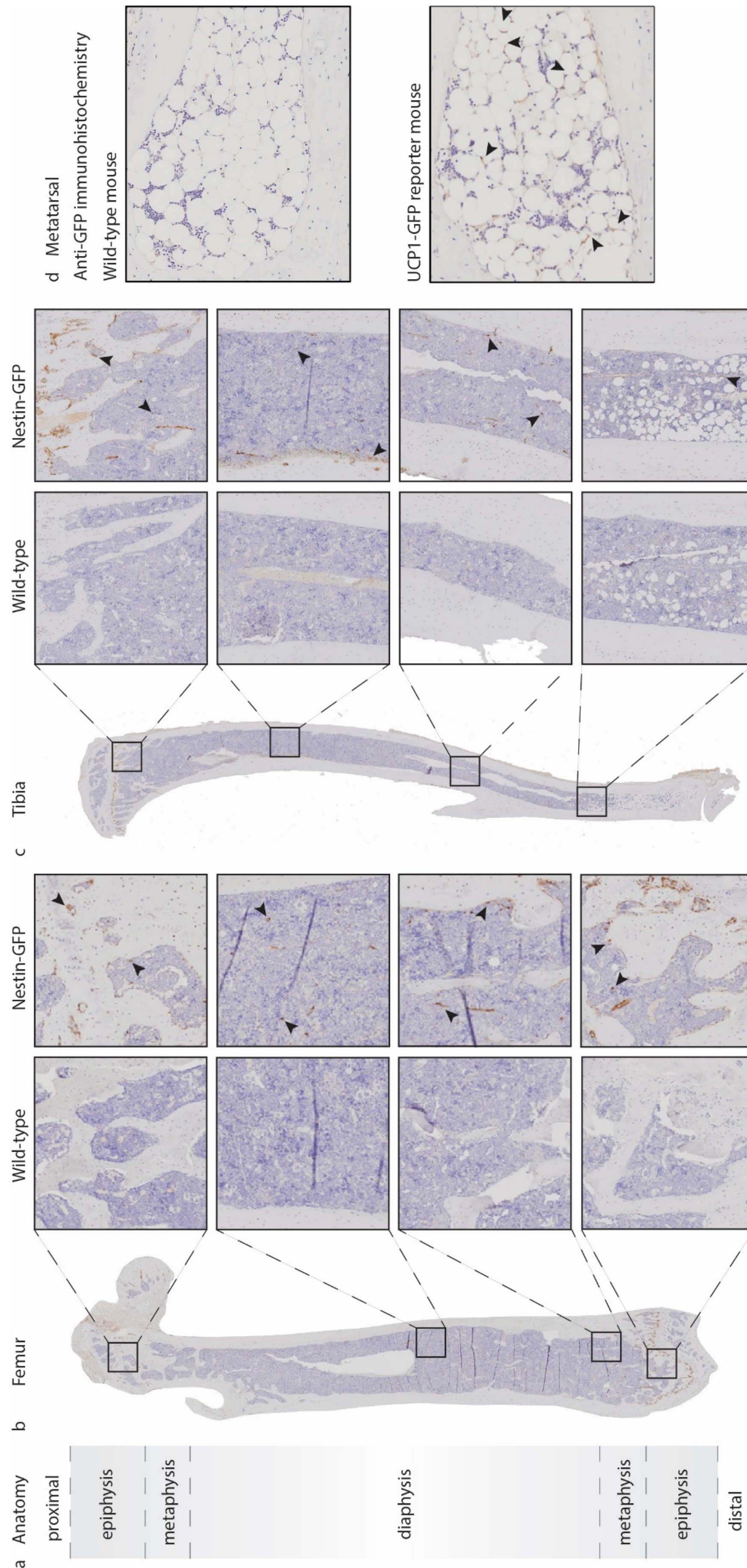

Figure 3 | Niches within the marrow are location specific, as illustrated by *Nestin* or *Ucp1* expression variation in long bones. **a** The anatomical organization of murine long bones originates from two distinct ossification centers. **b** Femur and **c**, tibia isolated from 13-week-old C57BL/6 female wild-type or transgenic mice expressing GFP under the nestin promoter (*Nestin*-GFP), were processed for histology and stained against GFP with chromogenic detection (brown). Nestin^{+} stromal cells (arrows) are located in the sinusoidal- and endosteal/arteriolar-defined niches of the red marrow at varying frequencies across the short and long axis. **d** Presence of GFP positive cells in metatarsals of transgenic mice with a *Ucp1*-GFP reporter. Figure produced by Dr. Josefine Tratwal and Dr. Shanti Rojas-Sutterlin. GFP: green fluorescent protein.

Regarding skeletal site-specificity, BMAT expansion occurs in a centripetal pattern, originating first in the very distal skeleton, then in the epiphyses and diaphysis of the long bones while appearing later in the axial skeleton that remains largely hematopoietic [109]. This may correspond with distinct waves of Osx^{+} stromal, primitive, and definitive cells organizing the developing BM [95]. Distal sites contain stable BMAds, also referred to as constitutive BMAds (cBMAds), which form early in development [110]. They appear just after birth, are large in size with predominantly unsaturated lipids and are not readily mobilized. In mice, these stable BMAds extend from the malleolus in the medullary canal of the tibia until the tibia-fibular junction [111], while in rabbits they form a core through the center of the medullary canal of the long bones with surrounding regulated BMAds (rBMAds) and hematopoietic marrow between the cBMAds and encompassing cortical bone [112]. In humans, the very first appearance of cBMAds is in the terminal phalanges of the fetus just before birth when the marrow is fully hematopoietic [11]. It is documented that the stable yellow marrow in the long bones first appears in the distal epiphyses and radiates from the mid- diaphyses mostly filling the medullary canal by adulthood with the exception of the proximal metaphysis that remains hematopoietic until old age [113]. The labile rBMAds fill the medullary canal just below the growth plate of the primary spongiosa and appear in the secondary ossification center [111]. They extend through the metaphysis, accumulating preferentially along the endocortical surface of the diaphysis in mice (both during aging and with BMA induction) [114]. These BMAds are smaller in size with mostly saturated lipids and are readily mobilized upon stimulation, for example, upon hematopoietic demand.

Reciprocity of bone marrow adipocytes and hematopoiesis

The paradoxical relationship between BMAds and hematopoiesis has been recently highlighted [115], [116], and their reciprocal relation in murine marrow quantitatively validated [74]. Understanding how BMAds regulate and influence hematopoiesis needs to be at least partially contextualized due to the heterogeneity of the BMAT tissue itself, as noted above. Dissection of the heterogeneity of BMAT within the context of the BMSC-BMAd differentiation axis has been propelled by single-cell RNA sequencing efforts conducted using mouse models, although technical limitations still prevent analysis of mature BMAd through this approach. A working model integrating these elements is presented in Figure 4 and detailed below. Specifically, adipocytic primed LepR^{+} cells have been found to be a major reservoir of pro-hematopoietic factors in the BM niche [79], [80], which have been further refined as LepR^{+}Osteolectin^{-} [117]. In another study, Baccin et al. identified a specific CAR subpopulation expressing an adipocytic-lineage gene signature (AdipoCARs) and found it to be a critical component of the perivascular hematopoietic niche [118]. A similar but more restrictive subpopulation comprising non-lipidated adipocyte precursors was identified through the sequencing of TdTomato^{+} bright endosteal cells in *Col2-Cre Rosa26(lsl-tdTomato)* mice [81]. In this mouse model, which labels all BMAds, TdTomato^{+} cells contained all CFU-F activity, and a fraction thereof resembled previously described CD45^{-}/Ter119^{-}/Sca1^{+}/PDGFRα^{+} multipotent stromal progenitors with hematopietic supportive capacities [119]. Furthermore, in the same study, a seemingly post-mitotic downstream population of marrow adipogenic precursors (MALPs) expressing LepR was found to reside abundantly in the pericyte position. TdTomato^{+}PDGFRα^{+} cells were demonstrated to function to support marrow vasculature and suppress bone formation. Further investigation is necessary to more completely define how the different stages of commitment along the marrow adipocyte differentiation axis correlate with hematopoietic support, and their loss with changes in the support of both long-term HSCs and a rapidly proliferating hematopoietic compartment. Moreover, the adipocytic trajectories and subpopulations of the hematopoietic niche in human BM are yet to be characterized. In transposing lessons learnt from murine models to human marrow, the strikingly different vascularization pattern and organization in

hematons as units of hematopoiesis may reveal significant differences in stromal cell trajectories and sub-specialization [120].

Functionally, the role of BMAds as active contributors to the regulation of hematopoiesis through paracrine and endocrine signaling is now widely accepted. BMAT was initially identified as a negative regulator of hematopoiesis. Naveiras et. al showed that adipocyte-rich BM from the tail vertebrae of mice had reduced frequency of HSCs and short-term progenitors together with impaired cycling capabilities when compared to adipocyte-free thoracic vertebrae [75]. Engraftment after irradiation was accelerated in the "fatless" *A-ZIP/F1* mouse model and through pharmacological inhibition of adipogenesis. This finding was corroborated by a separate group showing increased hematopoietic recovery through BADGE-inhibition of adipogenesis following chemotherapy [121], and through simvastatin-mediated inhibition of BMAT [122]. Rescue of hematopoiesis in *A-ZIP/F1* models was also reported [123]. Meanwhile, monocyte chemoattractant protein -1 (MCP-1) was found to be central in a feedback-loop mechanism between BMAds and BMSCs, causing fat accumulation, and is proposed to negatively regulate abundance of long-term HSCs [124]. On the other hand, cell-to-cell contact with BMAds was shown to impair granulopoiesis through suppression of granulocyte-colony stimulating factor (G-CSF) production and is proposed to be mediated by neurolipin 1(NRP 1) [125]. Further studies show the that inhibitory mechanisms of NRP1 in the context of hematopoiesis includes apoptotic induction in HPCs, downregulation of CXCR4 expression, and secretion of TGFβ1 [126]. Plasminogen activator inhibitor-1 (PAI-1), an adipokine secreted by BMAds and $CD45^-CD31^-Ter119^-Sca1^+CD24^-$ $PDGFR\beta^+$ adipocyte-progenitor cells in the pericyte position was found to be one of the factors contributing to the inhibition of hematopoietic regeneration post-transplantation in control and in diet-induced obese mice [127]. An increase in BMAT through aging and obesity was also demonstrated to impair hematopoiesis in mice [100]. In this model, dipeptidyl peptidase-4 (DPP4) was shown to have a role in the delay in fracture healing and has been proposed to have a inhibitory role in hematopoietic recovery, as previously shown in a different context [128].

CXCL12 is a critical factor for HSCs, primarily secreted by perivascular stromal cells [67]. Mattiucci et al. demonstrated that human mature BMAds express CXCL12 and are able to maintain HSCs in long-term co-culture, albeit to a much lesser degree than undifferentiated primary BMSCs [129]. This result from human primary BMAds is congruent with data showing that OP9 and C3H10T1/2 murine BMSCs, when differentiated into adipocytes, can support primitive hematopoietic progenitors but lose the capacity to robustly support short term progenitor expansion *in vitro* [130]–[132]. Altogether, these data suggest that mature BMAds may have a role in steady-state HSC maintenance. Adiponectin, which as discussed above is secreted by stromal cells throughout the BMSC-to-BMAd differentiation axis, has also been demonstrated to stimulate HSC proliferation and multipotency through the p38 MAPK pathway. HSCs pre-treated with adiponectin showed improved hematopoietic reconstitution potential after transplantation in lethally irradiated mice [133]. Conversely, adiponectin deficiency results in defective hematopoietic recovery in mice post-chemotherapy [134]. In another study, the role of the BMSC-to-BMAd differentiation axis in supportive native hematopoiesis was further highlighted, in total lipodystrophic *PPARG*$^{\Delta/\Delta}$ and *AZIP*$^{tg/+}$ mouse models, wherein extramedullary hematopoiesis associated to CXCR4 loss in HPCs was observed together with altered myeloid and lymphoid populations and expansion of the osteogenic compartment within the marrow cavity. Notably, lipodystrophy-associated inflammation, which is present on the *PPARG*$^{\Delta/\Delta}$ but not the *AZIP*$^{tg/+}$ model, could not explain the phenotype [135]. Furthermore, BMAd deletion was found incomplete in *AZIP*$^{tg/+}$ mice, and pharmacological inhibition of BMAd formation through PPARg inhibtor BADGE, or more consistently via GW9662, was associated with increased hematopoietic recovery in aplastic anemia

models via direct T-cell inhibition [136]. Meanwhile *Ptrf*$^{-/-}$ mice, a model of congenital generalized lipodystrophy 4, selectively inhibits the formation of rBMAds; however the effect on hematopoiesis has not yet been reported [110].

Furthermore, *Scf* deletion in the adipocyte lineage in adiponectin-expressing cells was shown necessary for the survival and maintenance of HSCs [123]. Zhou et al. demonstrated that after myeloablation, SCF from adiponectin-expressing cells, which were equated to BMAds, mediates hematopoietic regeneration. The absence of this factor led to HSC deficiency and reduced animal survival [123]. This was substantiated by another study in which BMAT-derived SCF was found to be essential in both steady-state and metabolic stress conditions [137]. Concurrently, the increase in serum adiponectin that occurs with BMAT accumulation is likely involved in normal and pathological regulation of hematopoiesis [138]–[140].

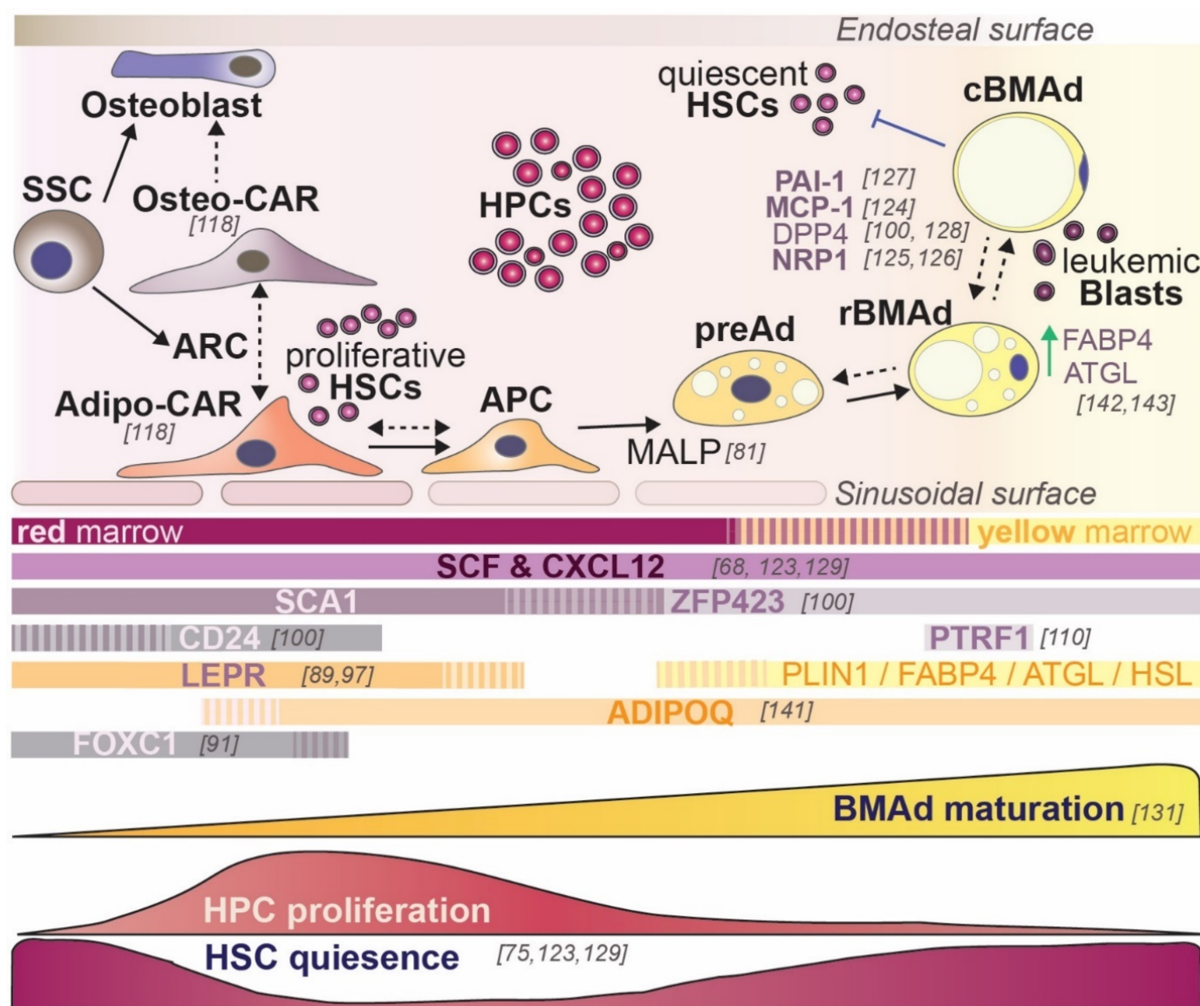

Figure 4| Combined in vitro and in vivo findings (from murine and partly human data) suggests the following working model for the relationship between hematopoeisis and adipogenesis within the bone marrow. *References are given for the main findings and non-bolded names indicates further research is required for placement on the BMAd differentiation axis.* Skeletal stem cells (SSCs) with multilineage capacity, reside at the apex of the bone marrow stromal cell (BMSC) organization hierarchy [101], [102]. SSCs give rise to the osteogenic lineage and to CXCL12-expressing adventitial reticular cells (CAR or ARCs), the precursors to the adipogenic lineage (adipogenic progenitor cells, APCs) within the BM. Adipo-CAR cells reside on the subendothelial luminal surface of BM sinusoids, forming a perivascular niche [118]. Transgenic mouse

models have shown that stem cell factor SCF and CXCL12 are expressed from the stage of perivascular stromal cells, regulating hematopoietic stem cell (HSC) proliferation and hematopoietic progenitor cell (HPC) expansion through their respective receptors, CD117 and CXCR4 [68], [89]. Adiponectin labels the BM adipogenic lingeage from the stage of AdipoCAR cells while leptin receptor (LepR) traces SSCs to Adipo-CAR cells [118], [123], [141]. Perilipin (*Plin1*) is expressed from the time of lipid droplet formation in BM preadipocytes (preAd) arising from APCs or marrow adipogenic precursors (MALPs), which also express LepR [81]. The strongest hematopoietic support in the form of HPC proliferation is seen in these perivascular stromal populations. Once $Sca1^+$ APCs advance to $Zfp423^+$ preAds, these cells can no longer revert to an earlier differentiation stage [81], [100].Upon further BM adipocyte (BMAd) maturation, the HPC supportive capacity is gradually lost while HSCs are preferrentially mantained in a quiescent state in the adipocyte rich marrow [75], [123], [129]. In a *Ptrf* knock-out model, the uni-locular constitutive BMAds (cBMAds) remain while mutli-locular regulated BMAds (rBMAds) are lost, thus representing a potential marker for distinguishing these BMAd subtypes [110]. Whether there is a reversible differentiation process between rBMAds and cBMAds sharing a common intermediate progenitor or whether there is heterogeneity between these two separate terminal maturation stages is unknown. Furthermore, while malignant leukemic HSCs remodel BMAds by upregulating FABP4 and ATGL, the precise interaction between leukemic HSCs and the BMAd differentiation axis also warrants further investigation [142], [143]. *Referred proteins and corresponding human/mouse Ensembl Gene Ids*: SCF (*KITLG/Kitl);* CXCL12 (*CXCL12/Cxcl12)*; CD117 (*Kit);* CXCR4 (*CXCR4/Cxcr4)* Sca1 (*Ly6a)*; Zfp423 *(Zfp423)*; CD24 (*Cd24a);* PTRF (*Ptrf);* LepR (*Lepr);* PLIN1(*Plin1);* FABP4 (*FABP4);* ATGL (*ATGL)*; HSL (*HSL);* Adiponectin (*Adipoq)*; Foxc1 (*Foxc1*); PAI-1(*SERPINE1);* MCP-1(*CCL2*) DPP4 (*Dpp4);* NRP1 (*NRP1*).

Interpretation of these studies and their apparently paradoxical results needs to be integrated within the context of the atypical early expression of some mature adipocyte markers such as adiponectin in BMSCs. In fact, it has been shown that non-lipidated BM stromal subpopulations express adiponectin, which is otherwise considered as a marker of mature, terminally differentiated adipocytes in peripheral tissues [81]. Expression of a Cre recombinase transgene under the control of the adiponectin promoter in *Adipoq*-Cre^+*; R26*$^{tdTomato/+}$ mice leads to reporter expression in the vast majority of CAR cells, with a pattern similar to that reported from LepR reporters from postnatal day 1 [97], [141]. Prior works have found only a minority of $LepR^+$ BMSC cells to excise Cre in *Adipoq*-Cre/ER*; R26*^{tdTomato} mice [123], possibly due to lower efficiency of recombination on the ER inducible model or to adipose-specific necrosis in tamoxifen treated animals [144]. Adiponectin expression within the BM thus cannot be equated to terminally differentiated adipocytes as in peripheral adipose tissues. Moreover, $Td\text{-}Tomato^+$ cells from induced *Adipoq*-Cre/ER*; R26*^{tdTomato} mice retain multilineage CFU-F forming capacity with very limited *in vivo* osteogenic capacity, indicating that Adiponectin is expressed in the BM prior to post-mitotic, irreversible commitment to BMAd [123]. The plasticity of this fate commitment is yet to be carefully elucidated. Moreover, the term “adipocyte” or BMAd should be used carefully and with precision in this context. Hemopathologists refer to BM “adipocytes” as lipidated mature adipocytes, which determine the denominator of hematopoietic cellularity measurements [74], while stem cell biologists often refer to BM “adipocytes” as stromal bone marrow cells committed to the adipocyte lineage, irrespective of their maturation state [81].

Further, genetic models with manifest BMAT depletion such as *Kit*$^{W/W\text{-}v}$ and *Kit*$^{Sl/Sl\text{-}d}$ are also non-selective. They have lesser metabolic phenotypes than lipodystrophic mice (eg. AZIP/F) but have an intrinsic hematopoietic defect [145] and thus should be interpreted with caution regarding the hematopoietic supportive function of BMSC and downstream adipocyte populations. In the abnormal hematopoietic microenvironment of *Sl/Sl-d* mutant mice expressing only the soluble form of SCF, HSCs were depleted indicating need for cell-cell contact or dose-dependent SCF availability perhaps via Notch-mediated signaling [146]–[148]. BMAT has been genetically ablated in kit-deficient mice. Specifically, loss of function mutations in kit receptor or kit ligand resulted in reduction of BMAd and precursors in long

bones of *Kit*$^{W/W-v}$ and *Kit*$^{Sl/Sl-d}$ mice respectively and a modified lipid composition of the stroma [149], [150]. Very interestingly, this model uncouples the increase of BMAd from defects in bone formation [145]. Tissue specific targeting of osteogenic precursors (doxycycline-*inducible mT/mG;osx-cre;β-cat*$^{f/f}$ KO mice) has demonstrated the relevance of the endogenous Wnt/β-catenin axis in the inhibition of BMAd fate from BM multipotent stromal progenitors [151] probably through Wnt10b availability [152]. Current genetic models of BMAd depletion and other models of bone marrow adiposity (BMA) are discussed in [153].

In conclusion, the study of BMAd and hematopoiesis is limited by the absence of specific models for mature BMAd deletion, both because of the strong systemic metabolic phenotype imposed by generalized lipodystrophy and because of the early expression of adipocyte markers, especially adiponectin, in BMSC populations (e.g. CAR cells, see Figure 4). The validation of a step-wise BMAd commitment trajectory that is specific to the BMAd lineage is therefore urgently needed to generate the genetic tools necessary to study the reciprocal relationship between hematopoietic proliferation and the adipocytic differentiation axis in the main hematopoietic organ of the adult. The increase in bone formation upon hematopoietic ablation in lipodystrophic mice and *Kit*$^{W/W-v}$ mice already points to the complexity of this relationship and the three-way reciprocal cross-talk between the bone, fat and hematopoietic compartments within the marrow. The complexity of this interaction emphasizes the importance of systematically quantifying all three compartments, and defining BMAd maturation state beyond simple adipocyte lineage commitment, so as to understand coregulation in mechanistic studies.

Malignant hematopoiesis

Acute myeloid leukemia (AML) is characterized by the generation of dysfunctional leukemic blasts that gradually substitute the hematopoietic stem and progenitor cell populations by imposing a maturation block and impaired myelo-erythropoeisis together with a survival advantage of the malignant clone. It has been recently shown that the neoplastic AML blasts inhibit the proliferation of normal human CD34^{+} HPCs and prevent BMAd differentiation. Specifically, forced BMAd differentiation with PPARγ agonist GW1929 rescued myelo-erythropoeisis *in vitro* and *in vivo* and reduced the colony forming capacity of leukemic cells *in vitro* [154], [155]. Specifically, neoplastic cells in AML activate lipolysis via increased phosphorylation of hormone sensitive lipase (HSL) in BMAd and fatty acid binging protein 4 (FABP4)-mediated transfer of free fatty acids (FFAs) to AML blasts, impairing the BMAd niche by blast propagation [116], [142]. Notably, BMAd remodeling did not happen on the tail, described as the first and most stable site of cBMAT upon skeletal development [75], [154]. Blocking lipid transfer through inhibition of FABP4 increased survival of leukemic mice [142]. The FABP4/IL-1alpha axis has also been implicated in direct FFA transfer from BMAd to prostate cancer cells in the context of metastatic bone disease [156], [157]. Congruently, another study found that smaller BMAds have been associated with a worse prognosis in AML, both in terms of refractory disease to first induction chemotherapy and of reduced overall survival [143]. *In vitro* exposure of AML lines (K562, HL-60, THP-1) or primary human AML blasts to conditioned media from small BMAds supported AML leukemic cell proliferation [143]. This phenomenon was interpreted in the context of ATGL-mediated lipolysis contributing to FFAs and fatty acid β-oxidation (FAO), similarly to a murine model of acute monocytic leukemia [158]. A follow-up study has shown that GDF15 produced by AML blasts is responsible for this adipocyte remodeling, likely via transcriptional inhibition of Foxc1, which can be prevented by treatment with TRPV4 activator 4a-phorbol 12,13-didecanoate [153]. This both restored fully lipidated BMAds *in vivo* and slowed tumor growth. A more recent study further reported that AML-BMSCs have increased adipogenic potential and improved the survival of leukemia progenitor cells. Targeting SOX9 in these cells decreased their differentiation capacity and their ability to support AML progenitor cells [160]. Indeed, BM microenvironments of adipocyte-rich (cMAT)

versus adipocyte-poor (rMAT) were shown to imprint niche-specific features to leukemic cells associated with modified survival, metabolism, and cell-cycle progression related to chemo-resistance in the context of chronic myeloid leukemia and acute lymphoblastic leukemia (ALL), at least in part due to protection from oxidative stress [161]–[163]. Other hematological malignancies are also associated with BMAT. Multiple myeloma (MM), characterized by clonal proliferation of transformed antibody-producing plasma cells, resides in close contact with BMAds [164]. While adiponectin has been shown to inhibit MM, mature BMAds have been shown to support tumor growth and even protect MM cells from chemotherapy-induced apoptosis [165]–[170]. Interestingly, a characterization study on primary human BMAds from proximal femoral metaphysis, a site where cBMAT is predominant, has found that BMAds can have defective lipolytic function and thus orient towards a cholesterol-based metabolism [171]. This observation further highlights how AML blasts might influence and shift the metabolic activity of BMAds to provide a more supportive malignant microenvironment (simplified model in Figure 4), namely by directly affecting FFA transfer and FAO. Whether or not the BMAd niche serves as a similar sanctuary for non-malignant HSCs needs further investigation.

BMAds have been shown to play an active role in supporting neoplastic cells in the BM niche and could be considered as potential therapeutic targets. Genetic alterations (eg. *Dicer1, Sbds*) in the stromal HSC microenvironment have been demonstrated to have the potential to drive myeloproliferative and myelodysplastic syndromes (MDS), secondary leukemia, and AML in murine models [172]–[174], highlighting the importance of the HSC niche contribution to the pathogenesis of hematological malignancies. BMSCs derived from ALL patients were shown to have altered BMP4 production and increased adipogenic capacity [175], while BMSCs from MDS patients were shown to have a reduced adipogenic signature [176].

Overall, these findings highlight the capacity of malignant hematopoietic cells to remodel mature BMAds, actively increasing lipolysis to selectively favor tumor growth and suppress myelo-erythroid maturation. Data on the polarization of earlier BMSC progenitors towards or away from the adipocyte lineage is less abundant. Conflicting results may indicate either disease-specificity or limited understanding of the hematopoietic support function associated to the individual steps of differentiation along the BMAd differentiation axis.

In conclusion, BMAds are active participants of the hematopoietic microenvironment. BMAds exert regulatory functions on the hematopoietic process through the secretion of specific factors, such as SCF, CXCL12, PAI-1, MCP-1, DPP4 and NRP1 influencing the differentiation of derived HSCs and downstream hematopoietic progenitors [67], [89], [100], [123], [124], [126], [127], apart from providing spatial support. Based on seemingly contradictory reports, BMAds almost certainly exhibit functional heterogeneity that is highly dependent on niche localization (endosteal/sinusoidal/perivascular), differentiation stage (adipocyte progenitor/preadipocyte/mature adipocyte) and context (homeostasis versus stress hematopoiesis). Unraveling the complex relationship between BMAds and hematopoiesis should account for and aim to dissect this heterogeneity to provide a better understanding of the BM microenvironment and hematological disease

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