

# Osteogenic niche in the regulation of normal hematopoiesis and leukemogenesis

Phuong M. Le,<sup>1</sup> Michael Andreeff<sup>2</sup> and Venkata Lokesh Battula<sup>2,3</sup>

<sup>1</sup>Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; <sup>2</sup>Section of Molecular Hematology and Therapy, Leukemia Department, The University of Texas MD Anderson Cancer Center, Houston, TX and <sup>3</sup>Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA



## ABSTRACT

The bone marrow microenvironment, also known as the bone marrow niche, is a complex network of cell types and acellular factors that supports normal hematopoiesis. For many years, leukemia was believed to be caused by a series of genetic hits to hematopoietic stem and progenitor cells, which transform them to preleukemic, and eventually to leukemic, cells. Recent discoveries suggest that genetic alterations in bone marrow niche cells, particularly in osteogenic cells, may also cause myeloid leukemia in mouse models. The osteogenic niche, which consists of osteoprogenitors, preosteoblasts, mature osteoblasts, osteocytes and osteoclasts, has been shown to play a critical role in the maintenance and expansion of hematopoietic stem and progenitor cells as well as in their oncogenic transformation into leukemia stem/initiating cells. We have recently shown that acute myeloid leukemia cells induce osteogenic differentiation in mesenchymal stromal cells to gain a growth advantage. In this review, we discuss the role of the osteogenic niche in the maintenance of hematopoietic stem and progenitor cells, as well as in their transformation into leukemia cells. We also discuss the signaling pathways that regulate osteogenic niche-hematopoietic stem and progenitor cells or osteogenic niche-leukemic stem/initiating cell interactions in the bone marrow, together with novel approaches for therapeutically targeting these interactions.

## Introduction

Hematopoietic stem cells (HSCs) home to specific microenvironments in the bone marrow (BM) and receive signals that drive their fate under both normal and pathological conditions. So far, two predominant niches that differentially regulate HSCs through their non-hematopoietic compartments and levels of hypoxia have been identified.<sup>1,2</sup> The endosteal niche near the inner bone surface is populated by osteoblastic lineage cells, including osteoprogenitor cells, pre-osteoblasts, mature osteoblasts, and osteocytes, as well as mesenchymal stromal cells (MSCs) and osteoclasts, whereas the non-endosteal niche consists mainly of sinusoidal endothelial cells, pericytes, and non-myelinating Schwann cells. Both niches are highly vascularized yet associated with distinct subtypes of blood vessels that support either the bone-forming or sinusoidal domain.<sup>3</sup> Recent work from the Adams group also revealed a strong association between the osteogenic niche and a third vessel type that made up the transition zone in the developing bone. This subset seems to function upstream of both endosteal and sinusoidal endothelium, though more functionally related to the former, and connect the two vasculatures during the early stages of specialization.<sup>4</sup> Stromal cells in both niches share overlapping signatures; however, it has been suggested that endosteal MSCs support HSC quiescence whereas non-endosteal MSCs promote HSC proliferation.<sup>5</sup>

Acute myeloid leukemia (AML) is one of the most aggressive hematologic malignancies, characterized by increased numbers of myeloid precursors in the BM that fail to differentiate into more mature myeloid cells. Recent studies have

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## Correspondence:

vbattula@mdanderson.org

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highlighted complex tumor-host interactions within the BM during AML progression. Malignant cells compete with their normal counterparts for niche resources and occupancy, and disrupt normal hematopoiesis by inflicting a differentiation block, which often manifests itself as BM failure and pancytopenia.<sup>6,7</sup> In these conditions, leukemic cells seem to lose sensitivity to antiproliferative cues from the niche.<sup>8</sup> Under the expansion of leukemia, MSCs have shown signs of “reprogramming”.<sup>9-11</sup> In particular, the role of the osteoblast-rich region of the BM has been implicated in both AML chemoresistance and relapse.<sup>12,13</sup> Unraveling the mechanisms underlying osteogenic niche-mediated support to AML cells is key to identifying molecular targets in order to develop effective drug therapies. In this review, we focus on advances in our understanding of the osteogenic niche in the leukemic BM microenvironment and discuss the key components of this niche as therapeutic candidates in AML.

### Osteolineage cells regulate normal hematopoiesis

Non-random distribution of HSCs in the BM highlights the role of osteolineage cells in HSC maintenance. The physical association of HSCs with the endosteum correlates strongly with the colony formation and proliferative capacity of HSCs, and is primarily evident after BM transplantation.<sup>14,15</sup> Anatomical evidence has provided the basis on which the functional relationships between osteolineage cells and HSCs have continued to be unraveled. Osteoblasts secrete cytokines and growth factors including granulocyte-colony stimulating factor (G-CSF),<sup>16</sup> hepatocyte growth factor,<sup>17</sup> and osteopontin (OPN),<sup>18</sup> which have been shown to maintain the pool size of the CD34<sup>+</sup> progenitor population in the BM. Osteoblasts mediate HSC migration in and out of the BM, primarily through the CXCL12/CXCR4<sup>19</sup> and VCAM-1/VLA-4<sup>20</sup> axes, and under the influence of the sympathetic nervous system.<sup>21</sup> In a knockout mouse model lacking bone morphogenetic protein (BMP) receptor I, Zhang *et al.*<sup>22</sup> reported that an increase in HSC number was associated exclusively with a cell population that lined the long bone and had an osteoblastic phenotype. Similarly, Calvi *et al.*<sup>23</sup> demonstrated that increasing osteoprogenitor or pre-osteoblast activation by augmenting parathyroid hormone (PTH) signaling enriched Lin<sup>-</sup> Sca-1<sup>+</sup> c-Kit<sup>+</sup>, or HSC-like, cells *in vivo*. Interestingly, this HSC expansion occurred without substantially affecting the overall number of hematopoietic cells. These observations suggest that PTH-induced signaling in osteoprogenitor cells or pre-osteoblasts might play a selective role in maintaining HSC self-renewal but not in the proliferation of their committed progenitors. How osteoblasts regulate HSC quiescence has been rigorously investigated. Loss of ligand-receptor interactions, such as angiopoietin-1 receptor tyrosine kinase 2 (Ang-1/Tie2)<sup>24</sup> and thrombopoietin-MPL (TPO/MPL),<sup>25</sup> deregulates not only cell-cycle checkpoints but also coping mechanisms against extrinsic stressors, resulting in a reduction in slow-cycling hematopoietic cells. Stem-cell exhaustion and reduced self-renewal capacity after inhibition of Wntless (Wnt) signaling in osteoblasts further suggest that the mechanism underlying osteoblast-mediated regulation of HSCs does not follow a single axis.<sup>26</sup>

Surprisingly, osteoblast ablation, although associated with poorer HSC engraftment *in vivo*, does not lead to a massive loss of quiescent HSCs.<sup>27</sup> It has also been shown

that osteoblast deficiency in chronic inflammatory conditions, such as rheumatoid arthritis, does not affect the frequency of Lin<sup>-</sup> Sca-1<sup>+</sup> c-Kit<sup>+</sup> cells or their long-term repopulating potential.<sup>28</sup> Mice with conditional deletion of CXCL12<sup>29</sup> or stem cell factor (SCF)<sup>30</sup> in osteoblasts do not exhibit HSC defects. It is possible that osteoblastic regulation of HSCs overlaps with other regulatory pathways and hence is easily compensated. Different osteolineage members may also share common signals while differing in the degree of impact.<sup>31</sup> Together, these data suggest that osteolineage cells or more primitive cells such as MSCs orchestrate a diverse, though possibly non-essential, network of signals to maintain the stemness of HSCs and prompt hematopoietic activities, such as mobilization and expansion, in response to physiological needs.

### Altered osteogenic niche leads to myeloid leukemia in BM

It has been firmly demonstrated that mutations affecting the ability of HSCs to differentiate into mature hematopoietic cells transform HSCs into pre-leukemic cells, and ultimately to leukemic cells when additional mutations are acquired (Figure 1).<sup>32-34</sup> However, very little is known about the influence of other cellular components in the BM microenvironment on leukemic transformation of hematopoietic cells.

The Scadden group was the first to show that genetic alterations in osteolineage cells could lead to myelodysplastic syndromes (MDS) and leukemia. Deletion of *Dicer1*, a critical RNA processor and microRNA synthesizer, in *Osterix* (*Osx*)-expressing osteoprogenitor cells in a conditional knockout mouse model caused MDS and, on occasions, secondary AML.<sup>35</sup> These mice first developed severe cytopenia and myelodysplasia, which transformed into monoblastic AML in 4 out of 200 cases, presenting as invasive myeloid sarcomas, anemia, and monocyte-like blast expansion in the peripheral blood, spleen, and BM. Of interest, *Dicer1* was intact in the myeloblastic tumors, suggesting that dysfunctional osteoblast precursors could mediate clonal evolution in neoplastic formation. Similarly, constitutive activation of  $\beta$ -catenin in mouse osteoblasts resulted in a broad spectrum of dysfunctional hematopoiesis, including monocytosis, lymphocytopenia, and somatic mutations that resembled those of human AML in myeloid progenitors. Kode *et al.*<sup>36</sup> noted that both wild-type mice engrafted with long-term (LT) HSCs from  $\beta$ -catenin-mutant mice and  $\beta$ -catenin-mutant mice engrafted with healthy BM cells developed AML and died shortly after transplantation. These observations suggest that an altered osteogenic niche could induce permanent damage to LT-HSCs and transform them to pre-leukemic and/or leukemic cells. Kousteni *et al.* attributed this niche-induced carcinogenesis to the oncogenic role of FoxO members involved in bone formation, which, surprisingly, are known tumor suppressors.<sup>37,38</sup> This discovery sparks a debate about whether osteoblasts differentially regulate normal and malignant hematopoiesis. Recently, Dong *et al.*<sup>39</sup> also reported that mice with a mutant allele of protein tyrosine phosphatase SHP2 (*Ptpn11*) in osteoprogenitors or Nestin<sup>+</sup> MSCs could develop juvenile myelomonocytic leukemia-like myeloproliferative neoplasms (MPN). With concomitant mutations in HSCs, mice with mutated MSCs were twice as likely to progress from MPN to acute leukemia as were mice with altered endothelial cells. This study under-

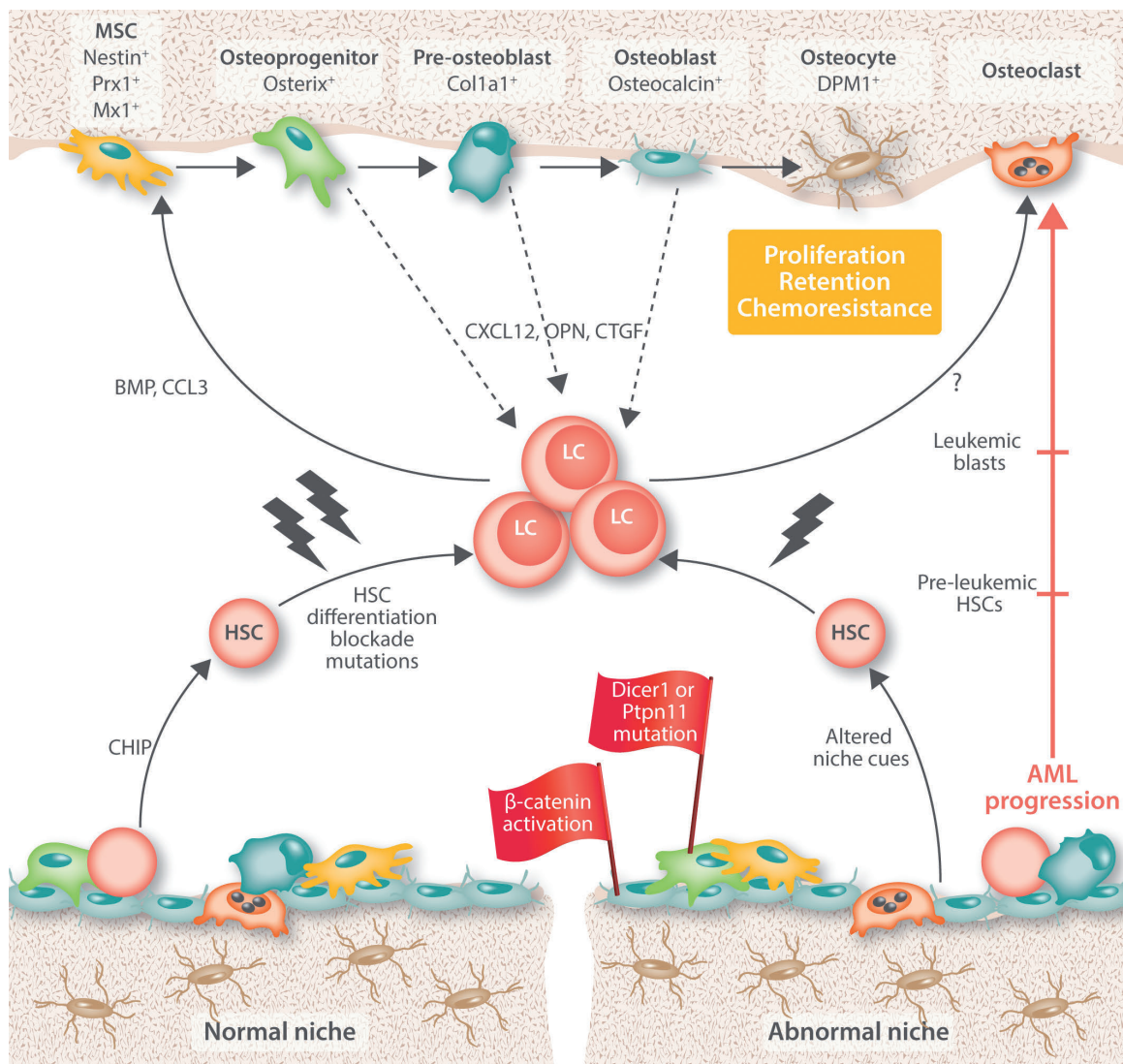
scores cell-type-specific leukemogenic effects of various niche components. While these findings in mice offer direct evidence for osteoblast-induced leukemogenesis, emerging reports of donor cell leukemia in humans (1-5% of all post-transplant leukemia relapses), also suggest the role of an oncogenic microenvironment driving secondary malignancy.<sup>40</sup> Collectively, it has been increasingly recognized that genetic aberrations in the endosteal compartment could be a key event in AML initiation and progression (Figure 1).

**AML induces osteogenic and osteolytic activity**

Numerous AML studies have emphasized the toxicity of leukemic expansion to BM niches. AML cells have been shown to alter BM niches by competing with HSCs

for niche support, thereby affecting normal hematopoiesis.<sup>7,41,42</sup> Whether the genomic landscape of non-hematopoietic components of BM niches changes has remained largely unexplored, and whether these alterations may drive AML initiation, progression, and resistance to chemotherapy is questionable.

Due to inconsistencies in methodology, cytogenetic analyses from different labs have led to a debate about the existence of chromosomal aberrations in leukemia patient BM-derived MSCs.<sup>10,43-45</sup> To explore global changes induced by AML in stromal cells, our group performed a large-scale comparison of proteomic, microRNA, and gene expression profiles between AML patient-derived (AML-MSCs) and healthy donor-derived BM MSCs. We found upregulation of multiple pro-proliferative and anti-



**Figure 1. Osteogenic niche in hematopoietic stem cell (HSC) maintenance and leukemogenesis.** Interactions between HSCs and osteogenic niche cells could happen in two ways. First, HSCs stay quiescent and self-renew when they are in osteogenic niche. When they acquire mutations under physiological stress, HSCs become pre-leukemic and eventually transform into leukemia blast cells. Alternatively, genetic abnormalities in osteogenic cells in the bone marrow could induce myeloid leukemia in non-mutated or in pre-leukemic HSCs. Second, leukemic cells could induce osteogenic differentiation in mesenchymal stromal cells (MSCs), which normally go through a series of differentiation steps to become fully mature osteoblasts or osteocytes. This feedback loop, involving bone remodeling, probably fuels leukemia progression. However, the extent to which acute myeloid leukemia (AML) cells induce osteogenic differentiation is not clear. BMP: bone morphogenetic protein; CHIP: clonal hematopoiesis of indeterminate potential; CTGF: connective tissue growth factor; HSC: hematopoietic stem cell; LC: AML cell; OPN: osteopontin.

apoptotic pathways and downregulation of RNA regulators previously implicated in survival and differentiation of leukemic cells.<sup>46</sup> Of particular interest, marked under-expression was observed for IGFBP5, an insulin-like growth factor binding protein that primarily inhibits osteoblast differentiation of MSCs.<sup>47</sup> Moreover, TP53 was increased in AML-derived MSCs, resulting in senescence. We also found that the leukemia genotype, in particular the presence of FLT3-ITD mutations and lack of p53, induce both shared and leukemia genome-specific alterations in MSCs.<sup>48</sup> These reports suggest that AML cells alter stromal development, and potentially their functionality.

Previously, Hanoun *et al.* had reported that AML primed MSCs to commit to osteoblastic lineage.<sup>49</sup> The endosteal surface of mice transplanted with MLL-AFL9 leukemic cells was packed with *Osx*-expressing osteoprogenitor cells yet lacking *Osteocalcin*-positive (*Osc*<sup>+</sup>) mature osteoblasts. It is important to note that despite this osteogenic potential, these mice showed deficient bone mineralization and a lack of terminally differentiated osteoblasts compared with healthy controls. These observations were confirmed independently both *in vitro* and *in vivo* by our group.<sup>50</sup> AML-MSCs displayed significantly higher alkaline phosphatase (ALP) expression and activity than did healthy donor-derived MSCs. In addition, when cultured in osteogenic differentiation medium, AML-MSCs differentiated to mature osteoblasts (alizarin red-positive) within two weeks compared with the three weeks needed for normal MSCs. Remarkably, gene expression analysis of normal MSCs co-cultured with different leukemic cell lines for five days revealed 2- to 10-fold upregulation of osteogenic markers, such as runt-related transcriptional factor (*Runx2*), *Osx*, *Opn*, and tissue non-specific ALP (*Tnap*), suggesting that this osteoprogenitor-priming pattern in MSCs resulted from AML exposure.<sup>50</sup> To validate AML-induced osteoblast differentiation *in vivo*, we created a human BM implant mouse model and assessed osteogenic potential of BM MSCs after four weeks of leukemia engraftment. Human MSCs obtained from these transplanted mice showed a 5- to 7-fold increase in *Osx* and *Runx2* expression compared with control mice.<sup>50</sup> These experimental data were consistent with *OSX* and *RUNX2* upregulation in BM biopsies of AML patients.

We also found that AML-MSCs became less multipotent since they differentiated poorly into adipocytes and chondrocytes, two mesodermal lineages that usually arise from MSCs; the Bhatia group confirmed this adipocyte suppression in the setting of AML by immunostaining within human BM and global transcriptome analysis of AML-MSCs.<sup>51</sup> Note that in the same study, gene sets poised towards osteoblast, but not adipocyte lineage were enriched in AML-MSCs, underscoring the need to understand the role of distinct mesenchymal fractions in AML progression.

We asked whether this osteolineage-specific priming provided any advantage for leukemic growth. Indeed, AML cells up-regulated connective tissue growth factor (CTGF) in MSCs and activated BMP signaling *via* Smad1/5 phosphorylation, both of which have been associated with persistence of tumors and poor prognosis in patients with acute leukemia.<sup>52-54</sup> Besides, AML-induced TNAP overexpression in MSCs was implicated in osteoblast-mediated protection of leukemia blasts against

apoptosis.<sup>55</sup> By unraveling a feedback loop between stroma functionality and AML expansion, our study has highlighted the dynamics of the endosteal niche in AML pathogenesis (Figure 2).

The reduced bone mineralization seen by Hanoun *et al.*<sup>49</sup> could have resulted from altered osteolytic activity.<sup>56</sup> A short-lived increase in osteoclasts and upregulation of CCL3, a pro-inflammatory cytokine with pro-osteoclastic action previously established in multiple myeloma,<sup>57</sup> was found in a murine model of blast-crisis chronic myeloid leukemia (CML) phenotype.<sup>56</sup> These acute leukemia-like mice showed a significant reduction in *Osc*<sup>+</sup> osteoblasts and thinning of bone structures that could not be reversed completely by inhibition of osteoclasts. Bone deposition and resorption are tightly coupled processes that maintain bone homeostasis; however, this evidence suggests that the leukemic condition distorts this balance. Of interest, excessive CCL3 production does not typically lead to osteolytic lesions or bone loss,<sup>58</sup> as seen in *Ptpn11*-mutated leukemic mice,<sup>39</sup> although overexpression of this protein is common in the BM of AML patients.<sup>56</sup> It is possible that monocyte differentiation into osteoclasts is defective in AML, yet the effects are masked by strong CCL3-driven recruitment of monocytes into the osteogenic niche.<sup>59</sup> The extent to which osteoblasts and osteoclasts work in tandem to reconstruct an inhospitable microenvironment under AML burden needs further investigation.

#### Osteoprogenitors or mature osteoblasts: true 'partners-in-crime' in AML progression?

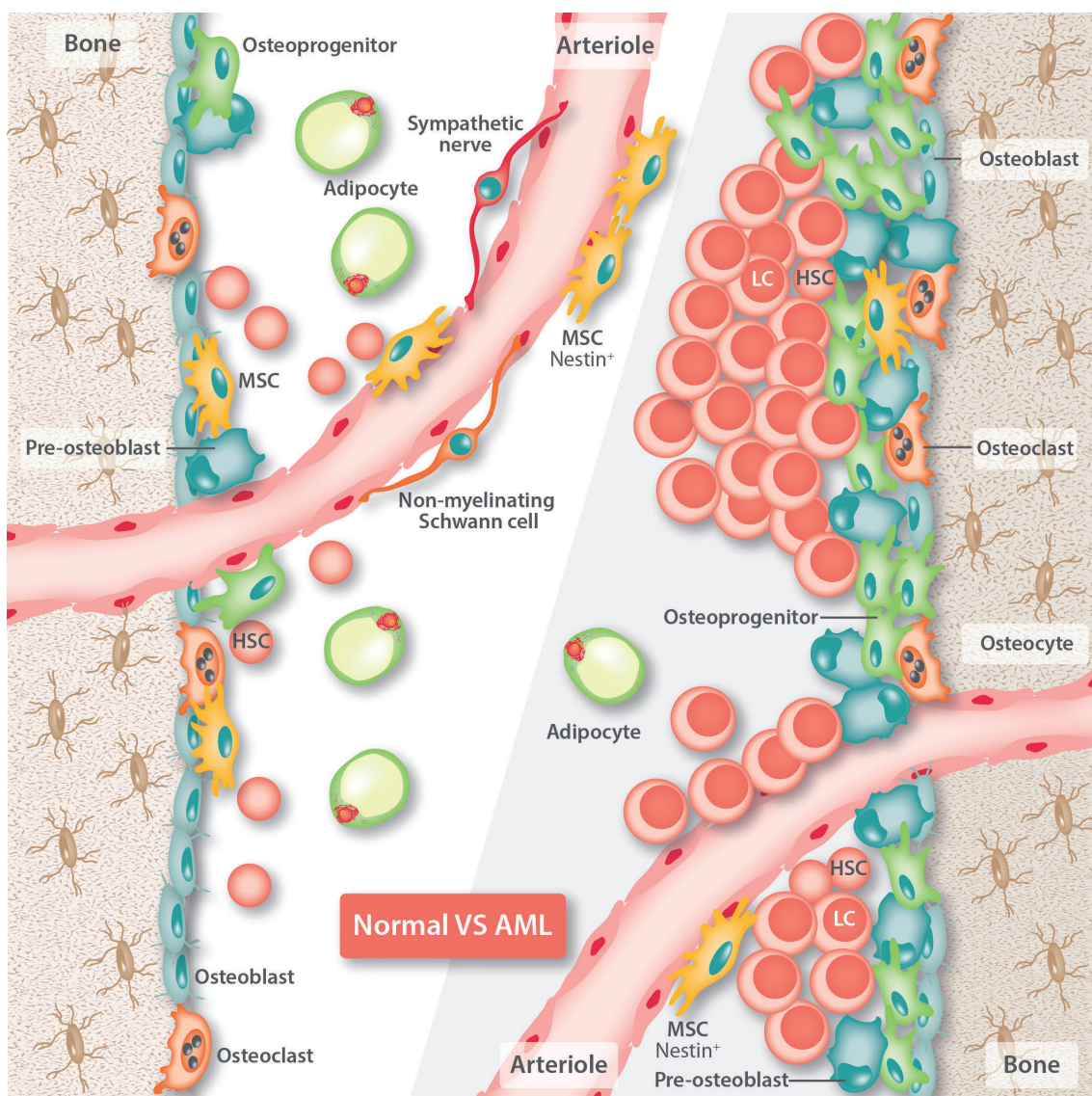
The osteogenic niche comprises a variety of cell types which differ in their maturation status, ranging from very immature multipotent MSCs to mature osteoblasts and osteocytes (Figure 1). However, the differentiation status of osteogenic cells supporting normal hematopoiesis or leukemogenesis is an emerging question that remains to be resolved. Several studies have shown that, compared with less mature osteoblasts, terminally differentiated osteoblasts regulate HSC lineage commitment, such as B lymphopoiesis and erythropoiesis, while having less effect on HSC proliferation.<sup>60-62</sup> Whether this functional stratification applies to the context of malignancy is poorly understood. Accumulating evidence has demonstrated that defective osteolineage cells are potent initiators of leukemia in the BM. These findings led to the question: which osteolineage cells, osteoprogenitors or mature osteoblasts, play a major role in promoting leukemogenesis?

As previously discussed, Raaijmakers *et al.*<sup>35</sup> were the first to show that hematopoiesis could go awry as a result of a genetic alteration in osteoprogenitors. The Scadden group emphasized the differential leukemogenic capacity of immature and mature osteoblasts by comparing AML phenotype in *Dicer1*<sup>fl/fl</sup> mice with *Osx*- versus *Osc*-driven Cre recombinase. Mice with a *Dicer1* defect in mature osteoblasts did not exhibit any hematologic problem besides bone-related deformities. Similarly, Dong *et al.*<sup>39</sup> confirmed the distinct role of stage-specific osteoblasts in leukemic development by generating mice with *Ptpn11* mutations at various stages of MSCs: mesenchymal progenitor/stem cells, differentiated MSCs, *Osx*<sup>+</sup> osteoprogenitors, and *Osc*<sup>+</sup> mature osteoblasts. Of interest, the leukemogenic effect of this abnormality was observed in mice bearing the mutated form of either primitive MSCs

or bone progenitor cells, but not more differentiated osteoblasts. These data are consistent with our observations that AML-MSCs show characteristics of osteoprogenitors but not of mature osteoblasts. AML-MSCs express early-stage osteoblast markers, including *osterix*, *RUNX2*, and *Col1a1*, but not mature osteoblast markers such as osteocalcin.<sup>50</sup> In addition, functional assays revealed that AML-MSCs stained positive for ALP enzyme activity but were negative for alizarin red S staining.<sup>50</sup> These observations suggest that AML-MSCs can differentiate into committed osteoprogenitors, but not mature osteoblasts. These data were also validated by co-culture of AML cell lines with normal BM-MSCs *in vitro* and by different AML mouse models.<sup>50,63</sup> These findings

also do not contradict the observations of Frisch *et al.*,<sup>56</sup> Geyh *et al.*<sup>64</sup> and Krevvata *et al.*<sup>65</sup> since the osteoblasts inhibited in these studies were marked by osteocalcin. Using intravital microscopy, Duarte *et al.* also showed a significant depletion of Col2.3 promoter-expressing mature osteoblasts in areas with a high level of AML infiltration.<sup>66</sup> Collectively, stalling the maturation of osteoblast precursors appears to be a key step in AML initiation and progression (Figure 2).

This differentiation blockade could be mediated by different AML-derived factors. Kumar *et al.*<sup>63</sup> reported upregulation of DKK1, a negative regulator of osteogenesis, when co-culturing AML-derived exosomes with BM MSCs. Of particular interest, a short-term dose of DKK1

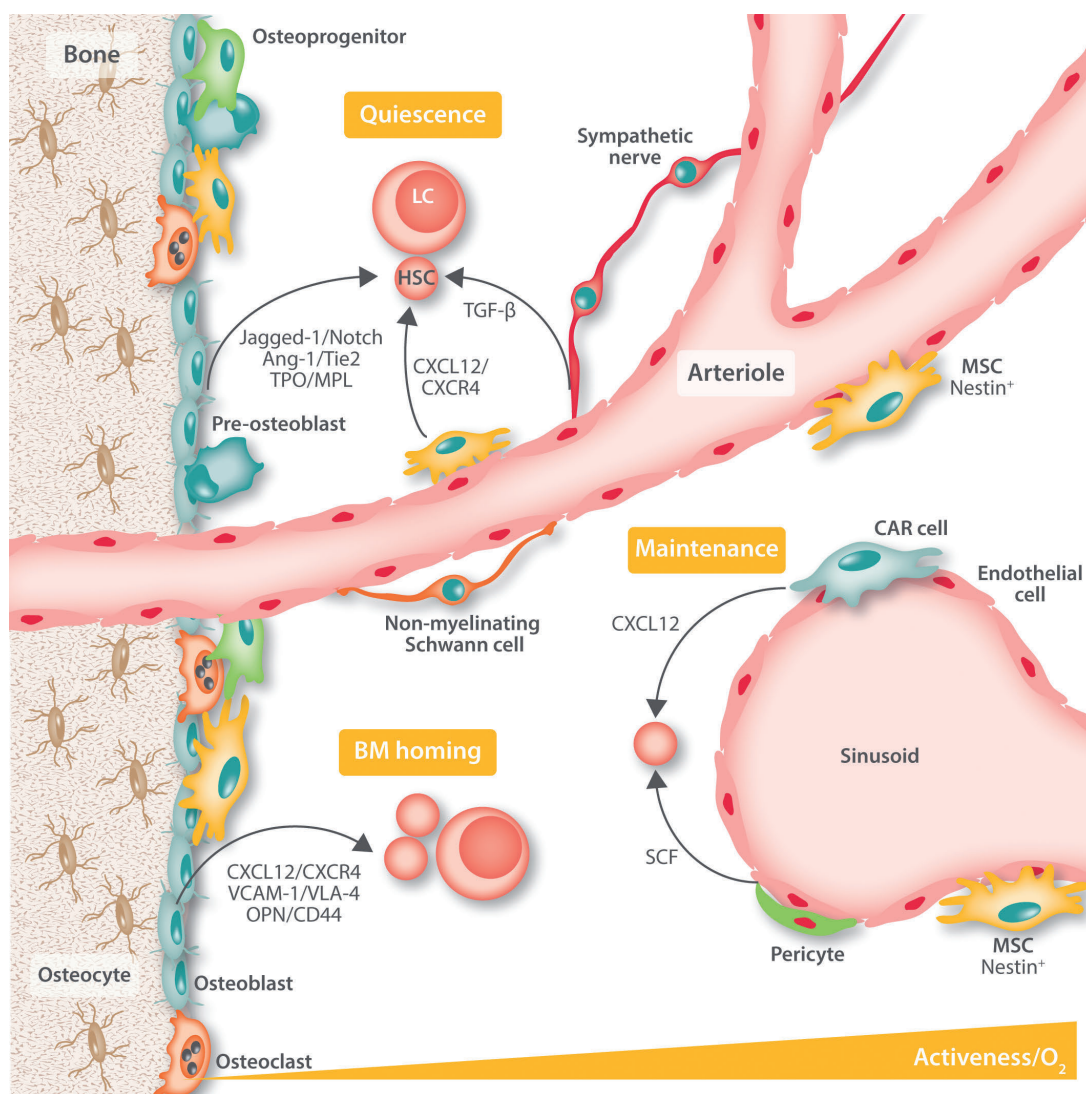


**Figure 2. Schematic representation of normal versus acute myeloid leukemia (AML)-bone marrow (BM) microenvironment.** Normal BM consists of osteoprogenitor cells, pre-osteoblasts, mature osteoblasts, and osteocytes, mesenchymal stromal cells (MSCs) and osteoclasts at endosteal niche and endothelial cells, pericytes, and non-myelinating Schwann cell at non-endosteal niche. In addition to these cell types, adipocytes are present throughout the BM cavity. Hematopoietic stem cells (HSC) are present in both niche areas and gain support from stromal cells to stay quiescent and self-renew, whereas in AML BM, leukemic blasts displace HSCs from the protective niche area and occupy this sanctuary, thereby affecting normal hematopoiesis. In addition, AML cells create or expand the existing niche by inducing osteogenic but inhibiting adipogenic differentiation in MSCs. However, there are no reports suggesting higher bone volume in AML patients. Therefore, it is possible that induction of osteogenic differentiation is halted at the osteo-progenitor or pre-osteoblastic stage.

inhibitor promoted terminal differentiation of these osteolineage-primed MSCs *in vitro* and improved survival of mice engrafted with AML. This suggests a tight coupling of AML development with impaired maturation of osteoprogenitors. The fact that disruption of miRNA processing in immature, but not mature, osteoblasts could trigger AML development<sup>65</sup> implies that deregulation of the maturation process at the post-transcriptional level might play a role in its failure. It would be interesting to further investigate the function of the miR-29 family, whose members are commonly down-regulated in AML blasts<sup>67</sup> while engaging Wnt signaling antagonists, such as DKK1, in a feedback loop to promote osteolineage development.<sup>68</sup> Another potential mediator is IL-1 $\beta$ , a pleiotropic cytokine produced abundantly by AML blasts<sup>69</sup> that has been shown

to suppress osteogenesis of MSCs in periodontal tissue at a high physiological level.<sup>70</sup> Whether one or more pathways are involved in causing this lack of osteoblast maturation remains to be elucidated.

Intriguingly, the effect of maturing osteoblasts on leukemia progression may be context-dependent and disease-specific. Schepers *et al.*<sup>71</sup> showed that development of CML-like MPN induced by the BCR/ABL oncogene led to the expansion of a mixture of immature and mature osteoblasts that formed BM fibrosis and had decreased capacity to support HSCs. Krevvata *et al.*<sup>65</sup> reported a strong correlation between the decrease in mature osteoblasts in mice and aggravated engraftment of different acute leukemia cell lines. In this study, over-stimulating *Osc*<sup>+</sup> osteoblast production by inhibiting gut-derived



**Figure 3.** Key signaling pathways in the osteogenic niche that regulate the fate of hematopoietic stem cells (HSC) and leukemia stem/initiating cells (LSCs). HSCs home to bone marrow (BM) and receive maintenance signals from both endosteal and non-endosteal niches. Various cell types in the perisinusoid region, such as CXCL12-abundant reticular (CAR) cells and endothelial cells, maintain less quiescent HSCs via CXCL12 and stem cell factor (SCF). Non-myelinating Schwann cells and osteolineage cells, including mesenchymal stromal cells (MSCs), osteoprogenitors, and premature and mature osteoblasts, play a major role in retaining slow-cycling HSCs near the bone surface. LSCs exploit the same cues from the osteogenic niche to hibernate and evade chemotherapy. In acute myeloid leukemia (AML), the BM niches are relatively hypoxic, whereas, in normal BM the hypoxic regions are more restricted to HSC-residing areas. Ang-1: angiopoietin 1; CXCL12: C-X-C motif chemokine 12; CXCR4: C-X-C chemokine receptor type 4; LSC: leukemic stem/initiating cell; OPN: osteopontin; SCF: stem cell factor; TGF- $\beta$ : transforming growth factor  $\beta$ ; TPO: thrombopoietin; VCAM-1: vascular cell adhesion protein 1; VLA-4: very late antigen-4.

serotonin synthesis in mice bearing MLL-AF9-induced AML attenuated disease burden.<sup>65</sup> Conversely, using the same inducible model, Krause *et al.*<sup>8</sup> showed that increasing osteoblastic activity through PTH activation augments leukemic expansion in AML while inhibiting CML-like MPN.<sup>9</sup> These contrasting data are, at least in part, due to the heterogeneity of osteolineage cells that are characterized differently across studies. However, these results may also reflect the intrinsic differences in osteoblast-leukemia interaction between acute and chronic myeloid malignancies.

### Deregulated signaling network in the osteogenic niche offers promising therapeutic targets

In the past decade, huge strides forward have been made in AML induction therapy by combining chemo- and targeted therapies, yet this approach has offered limited success in preventing disease recurrence. It is assumed that the osteogenic niche shields slow-cycling AML cells from cell cycle-dependent treatment just as it maintains HSC quiescence (Figure 3); however, that is just

the tip of the iceberg. The larger implication is that AML induces osteogenic dysfunction and disrupts the signaling network associated with the osteogenic niche (Table 1). This transformation of the niche could in turn fuel leukemia persistence and resistance to therapy. It is, therefore, critical to identify and target less visible threats underlying AML-osteogenic niche interactions to achieve more profound treatment efficacy.

Current approaches revolve mainly around disrupting BM homing axes, notably CXCL12/CXCR4. CXCR4 overexpression is common both at diagnosis<sup>72</sup> and after chemotherapy,<sup>73</sup> and correlates with poor prognosis in AML patients.<sup>74,75</sup> CXCR4 inhibition prevents AML anchorage and promotes mobilization of leukemic stem/initiating cells (LSCs) out of the endosteal niche, thereby increasing their vulnerability to chemotherapy. Pre-clinical and clinical studies of CXCR4 antagonists have shown encouraging results, demonstrating that these agents not only sensitize AML cells to chemotherapy, but also reverse stroma-mediated antiapoptotic effects.<sup>76,77</sup> Although the first generation of CXCR4

**Table 1.** Dysregulation of signaling network and potential therapeutic targets associated with osteogenic niche in acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPN) progression.

Role in normal hematopoiesis	Pathway	Deregulated feature	Prognostic marker?	Contribution to leukemogenesis	Ref
HSC homing and retention in BM osteogenic niche	CXCL12/CXCR4	↑CXCR4	Yes	→ BM homing of AML LSCs → Prosurvival/antiapoptotic pathways	73-77 81-84
	VCAM-1/VLA-4	↑ VLA-4	Yes/No	→ BM homing of AML LSCs → Stroma-mediated chemoresistance <i>via</i> NF-κB activation	89-92
	OPN/CD44	↑ OPN ↑ CD44v	Yes	→ BM homing of AML LSCs	88,112
HSC maintenance	Jagged-1/Notch	↑ ↑ Jagged-1 and Notch receptor with limited autonomous Notch activation	TBI	<i>Activation effect is context-dependent:</i> ↑ ↓ AML expansion <i>in vitro</i> ↓ AML progression (crosstalk unspecified) ↑ AML progression in crosstalk with β-catenin/FoXO1 in osteoblasts	38,99,100,113
	TGF-β	↓Activation <i>via</i> fusion gene	TBI	<i>Loss of TGF-β signaling is favorable though not required for AML initiation. Activation effect is context-dependent:</i> → LSC quiescence and chemoresistance ↑ AML progression & ↓ MPN progression <i>via</i> PTH activation in osteoblasts ↑ AML progression <i>via</i> CTGF upregulation in MSCs	8,11,50,114
	BMP	↑Activation <i>via</i> fusion gene ↑Activation <i>via</i> osteogenic priming by AML	TBI	↑ AML progression in crosstalk with TGF-β/CTGF in MSCs	50,53
Bone and hematopoietic homeostasis	SNS	Neuropathy	TBI	↑AML progression <i>via</i> osteogenic priming in MSCs ↑MPN progression <i>via</i> deficient sympathetic stimulation of MSCs	49,101
TBI	CCL3	↑Proinflammatory cytokines	TBI	MPN progression <i>via</i> HSC displacement	39

**In AML cells; In osteolineage cells; ↑Increase; ↓Decrease; →Mediate;** TBI: to be investigated; HSC: hematopoietic stem cells; CXCL12: stromal derived factor-1; CXCR4 C-X-C: chemokine receptor 4; VCAM-1: vascular cell adhesion protein-1; VLA-4: very late antigen-4; OPN: osteopontin; TGF-β: transforming growth factor β; BMP: bone morphogenetic protein; SNS: sympathetic nervous system; CCL3: chemokine ligand 3; BM: bone marrow; LSC: leukemic stem/initiating cells; NF-κB: nuclear factor kappa-light-chain enhancer of activated B cells; PTH: parathyroid hormone; CTGF: connective tissue growth factor.

inhibitors, such as AMD3100 (plerixafor) and AMD3465, show anti-leukemic effects only synergistically with chemotherapy and their action is rather transient, a second generation that has potential as monotherapy is emerging.<sup>78,79</sup> Of note, the clinical benefit of CXCR4 blockade could be further optimized given the role of differentiating osteoblasts in shielding AML cells from CXCL12-mediated apoptosis in hypoxia.<sup>12</sup> Though such induction of apoptosis<sup>80</sup> is controversial,<sup>79,81-83</sup> it cannot be excluded that the interplay between hypoxia-mediated protection of leukemic cells typically found in AML BM<sup>84,85</sup> and niche components may tip the balance between CXCL12-mediated pro-survival and apoptosis. This possibility is noteworthy given CXCR4 is a well-established target of HIF-1 $\alpha$ , and therefore hypoxia.<sup>86</sup> The role of HIF-1 $\alpha$  in survival and maintenance of CML has also been described.<sup>87</sup> The Bonnet group found that HIF-2 $\alpha$ , another factor that is regulated by hypoxia, plays a crucial role in regulation of the long-term re-populating ability of CD34<sup>+</sup> umbilical cord blood cells. In addition, their data demonstrate that inhibition of HIF-2 $\alpha$  in primary AML cells inhibits their proliferation and sensitizes them to endoplasmic reticulum stress-induced apoptosis by upregulation of reactive oxygen species.<sup>85</sup>

Similarly, chemosensitization and reduced AML engraftment could be achieved in mouse models with the use of CD44 specific antibody<sup>88</sup> and VLA-4 blocking agents, such as natalizumab<sup>89</sup> and AS101.<sup>90</sup> Extramedullary BM models by Chen *et al.*<sup>91</sup> and Jacamo *et al.*<sup>92</sup> demonstrated that AML and stromal cells interact *via* VLA-4 and VCAM1 to activate downstream NF $\kappa$ B signaling in both cell types. Blockade of these interactions resulted in inhibition of stroma-induced chemoresistance in AML cells.<sup>91,92</sup> Besides, antagonizing these adhesion molecules has been found to relieve differentiation block in blasts,<sup>88</sup> a clinical benefit also seen when epigenetic modifiers<sup>93</sup> or FLT3 and IDH1/2 inhibitors<sup>94,95</sup> were used to treat AML. This mobilizing approach, while preferentially mobilizing AML cells, carries the risk of moving HSCs out of their protective BM niche, among other adverse effects.<sup>96-98</sup> Further randomized trials are needed to determine whether targeting AML homing axes is safe and how to optimize this chemosensitizing approach without impairing normal hematopoiesis.

There has been growing evidence to indicate another promising strategy: to target osteoblast function. Leukemia-stroma contact potentiates osteoblast differentiation in MSCs, which counteracts apoptogenic cues and promotes proliferative signals from the microenvironment to leukemic cells.<sup>12,50,55</sup> It has also been shown that abnormal signaling pathways and crosstalks that take place specifically in osteoblasts could induce or aggravate AML phenotype.<sup>8,38</sup> Manipulating signals from osteolineage cells would, therefore, render the osteogenic niche hostile to AML cells and abrogate the feedback loop fueling their perpetual life cycle. Indeed, modulation of mature osteoblast numbers by inhibiting gut-derived serotonin synthesis results in leukemia regression, providing a 'proof of concept' for this approach.<sup>65</sup>

The fact that stage-specific osteolineage cells have distinct functions and may differentially regulate normal and malignant hematopoiesis makes them an even more attractive target, especially with regard to their involvement in pleiotropic signaling pathways that support

HSCs, such as Notch or TGF- $\beta$ . For example, despite already being known for its tumor-suppressor role in AML,<sup>99,100</sup> Notch activation has been reported to be leukemogenic when synergizing with activating  $\beta$ -catenin/FoxO signaling in *Col1a1*<sup>+</sup> pre-osteoblasts.<sup>58</sup> This observation suggests targeting FoxO signaling in pre-osteoblasts may be beneficial to patients with constitutive activating  $\beta$ -catenin mutation. As the effects of myeloid leukemia on cell differentiation along the osteolineage unfold, more leukemia modulators might be identified, and these will facilitate patient stratification and prevent treatment failure.

Restricting osteogenic capacity of MSCs could also be a therapeutic option. This strategy potentially limits the OPN reservoir of the BM, further preventing AML cells from hiding in the osteogenic niche and evading chemotherapy. Maintaining the primitive MSC pool *via*  $\beta$ 2- and  $\beta$ 3-adrenergic agonists has shown multiple advantages in managing AML and MPN: rescuing healthy HSCs in the osteogenic niche with HSC maintenance factors and preventing LSCs from crowding out these normal residents.<sup>49,101</sup> Studies have further shown that the bone surface and periarteriolar region are prone to inflammation during the early stage of osteogenic niche remodeling.<sup>59,56</sup> This can be ameliorated by blocking receptors of pro-inflammatory cytokines, e.g. *via* CCL3 receptor antagonists.

Alternatively, promoting adipocyte differentiation of MSCs has been demonstrated to be a viable strategy to improve disease management by rescuing at least the generation of myeloid-erythroid lineages.<sup>51</sup> However, the long-term efficacy of this pro-adipogenesis therapy remains to be tested given the debatable evidence about the role of adipocytes in AML seen so far. Different groups reported on adipocyte re-programming in which AML blasts exploit these energy reservoirs through lipolysis to fuel uncontrolled expansion.<sup>102-104</sup> On the other hand, Lu *et al.*<sup>105</sup> only found a statistically significant correlation between AML patients' poor prognosis and an increase in small adipocytes, but not the decrease in large- or medium-sized ones. This finding suggests that lipid transfer may not be the only mechanism through which adipocytes aggravate leukemia burden. It cannot be excluded that, as previously shown in acute lymphoblastic leukemia,<sup>106,107</sup> adipocytes may acquire a chemoprotective role in the setting of AML.

Recent discoveries provide evidence that mitochondria are transferred from BM stromal cells to leukemia cells which influence leukemia progression.<sup>108</sup> These studies demonstrate that mitochondria are transferred *via* tunneling nanotubes (TNTs) or extracellular vesicles resulting in enhanced ATP production through increased oxidative phosphorylation (OXPHOS) which translates into higher drug resistance in AML cells and relapse after chemotherapy.<sup>109,110</sup> Therefore, inhibition of mitochondrial transfer by targeting TNT formation or inhibiting OXPHOS is currently being considered as novel therapeutic strategies in AML therapy.

## Conclusions and emerging questions

Findings on BM stroma-mediated chemoprotection in AML since the early 2000s have paved the way for a wave of new insights into leukemia-BM niche interactions, hence re-defining the paradigm of leukemic devel-



opment and response to therapy. Although its fundamental role in HSC maintenance is still debatable, the osteogenic niche stands out as a pivotal sanctuary for LSCs and the cradle of blast production. Putting this into perspective, we foresee that AML-induced genetic changes and osteogenic priming in MSCs illustrate not only the long-standing multiple-hit hypothesis of carcinogenesis but also the newly-coined microenvironment-induced oncogenesis. Importantly, the role of the BM niches in the development of MDS and AML from clonal hematopoiesis of indeterminate potential is still completely unknown.<sup>111</sup> Many questions emerge, for instance, regarding the differential role of stage-specific osteolineage cells in AML progression, the uncoupling between osteogenesis and osteoclastogenesis, or the net therapeutic benefits of LSC dislodgement at the expense of HSC homelessness. Recent advances in our understanding of this osteoblast-rich region in AML progression provide a convincing premise with which to build the next genera-

tion of AML therapy to target the osteogenic niche. However, further studies are needed to clarify the self-reinforcing loop between AML and the osteogenic niche, with the goal of inducing deep remissions and controlling long-term disease.

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