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# Hematopoietic stem cell stretches and moves in its bone marrow niche

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ABSTRACT

Hematopoietic stem cells are the most illustrious inhabitants of the bone marrow. Direct visualization of endogenous hematopoietic stem cells in this niche is essential to study their functions. Until recently this was not possible in live animals. Recent studies, using state-of-the-art technologies, including sophisticated in vivo inducible genetic approaches in combination with two-photon laser scanning microscopy, allow the follow-up of endogenous hematopoietic stem cells' behavior in their habitat. Strikingly, the new findings reveal that quiescent hematopoietic stem cells are more mobile than previously thought, and link their retained steady state within the niche to a mobile behavior. The arising knowledge from this research will be critical for the therapy of several hematological diseases. Here, we review recent progress in our understanding of hematopoietic stem cell biology in their niches.

## 1. Introduction

### 1.1. Hematopoietic stem cells

The bone marrow is, presently, well-established as the primary postnatal site of new blood cells formation, generating approximately 10 billion leukocytes, 200 billion red cells, and 400 billion platelets daily during our whole life (Yoder, 2004). Nevertheless, this bone marrow's capacity was only first experimentally discovered at the second half of the 19th century by a German pathologist Ernst Neumann (Cooper, 2011). He also proposed the controversial, at that time, concept that one cell type may originate all other blood cells in the bone marrow (Cooper, 2011). This pioneer theory introduced the field of hematopoietic stem cell biology. Early works from the 50 s demonstrated that transplantation of bone marrow cells could protect the organism from some of the damages caused by irradiation, avoiding hematopoietic failure (Jacobson et al., 1950; Lorenz et al., 1951; Jacobson et al., 1951; Main and Prehn, 1955), suggesting the existence of a cell with reconstitutive ability in the middle of bone marrow cells. In the 60 s, James Till, Ernest McCulloch, and their colleagues brought the initial experimental proof of the existence of hematopoietic stem cells. They demonstrated that there were cells in the bone marrow with capacity to generate all blood cell types and make more of themselves (Till and Mc, 1961; Wu et al., 1968; Becker et al., 1963; Siminovitch et al., 1963; Wu et al., 1967; Wolf and Trentin, 1970). Since then, in the clinic, intravenous transplantation of bone marrow cells has proven to be effective to treat patients with several blood-related diseases, such as leukemia (Thomas et al., 1957; Mathe et al., 1959, 1963). In leukemic patients, bone marrow transplantation has revolutionized therapeutic options, and now is widely used in the clinic, allowing bone marrow cells from healthy donors to repopulate the bones of patients with leukemia after aggressive chemotherapy (Wayne et al., 2010; Yamamoto et al., 2020; Khaddour et al., 2020; Sanchez-Aguilera and Mendez-Ferrer, 2017; Singh and Cancelas, 2020).

Nowadays, hematopoietic stem cells can be isolated from the bone marrow highly enriched by using multiple specific molecular markers (Laurenti et al., 2008). Scientists are constantly searching for new markers to isolate subsets of purified hematopoietic stem cells. It is well

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accepted that stem cells capable of hematopoietic reconstitution are positive for Sca-1, a membrane glycoprotein (Okada et al., 1992) and c-Kit, a tyrosine kinase receptor (CD117), concomitantly being negative for lineage markers (Lin-), including Gr-1, Ter119, Mac-1, B220, CD4 and CD8 (Challen et al., 2009; Kiel et al., 2007a; Dykstra et al., 2006). Additionally, these characteristics are combined with strategies established by different groups to isolate purified hematopoietic stem cells (Kiel et al., 2007a, b), such as their status of expression of Thy1.1, Flk2, CD34, Endoglin (CD105) (Chen et al., 2002), Tie-2 (Arai et al., 2004), endothelial protein C receptor (EPCR) (Balazs et al., 2006), CD244, CD48, and/or CD150 (Kiel et al., 2007a). The exclusion of fluorescent dyes is an additional method that has proven advantageous to select for cells enriched with hematopoietic stem cells activity (Okada et al., 1992; Challen et al., 2009; Goodell et al., 1997; Pearce et al., 2004).

One obstacle in the hematopoietic stem cells' isolation is that the number of available compatible bone marrow donors still limits the usage of hematopoietic stem cells for transplantation. Although hematopoietic stem cells are maintained throughout all our life in their niche in vivo, we still are unable to multiply and expand effectively hematopoietic stem cells in vitro under suitable conditions. Therefore, a deeper understanding of hematopoietic stem cells biology will be essential for the better efficiency of bone marrow transplantation in the future.

In this review, we discuss the recent progress in our understanding of hematopoietic stem cell biology in their niches, focusing on hematopoietic stem cells' heterogeneity and interactions with other cells in the context of recent findings. Furthermore, we shed light on the gaps in the field and highlight important open questions.

### 1.2. Hematopoietic stem cells within the bone marrow niche

Hematopoietic stem cells reside predominantly within the bone marrow (Birbrair and Frenette, 2016a). The hematopoietic stem cells' bone marrow niche regulates the behavior of those cells (Schofield, 1978). Hematopoietic stem cell fate is decided by the pro-quiescence, pro-renewal, or pro-differentiation intrinsic and extrinsic regulators inside the niche (Rashidi et al., 2014). Multiple genetically engineered mouse models have been extensively used to explore the complexity of the hematopoietic stem cell niche within the bone marrow. These investigations established diverse components as niche-supporting cells for hematopoietic stem cells, providing many molecules, such as cytokines, to control hematopoietic stem cell function (Asada et al., 2017a). Experimental proof has revealed that intervention in the key niche regulators may lead to various hematologic pathologic processes (Birbrair and Frenette, 2016a; Borges et al., 2017). Thus, understanding hematopoietic stem cells' behavior in their niche, as well as their interactions with other niche constituents, is of crucial significance.

Direct visualization of hematopoietic stem cells in their niche is necessary to study their activity *in vivo*. This was possible with the advancement of deep confocal microscopic imaging that helped determine hematopoietic stem cell niche architecture. Several studies analyzed the localization of hematopoietic stem cells relative to distinct niche components (Kunisaki et al., 2013; Asada et al., 2017b; Acar et al., 2015). In most studies, the hematopoietic stem cells behavior was analyzed in bone marrow biopsies, in which hematopoietic stem cells can be precisely identified using a combination of molecular markers by immunohistochemistry (Kunisaki et al., 2013; Asada et al., 2017b; Acar et al., 2015). Nevertheless, remains the open question whether hematopoietic stem cell behavior is the same within the bones of live animals. Other works analyzed the behavior of pre-labeled hematopoietic stem cells in recipient live mice (Lo Celso et al., 2009; Lewandowski et al., 2010; Takizawa et al., 2011). Nevertheless, it is not clear whether the non-physiological behavior of these introduced hematopoietic stem cells is the same as of endogenous stem cells. Additionally, for the efficiency of transplantation, recipient animals receive treatments that affect the bone marrow microenvironment, bringing the possibility of changes in hematopoietic stem cell behavior due to niche disruption.

Now, in a recent article in *Cell Stem Cell*, Upadhaya and colleagues demonstrated elegantly how endogenous adult hematopoietic stem cells behave in the bone marrow in live animals (Upadhaya et al., 2020). Using state-of-the-art technologies, including sophisticated *in vivo* inducible genetic approaches, such as lineage-tracing Cre/loxP mediated technologies, in combination with two-photon laser scanning microscopy, the authors selectively followed the behavior of single adult hematopoietic stem cells for several hours. The authors analyzed the bone marrow of a mouse model in which specifically endogenous hematopoietic stem cells produce red fluorescence, Pdzk1ip1-CreER/TdTomato mice. Behaviors of hematopoietic stem cells and macrophages, which were detected by their autofluorescence, were compared. These experiments revealed that hematopoietic stem cells present a constantly changing not-rounded shape extending cytoplasmatic projections, in contrast to the perfectly round cells as previously thought. Surprisingly, hematopoietic stem cells moved 7.5 times more than resident macrophages in steady state conditions (Upadhaya et al., 2020) (Fig. 1). Importantly, the authors confirmed that Pdzk1ip1-expressing cells were *bona fide* hematopoietic stem cells by confirming that the investigated cells were also Fgd5+ in Pdzk1ip1-CreER/TdTomato/Fgd5-ZsGreen mice. Upadhaya and colleagues also reported, as previously known, that hematopoietic stem cells are located in the perivascular space, and physically interact with stem cell factor (SCF)-expressing pericytes in the bone marrow. Strikingly, mobilization of the hematopoietic stem cells from the bone marrow niche by drugs that block C-X-C chemokine receptor type 4 (CXCR4) receptor and integrin signaling inhibited hematopoietic stem cell mobility as well as its form fluctuations within the niche (Upadhaya et al., 2020) (Fig. 2). This study reveals that hematopoietic stem cells are more mobile than previously thought, and links their retained steady state within the niche to a mobile behavior. Here, we discuss the findings from this work and evaluate recent advances in our understanding of the hematopoietic stem cell microenvironment.

## 2. Perspectives / future directions

### 2.1. Hematopoietic stem cells heterogeneity

Hematopoietic stem cells are not homogeneous. There have been shown subpopulations based on their life span (Yang et al., 2005), specific surface markers (Birbrair and Frenette, 2016b), differentiation capacities (Muller-Sieburg et al., 2012), and level of self-renewal (Ema et al., 2005). Although great advances were made regarding our knowledge of the bone marrow niche components, how extrinsic regulators act on hematopoietic stem cell subsets remains completely unknown. Interestingly, Upadhaya and colleagues analyzed only about one-fifth of hematopoietic stem cells, as this is approximately the amount labeled in Pdzk1ip1-CreER/TdTomato mice (Upadhaya et al., 2020). It remains unclear whether in these transgenic mice a subpopulation of rapidly moving hematopoietic stem cells is selected or whether all hematopoietic stem cells display approximately the same rate of movement. Future studies should study the behavior of not-expressing Pdzk1ip1 hematopoietic stem cells.

Hematopoietic stem cells modify their differentiation capacity during aging, losing gradually their self-renewal ability, becoming increasingly myeloid-biased (Mendelson and Frenette, 2014; Pang et al., 2011). The changes perceived in old hematopoietic stem cells were speculated to be exclusively due to hematopoietic stem cell-intrinsic alterations (Geiger et al., 2013; Birbrair et al., 2013). Nonetheless, recent results show the critical function of several extrinsic molecules inducing hematopoietic stem cell aging as well (Nakamura-Ishizu and Suda, 2014). It will be interesting to explore how hematopoietic stem cells' behavior changes in live animals with aging, and whether myeloid-biased hematopoietic stem cells behave differently from the others.

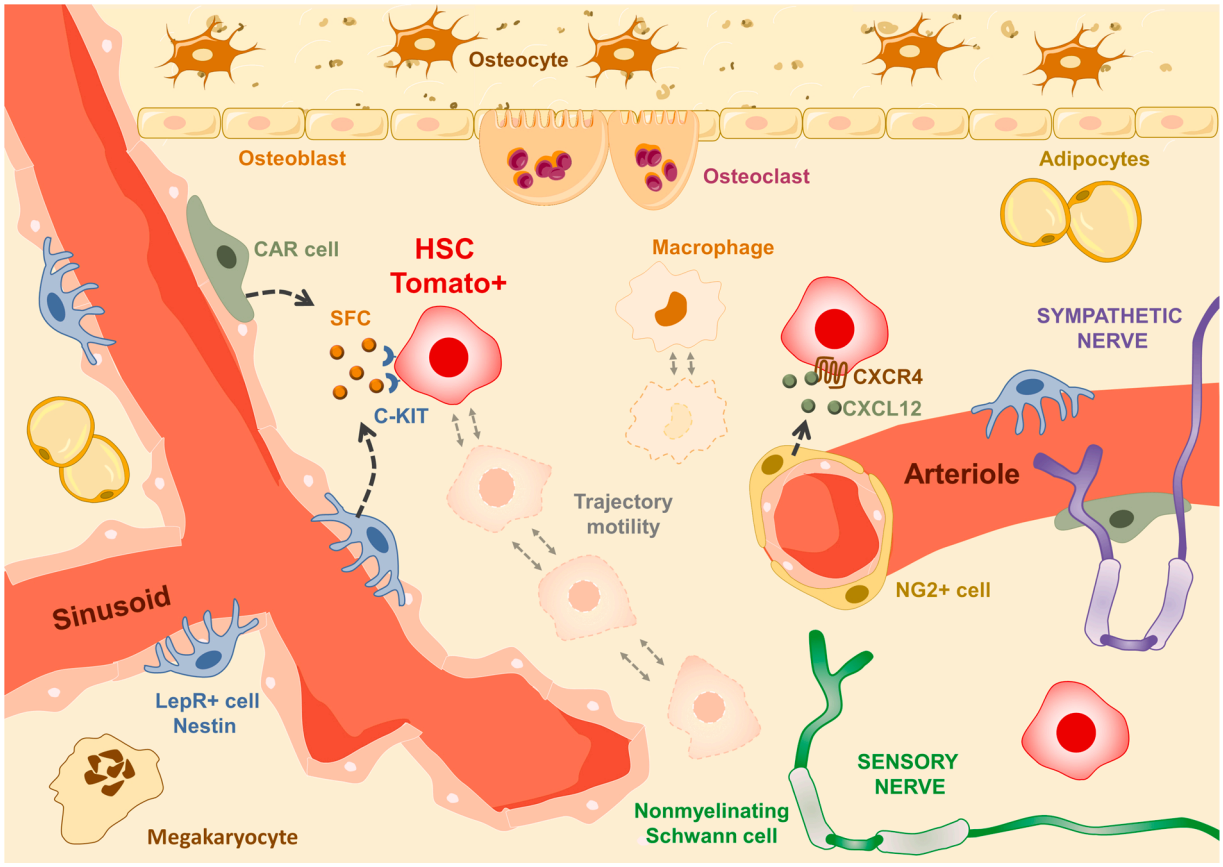


**Fig. 1.** Schematic illustrating hematopoietic stem cell movement within the bone marrow niche.
Hematopoietic stem cells (in red) present dynamic morphology (non-spherical) and complex motile behavior when compared to sessile resident macrophages (in brown) within the bone marrow cavity. Upadhaya and colleagues demonstrated that hematopoietic stem cells' displacement velocity is 7.5 times faster than macrophages (Upadhaya et al., 2020).

### 2.2. Other hematopoietic stem cell niches

During embryonic development, hematopoiesis occurs at specific anatomical sites that change with the developmental age (Khan et al., 2016; Al-Drees et al., 2015; Palis et al., 1999; Tavian and Peault, 2005). This happens because of the migration of hematopoietic stem cells throughout the embryo (Bowman and Zon, 2009). The hematopoietic activity starts in the extraembryonic yolk sac at embryonic day 7.5; then, at day 9, it advances to the dorsal aorta-gonad-mesonephros (AGM region), the para-aortic splanchnopleura, and chorioallantoic placenta (Rhodes et al., 2008); at day 10, it arrives to vitelline and umbilical arteries, spleen, skeletal muscle surrounding the developing long bones, and the fetal liver, where hematopoietic stem cells expand exponentially (Al-Drees et al., 2015; Palis et al., 1999; Swain et al., 2014; Tanaka et al., 2015; Medvinsky et al., 1993, 2011; Baron, 2005; Baron et al., 2012; Barminko et al., 2016; Kumaravelu et al., 2002; Muller et al., 1994; Medvinsky and Dzierzak, 1996; Sugiyama and Tsuji, 2006; Lux et al., 2008). Lastly, at day 15, hematopoietic stem cells from the fetal liver move through the circulation to the bone marrow cavity, which turns into the dominant niche for hematopoietic stem cells throughout the whole adult life (Khan et al., 2016; Medvinsky et al., 2011). Hematopoietic stem cells in adults can also appear outside the medullary spaces. This phenomenon is termed extramedullary hematopoiesis. was reported in adults in the periosteum, spleen, liver, heart, kidney, adrenal glands, fatty tissue, intra-spinal tissue, para-vertebral regions, pre-sacral region, nasopharyngeal region, paranasal sinuses, and in multiple types of cancers (Sohawon et al., 2012; Johns and Christopher, 2012; Tsamandas et al., 1995; Vassiliou et al., 2012; Macki et al., 2013; Inra et al., 2015; Bozzini et al., 1970; Bowen et al., 2015; Schnuelle et al., 1999; Woodward et al., 2000; Lewis et al., 1994). Although it normally indicates a pathologic state of the organ, recent works show the extramedullary hematopoiesis may occur under physiologic conditions as well. Elegant studies have shown the presence of hematopoietic stem cells in the pulmonary microenvironment under physiologic circumstances (Borges et al., 2017; Lefrancais et al., 2017). Future studies using modern technologies such as two-photon laser scanning microscopy adapted to the specific organs will reveal how hematopoietic stem cells behave in these extramedullary niches.

### 2.3. The quiescent state

The definition of quiescence emerged from the perception that each cell in a population proliferates at its own rate (Cheung and Rando, 2013). Thus, cells that are in a non-proliferative state are termed quiescent, even under certain stimuli they can enter the cell cycle and start proliferating. Unicellular organisms, which survive in adverse habitats, enter the quiescent state to not be extinct (Gray et al., 2004). Similarly, stem cells exist in a quiescent state throughout our life to keep for as long as possible a reserve pool. Despite quiescence being considered as a dormant static state, quiescence seems to portray a state in which the stem cell is ready to be activated. Upadhaya and colleagues demonstrate that quiescent hematopoietic stem cells are not so "dormant", being rather "awake" based on the movement that they present within the niche (Upadhaya et al., 2020). The reason for this augmented mobility of hematopoietic stem cells should be examined in future studies. It is interesting to explore the molecular mechanisms involved in this movement. It remains uncertain whether this migration is caused by active molecules that promote hematopoietic stem cell

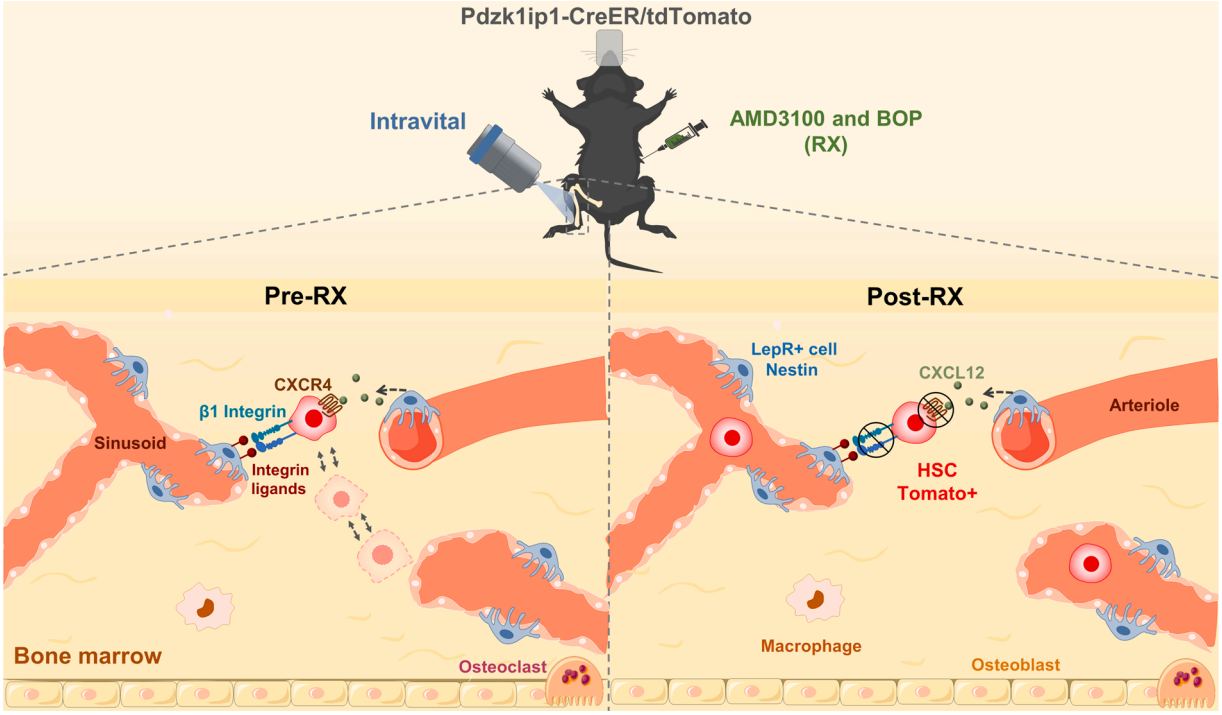


**Fig. 2.** Hematopoietic stem cell retained steady state within the bone marrow niche is linked to a mobile behavior.
Mobilization of the hematopoietic stem cells from the bone marrow niche by drugs, that block CXCR4 (plerixafor, AMD3100) and integrin signaling [N-(Benzenesulfonyl)-L-prolyl-L-O-(1-pyrrolidinylcarbonyl) tyrosine, (BOP)] (AMD3100 + BOP, RX), inhibits hematopoietic stem cell mobility as well as its form fluctuations within the niche (Upadhaya et al., 2020).

mobility or by the lack of specific anchoring factors. Are hematopoietic stem cells searching for a higher gradient of specific limited factors within the niche? Are other quiescent stem cells also behaving like hematopoietic stem cells in live mice? Also, as circadian rhythms influence hematopoietic stem cells (Mendez-Ferrer et al., 2009), it will be attractive to examine whether hematopoietic stem cell behavior varies during light cycles.

### 2.4. *Interactions within the bone marrow niche*

The bone marrow microenvironment defines the hematopoietic stem cell fate (Rashidi et al., 2014). Experimental data has revealed that small changes in niche regulatory mechanisms affect directly hematopoietic stem cells (Birbrair and Frenette, 2016b). Understanding exactly how hematopoietic stem cells are controlled by their niche is of fundamental importance. Upadhaya and colleagues showed the proximity of hematopoietic stem cells to the perivascular zones (Upadhaya et al., 2020), as it has been previously reported (Birbrair and Frenette, 2016b). Nevertheless, the perivascular niche itself is complex. Perivascular cells have been distinguished as essential components of the hematopoietic stem cell microenvironment (Mendez-Ferrer et al., 2010; Pinho et al., 2013), and *in vivo* genetic elimination of those cells from the bone marrow directly affects hematopoietic stem cells (Mendez-Ferrer et al., 2010). There are two main subpopulations of bone marrow perivascular cells in regards to their vascular positions: sinusoidal and arteriolar pericytes (Kunisaki et al., 2013; Nobre et al., 2021). Most of the quiescent hematopoietic stem cells reside closer to arterioles (Kunisaki et al., 2013). Upadhaya and colleagues did not determine whether their analyzes were done in the sinusoidal or arteriolar niches (Upadhaya et al., 2020). Future studies should explore whether hematopoietic stem cells behave differently in these two central niches within live mice.

Upadhaya and colleagues showed that the blockade of C-X-C motif chemokine 12 (CXCL12) signaling abrogates hematopoietic stem cell movement in the niche (Upadhaya et al., 2020). It is not clear, however, whether this is caused by a direct or indirect effect of the drug. Is the drug acting directly on hematopoietic stem cells or on a niche component? Interestingly, sinusoidal and arteriolar niches contribute with different cytokines for the maintenance of hematopoietic stem cells. CXCL12-derived from the arteriolar niche is essential for hematopoietic stem cells, but not the one derived from the sinusoidal niche. Thus, it would be important to analyze hematopoietic stem cell behavior in response to CXCL12 deletion only from arteriolar pericytes. In contrast, SCF from the sinusoidal niche, but not from the arteriolar, seems to be essential for hematopoietic stem cell functioning. Thus, future experiments should address how distinct niche regulatory molecules affect hematopoietic stem cells' behavior in live animals.

Modern technologies provide the possibility of eliminating single cells from the tissue microenvironment and analyzing the behavior of the remaining cells (Berthiaume et al., 2018; Santos et al., 2019; Prazeres, 2020; Coimbra-Campos, 2021; Sena, 2021). Thus, it is possible to explore the effect of eliminating single components of the niche by using targeted two-photon irradiation and analyzing the effect on hematopoietic stem cells' behavior by two-photon laser scanning microscopy. Alternatively, it will be interesting to evaluate what is the effect of the death of one hematopoietic stem cell on other neighboring hematopoietic stem cells. Thus, longitudinal imaging studies may advance significantly our knowledge on hematopoietic stem cell biology in the future.

Our better understanding of hematopoietic stem cells' behavior in their normal bone marrow microenvironment leads to questions on how these cells behave in the bone marrow in different pathologies. Changes in the normal bone marrow niche may activate the appearance of preleukemic microenvironments (Konopleva and Jordan, 2011). How leukemic stem cells may affect this hematopoietic stem cell behavior, as well as how the leukemia stem cells themselves behave in live animals within their niches remains to be discovered.

## 3. Conclusion

In conclusion, the study by Upadhaya and colleagues reveals how the most illustrious residents of the bone marrow behave within their niche in live animals (Upadhaya et al., 2020). However, our understanding of the hematopoietic stem cells' behavior in their niches still remains limited, and the complexity of interactions with all niche components should be elucidated in future studies. Despite the powerful experimental transgenic models that provide proof of concept for the hematopoietic stem cell biology within the bone marrow, we are still lacking direct demonstration of hematopoietic stem cell behavior within the human bone marrow cavity. The main question for the future is whether we can translate mice research into humans. Improving the availability of human bone marrow biopsies will be essential to reach this aim. The creation of bone marrow organoids from human induced pluripotent stem cells (iPSCs) may in the future support the data provided by elegant mouse studies.

## Declaration of Competing Interest

The authors indicate no potential conflicts of interest.

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