

REVIEW

Modeling the human bone marrow niche in mice: From host bone marrow engraftment to bioengineering approaches

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Xenotransplantation of patient-derived samples in mouse models has been instrumental in depicting the role of hematopoietic stem and progenitor cells in the establishment as well as progression of hematological malignancies. The foundations for this field of research have been based on the development of immunodeficient mouse models, which provide normal and malignant human hematopoietic cells with a supportive microenvironment. Immunosuppressed and genetically modified mice expressing human growth factors were key milestones in patient-derived xenograft (PDX) models, highlighting the importance of developing humanized microenvironments. The latest major improvement has been the use of human bone marrow (BM) niche-forming cells to generate human–mouse chimeric BM tissues in PDXs, which can shed light on the interactions between human stroma and hematopoietic cells. Here, we summarize the methods used for human hematopoietic cell xenotransplantation and their milestones and review the latest approaches in generating humanized BM tissues in mice to study human normal and malignant hematopoiesis.

The hematopoietic niche

The hematopoietic system is a hierarchy of multiple committed lineages originating from hematopoietic stem cells (HSCs; [Velten et al., 2017](#)), whereas the bone marrow (BM) HSC niche is a spatial environment in which the HSC pool resides and is maintained by a balance of quiescence and expansion. This tightly controlled balance is regulated by multiple components of the BM niche, which are responsible for the shift between these two states. The BM is a highly vascularized tissue with a vast network of endothelial cells (ECs), which form a major component of the HSC niche. BM ECs are known to release cytokines, signaling mediators, and growth factors into the BM microenvironment, therefore regulating HSC quiescence, expansion, and activation ([Raynaud et al., 2013](#); [Ramasamy et al., 2016](#)). Another major component of the hematopoietic niche is the mesenchymal stromal cell (MSC) fraction. It is a heterogeneous cell population well characterized in mouse models using specific reporters and also known as a relevant component of the HSC niche in the human context ([Zhou et al., 2014](#); [Matsuoka et al., 2015](#)). This class of stromal cells has the potency to give rise to other BM components, as chondro-, adipo-, and osteolineage cells. The nervous system also plays a role in the BM niche, as neuroglial cells regulate HSC traffic and proliferation ([Spiegel et al., 2007](#); [Méndez-Ferrer et al., 2008](#);

[Yamazaki et al., 2011](#)). Finally, mature hematopoietic cells and cells from the immune system (megakaryocytes, macrophages, and T cells) also play distinct supportive functions for HSCs in the BM niche ([Fig. 1](#); [Chow et al., 2011](#); [Bruns et al., 2014](#); [Zhao et al., 2014](#); [Yu and Scadden, 2016](#)). Deregulation of HSC activity within the BM niche is a key factor in the development of hematological malignancies. Although leukemia is predominantly considered a genetic disease ([He et al., 2016](#); [Papaemmanuil et al., 2016](#)), several recent findings indicate that leukemic cells (myeloid malignancies in particular) also affect the function of BM niche components and vice versa, pointing toward the existence of an active cross talk between the two compartments ([Raaijmakers et al., 2010](#); [Frisch et al., 2012](#); [Seke Etet et al., 2012](#); [Hartwell et al., 2013](#); [Krause et al., 2013](#); [Schepers et al., 2013](#); [Kode et al., 2014](#); [Medyouf et al., 2014](#); [Schajnovitz and Scadden, 2014](#); [Chattopadhyay et al., 2015](#); [Dong et al., 2016](#); [Hoggatt et al., 2016](#); [Lin et al., 2016](#); [Zambetti et al., 2016](#); [Passaro et al., 2017b](#); [Sánchez-Aguilera and Méndez-Ferrer, 2017](#)). Therefore, characterization of the relationship between normal and malignant HSCs, as well as with the various components of the BM niche, is required to better understand the mechanisms of leukemogenesis and identify new potential targets that could be used for therapeutic strategies. As a result of the interaction of multiple

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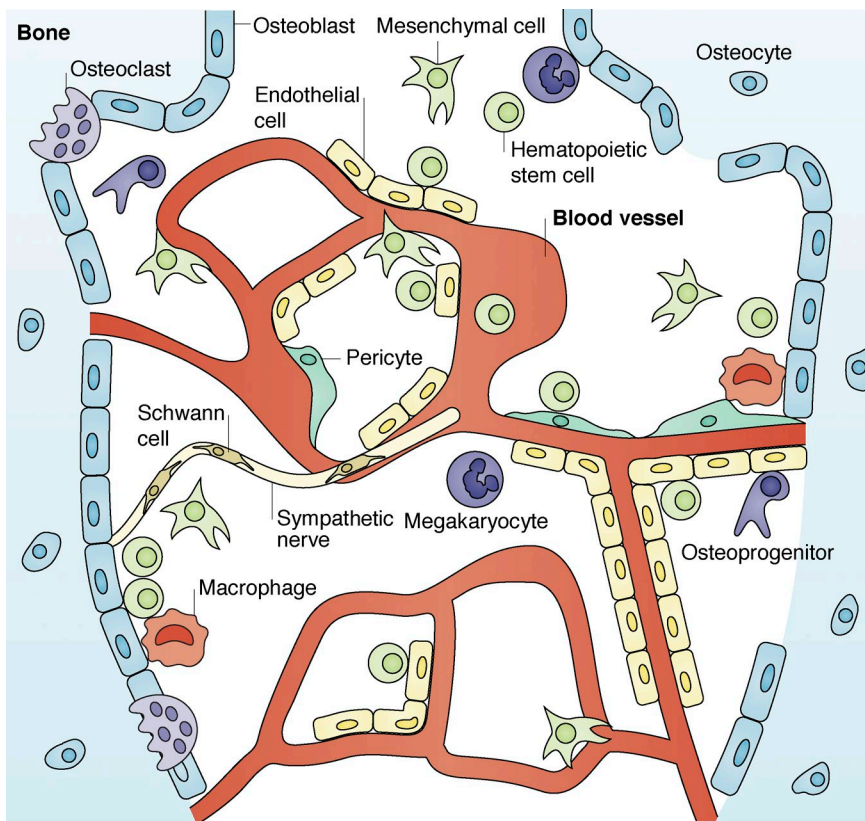


Figure 1. **The hematopoietic BM niche.** The BM is a heterogeneous environment composed of different types of cells. The two main architectural scaffolds of the tissue are the bone and the vessels, integrated in a complex network connected to nerve fibers. Associated with these structures are different types of cells, as depicted in the figure, regulating the tissue homeostasis and the normal HSC fate in healthy and disease states.

cellular components, the cytokine milieu, the presence of innervated vascular structures, and a variety of immune cells, the BM niche must be studied *in vivo*, as *in vitro* models are reductive and lack key functional components. Patient-derived xenograft (PDX) models provide the best system to study the interactions between the different components of the BM and the role the niche plays in various hematological malignancies.

Human hematopoietic xenotransplantation

Despite numerous obstacles and caveats (Theocharides et al., 2016), PDX models have proven their reliability in partially recapitulating features of human normal and malignant hematopoiesis (see Table 1 for a summary of the history of immunodeficient mouse development; Chelstrom et al., 1994; Vormoor et al., 1994; Baersch et al., 1997; Hogan et al., 1997; Steele et al., 1997; Dazzi et al., 1998; Wang et al., 1998; Borgmann et al., 2000; Rombouts et al., 2000; Nijmeijer et al., 2001; Medyouf, 2017; Yoshimi et al., 2017). These models have greatly improved our understanding of normal human stem cell biology, the concept of cancer stem cells (Lapidot et al., 1994; Bonnet and Dick, 1997), leukemic clonal heterogeneity (Clappier et al., 2011; Klco et al., 2014), clonal hierarchy (Woll et al., 2014; Mian et al., 2015), and the origins of relapse in leukemia (Shlush et al., 2017).

The initial studies showing engraftment in immunodeficient mice of human hematopoietic cells laid the foundations for xenotransplantation as a surrogate model to study hematological diseases (Kamel-Reid and Dick, 1988; McCune et al., 1988; Lapidot et al., 1992). Although severe combined immunodeficiency (SCID) mice were instrumental in deciphering the concept of initiating cells in human acute myeloid leukemia (AML; Lapidot et al.,

1994), the ever-improving non-obese diabetic (NOD)/SCID (NOD-SCID) model was essential in establishing the leukemic hierarchy of AML (Bonnet and Dick, 1997). Since then, and thanks to the use of IL-2 receptor (IL-2R) γ chain KO NOD/SCID (NSG) mice, the phenotypic heterogeneity of leukemia stem cells (LSCs) has been identified. It is indeed now well-accepted that despite the presence of LSCs in the CD34⁺CD38⁻ fraction, progenitors can also acquire LSC properties such as granulocyte-myeloid progenitor-like (CD34⁺CD38⁺CD45⁺) malignant cells (Goardon et al., 2011). Surprisingly, in some patients harboring mutations in *Nucleophosmin 1* gene, mature cells residing in the CD34⁻ fraction have been shown to possess LSC properties (Taussig et al., 2010; Quek et al., 2016). Chronic myeloid leukemia (CML) harboring the translocation t(9;22), which generates fusion protein *BCR-ABL1*, is the classical example of a stem cell disease where the LSCs originate from CD34⁺CD38⁻ HSCs (Nowell and Hungerford, 1961; Rowley, 1973; Heisterkamp et al., 1983; Giustacchini et al., 2017). Several immunodeficient mouse models have been used to study the properties of primary CML patient-derived LSCs, the most notable being NOD-SCID-based mouse models (Holyoake et al., 1999; Eisterer et al., 2005). Interestingly, the level of engraftment observed in these animals correlates with the disease state, with higher engraftment observed with primary samples obtained from the blast crisis phase rather than the chronic phase of the disease (Dazzi et al., 1998; Wang et al., 1998; Clarke and Holyoake, 2017).

Unlike in myeloid malignancies, whether acute lymphoblastic leukemia (ALL) is driven by a physically identifiable LSC population has been long debated. The use of PDX models has been a fundamental tool to shed light on this and to better define the

Table 1. Summary of immunodeficient mice useful in human hematopoietic xenograft studies

Strain name	Common name	Mutant alleles	Phenotype	HSC engraftment	Leukemic engraftment (AML/ALL)	References
B6.CB17-Prkdc ^{scid} /Szj	SCID	Prkdc ^{scid}	Leaky immunodeficient	+	+/+	Bosma et al., 1983; McCune et al., 1988; Mosier et al., 1988; Fulop and Phillips, 1990; Lapidot et al., 1992; Greiner et al., 1998
B6.129S7-Rag1 ^{tm1Mom} /J	Rag1KO	Rag1 ^{tm1Mom}	Nonleaky immunodeficient	+	+/+	Mombaerts et al., 1992; Shinkai et al., 1993; Greiner et al., 1998
B6(Cg)-Rag2 ^{tm1.1Cgn} /J	Rag2KO	Rag2 ^{tm1.1Cgn}	Nonleaky immunodeficient	+	+/+	Mombaerts et al., 1992; Shinkai et al., 1992; Greiner et al., 1998
NOD.CB17-Prkdc ^{scid} /J	NOD-SCID	Hc ⁰ ; Prkdc ^{scid}	NOD immunodeficient	+	+/+	Hesselton et al., 1995; Lowry et al., 1996; Pflumio et al., 1996
NOD.129P2(B6)-B2m ^{tm1Unc} /J	NOD/B2M	B2m ^{tm1Unc}	NOD, MHC class I negative	+	+/+	Koller et al., 1990; Feuring-Buske et al., 2003
B6.129S4-Il2rg ^{tm1Wjl} /J	IL-2R γ ^{null}	Il2rg ^{tm1Wjl}	T/B/natural killer cell deficient	+	Not assessed	Cao et al., 1995
C.Cg-Rag2 ^{tm1Fwa} Il2rg ^{tm1Sug}	BRG	Rag2 ^{tm1Fwa} ; Il2rg ^{tm1Sug}	BALB/c, immunodeficient, radiosensitive, humanized	++	Not assessed	Traggiai et al., 2004
NOD.cg-Prkdc ^{scid} Il2rg ^{tm1Sug}	NOG	Prkdc ^{scid} ; Il2rg ^{tm1Sug}	NOD, immunodeficient, radiosensitive, humanized	++	++/++	Ito et al., 2002; Yahata et al., 2003
NOD.Cg-Rag1 ^{tm1Mom} Il2rg ^{tm1Wjl}	NRG	Rag1 ^{tm1Mom} ; Il2rg ^{tm1Wjl}	NOD, immunodeficient, radiosensitive, humanized	+++	+++ / ++	Pearson et al., 2008; Brehm et al., 2010; Maykel et al., 2014
NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl}	NSG	Prkdc ^{scid} ; Il2rg ^{tm1Wjl}	NOD, immunodeficient, radiosensitive, humanized	+++	+++ / ++	Ito et al., 2002; Traggiai et al., 2004; Ishikawa et al., 2005; Shultz et al., 2005
NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzj	NSG-SGM3; NSGS	Prkdc ^{scid} ; Il2rg ^{tm1Wjl} ; Tg(CMV-IL3,CSF2,KITLG)1Eav	NOD, immunodeficient, radiosensitive, humanized; expresses 3 human cytokines	+++	++++ / +++	Nicolini et al., 2004; Wunderlich et al., 2010; Takagi et al., 2012; Yoshimi et al., 2017
C;129S4-Rag2 ^{tm1Flv} Csf1 ^{tm1(CSF1)Flv} Csf2Il3 ^{tm1(CSF2,IL3)Flv} Thpo ^{tm1(TPO)Flv} Il2rg ^{tm1.1Flv} /J	MITRG	Rag2 ^{tm1Flv} ; Csf1 ^{tm1(CSF1)Flv} ; Csf2Il3 ^{tm1(CSF2,IL3)Flv} ; Thpo ^{tm1(TPO)Flv} ; Il2rg ^{tm1.1Flv}	Immunodeficient, humanized; expresses 3 human cytokines	+++	++++ / not assessed	Rongvaux et al., 2014; Ellegast et al., 2016
C;129S4-Rag2 ^{tm1.1Flv} Csf1 ^{tm1(CSF1)Flv} Csf2/Il3 ^{tm1(CSF2,IL3)Flv} Thpo ^{tm1(TPO)Flv} Il2rg ^{tm1.1Flv} Tg(SIRPA)1Flv/J	MISTRG	Rag2 ^{tm1.1Flv} ; Csf1 ^{tm1(CSF1)Flv} ; Csf2/Il3 ^{tm1(CSF2,IL3)Flv} ; Thpo ^{tm1(TPO)Flv} ; Il2rg ^{tm1.1Flv} ; Tg(SIRPA)1Flv	Immunodeficient, humanized; expresses 4 human cytokines	+++	++++ / not assessed	Rongvaux et al., 2014; Das et al., 2016; Ellegast et al., 2016
NOD.Cg-Kit ^{W-41j} Prkdc ^{scid} Il2rg ^{tm1Wjl} /Waskj	NSGW41	Kit ^{W-41j} ; Prkdc ^{scid} ; Il2rg ^{tm1Wjl}	NOD, immunodeficient, humanized, human engraftment without irradiation	+++	Not assessed	Cosgun et al., 2014
NOD.Cg-Kit ^{W-41j} Tyr + Prkdc ^{scid} Il2rg ^{tm1Wjl} /Thomj	NBSGW	Kit ^{W-41j} ; Tyr +; Prkdc ^{scid} ; Il2rg ^{tm1Wjl}	NOD, immunodeficient, humanized, human engraftment without irradiation	+++	Not assessed	McIntosh and Brown, 2015
C57BL/6 Rag2 ^{null} Il2rg ^{null} NOD-Sirpa Kit ^{Wv/Wv}	BRGSK	Rag2 ^{null} ; Il2rg ^{null} ; Kit ^{Wv/Wv}	NOD, immunodeficient, humanized, human engraftment without irradiation	+++	Not assessed	Yurino et al., 2016
NOD.Cg-Foxn1 ^{em1Dvs} Prkdc ^{scid} Il2rg ^{tm1Wjl} /J	NSG-nude	Foxn1 ^{em1Dvs} ; Prkdc ^{scid} ; Il2rg ^{tm1Wjl}	NOD, immunodeficient, radiosensitive, humanized, hairless	Not assessed	Not assessed / +++	Wei et al., 2017

LSC concept in ALL. Xenograft studies have shown that the LSC compartment in ALL can be genetically heterogeneous within the same individual and can evolve during cancer progression (le Viseur et al., 2008; Clappier et al., 2011; Notta et al., 2011; Rehe et al., 2013; Elder et al., 2017). In a recent work, ALL xenografts have been instrumental to determine how the microenvironment is fundamental for dormancy, drug resistance, and LSC activity (Ebinger et al., 2016). Thus, PDX models have represented a reliable tool in defining and supporting the stochastic stem cell concept in ALL and in shifting the attention to the LSC function rather than LSC immunophenotype (Passaro et al., 2016).

Although PDX models have increased our understanding in the cell biology of human hematopoietic malignancies, challenges remain in the observed engraftment heterogeneity. Each patient sample shows a distinct initial phenotype, specific LSC frequency, and individual phenotype in disease-propagating cells during xenotransplantation. Moreover, predominantly in myeloid malignancies, the engraftment levels of patient leukemic cells vary considerably, with some patients engrafting at a very low level or not at all. Hence, it has been reported that in AML intermediate-risk patient groups, engraftment in immunodeficient mice can predict the clinical outcome of the patients, and indeed it is reported that patient samples able to engraft in immunodeficient mice correlate with a poorer overall survival (Pearce et al., 2006). Why some patients engraft and others do not is still to be determined. This might be related to the low proliferation and low LSC frequency of some leukemias and/or to the dependence of some leukemias on the human-specific factors that are not provided by the mouse microenvironment. In agreement with this, a recent study has provided data demonstrating improved engraftment of some patient leukemic cells when mice were kept alive for up to 1 yr (Paczulla et al., 2017), therefore demonstrating that low cell proliferation of leukemic cells in NSG mice and lower LSC frequency were at least part of the problem.

There have been attempts to manipulate the mouse microenvironment to mimic the human BM niche and provide functional support to human stem cells in “humanized” immunodeficient mice, but caveats still remain. The injection of human cytokines directly into mice produced a transient improvement in engraftment, but this effect was subdued over time (Dao et al., 1997; Lapidot et al., 1997). Alternatively, the use of transgenic expression of human SCF (hSCF), hGM-CSF, and hIL-3 (three poorly cross-reacting cytokines) into either the NOD-SCID or, more recently, the NSG mice has led to improvements in the expansion of normal myeloid cells. This method has also enabled the engraftment of patient samples that have historically been very difficult to study in vivo, such as those harboring Core Binding Factor oncogenes (*AML1-ETO*) or *CBFβ-MYH11* (Nicolini et al., 2004; Wunderlich et al., 2010) or samples from *CMML* and *JMML* patients (Yoshimi et al., 2017). Nevertheless, the high level of human cytokines produced in these mice causes the exhaustion of human normal hematopoietic stem and progenitor cells (HSPCs; Nicolini et al., 2004; Wunderlich et al., 2010). To circumvent this problem and obtain physiological levels of human cytokines, knock-in mice have been developed, such as the MIS TRG (Rongvaux et al., 2014; Das et al., 2016; Ellegast et al., 2016).

These mice were genetically engineered to express human macrophage-stimulating factor, IL-3, SIRPα, thrombopoietin, and GM-CSF to allow efficient human cell development (Rongvaux et al., 2014). These mice show higher engraftment of HSPCs derived from peripheral blood than conventional NSG mice (Saito et al., 2016) and support a robust engraftment of chromosome 16 inversion, favorable-risk group AML patients (Ellegast et al., 2016). Using a different approach, patient-derived MSCs have been co-injected with myelodysplastic syndrome (MDS) cells directly in the BM cavity of NSG-SGM3 mice (Medyouf et al., 2014), and this resulted in an increase in engraftment, highlighting the niche-MDS dependence and relationship. In the study by Medyouf et al. (2014), MSCs were detected for up to 3 wk in the mouse BM. However, a recent study has shown that hMSCs injected intra-BM were rapidly undetectable (even in the injected bone) 1 wk after injection and thus might not significantly improve human MDS engraftment compared with the control (no hMSC injected) mice (Rouault-Pierre et al., 2017a). It is worth noting the timing differences in tracing the luciferase signal in both studies. Medyouf et al. (2014) started tracing hMSCs from day 3 after injection, whereas Rouault-Pierre et al. (2017a) traced the MSCs from 4 h after injection.

In conclusion, the quality, robustness, and reliability of human hematopoietic myelolymphoid xenografts in genetically modified mouse models have been instrumental for the understanding of the pathogenesis of hematological malignancies (Table 1; Bosma et al., 1983; McCune et al., 1988; Mosier et al., 1988; Fulop and Phillips, 1990; Koller et al., 1990; Lapidot et al., 1992; Mombaerts et al., 1992; Shinkai et al., 1992, 1993; Cao et al., 1995; Hesselton et al., 1995; Lowry et al., 1996; Pflumio et al., 1996; Greiner et al., 1998; Ito et al., 2002; Feuring-Buske et al., 2003; Yahata et al., 2003; Nicolini et al., 2004; Traggi et al., 2004; Ishikawa et al., 2005; Shultz et al., 2005; Pearson et al., 2008; Brehm et al., 2010; Wunderlich et al., 2010; Takagi et al., 2012; Cosgun et al., 2014; Maykel et al., 2014; Rongvaux et al., 2014; McIntosh and Brown, 2015; Das et al., 2016; Yurino et al., 2016; Wei et al., 2017). However, further development is still required to fully model these human hematopoietic diseases in vivo (Rouault-Pierre et al., 2017a,b; Wei et al., 2017).

Development of ectopic mature bone formation

Over the last few decades, research in bone grafts and prosthetic devices has been undertaken with the key aim of supporting the bone-healing process after injury. The use of fresh bone tissue as grafts is a well-understood field, as these grafts provide osteogenic cells, osteoinductive signals, and osteoconductive physical structures. However, bone graft substitutes are often required, and therefore, several bone implantable approaches have been developed incorporating one or all of the following properties: proper mechanical and osteoconductive properties, cytokine and growth factor carrier for proper delivery and induction of bone formation, and osteogenic cell carriers for proper bone formation (Greenwald et al., 2001; Civantos et al., 2017).

In a 1965 landmark study, evidence for the inductive properties of bone after ectopic implantation was described. In this study, the author shows the autoinduction of bone formation by the implanted material, naming the bony structures formed

in ectopic regions: “ossicles.” These ossicles showed sequential development of cartilage and bone tissue, with mature bone and BM tissues formed, a feature the author hypothesized as a possible new active site of hematopoiesis (Urist, 1965).

Since then, ectopic implantation approaches have been instrumental for the study and assessment of the osteoinductive property of cytokines, growth factors, and carrier materials. Remarkably, growth factors such as BMP-2 and BMP-7 (bone morphogenetic proteins) implanted in combination with suitable carrier materials in ectopic regions induce the formation of bone with mature, vascularized BM tissue (Abarrategi et al., 2008, 2009; El Bialy et al., 2017). Ectopic models have also helped to define the osteoinductive property of some ceramic materials, although the molecular mechanisms still remain unknown (Barradas et al., 2011) and require the empirical testing of the *in vivo* osteoinductive property for each different implantable ceramic material (Fig. 2; Mankani et al., 2001, 2004; Arinzech et al., 2005).

The implantation of cell carrier materials in subcutaneous, muscle, or kidney capsule bone ectopic scaffolds has been thoroughly reported (Scott et al., 2012), and this has helped to define the osteogenic activity of different types of cells (Nusspaumer et al., 2017). Subcutaneous implantation approaches incorporating MSCs, and the subsequent bone formation assessment, are considered to be one of the key assays that defines the multipotent nature of these cells (Bianco et al., 2013; Bianco and Robey, 2015). As a paradigmatic example, the kidney capsule and subcutaneous implantation approaches, combined with materials and growth factors, helped to identify a specific subset of mouse MSCs with defined phenotype and skeletal stem cell properties, located in the bone growth plate. These cells were able to differentiate to cartilage and bone tissues and had the capacity to generate ossicles with mature hematopoietic BM tissue (Chan et al., 2015). Similarly, the hematopoietic function of ossicles and the chimerism of the newly formed tissues between the host cells and implanted cells has also been well described (Shih et al., 2017).

Bone differentiation of subcutaneously implanted MSCs can be improved using different methods. For example, it is known that parathyroid hormone (PTH) administration increases BM HSC engraftment (Adams et al., 2007; Adams and Scadden, 2008) and stimulates osteoblast formation (Uusi-Rasi et al., 2005). Therefore, the exogenous administration of PTH in implanted mice has been tested to induce the differentiation of MSCs, giving rise to ectopic bone formation and further homing of HSCs in the subcutaneous ossicles (Schneider et al., 2003; Song et al., 2010). Using a different approach, scaffold-seeded MSCs can be differentiated *in vitro* to cartilage before implantation in a process called chondrogenic priming, which represents another possible avenue for ossicle formation (Freeman and McNamara, 2017).

Subcutaneous humanized niche approaches in mice

Recent advances in bioengineering have enabled the use of material scaffolds to create a humanized microenvironment that acts as a framework to support cell proliferation and differentiation and study cell–cell interactions with the aim of maintaining implanted cellular phenotypes and function. hMSCs are used to create a layer of stroma on the carrier material, which provides

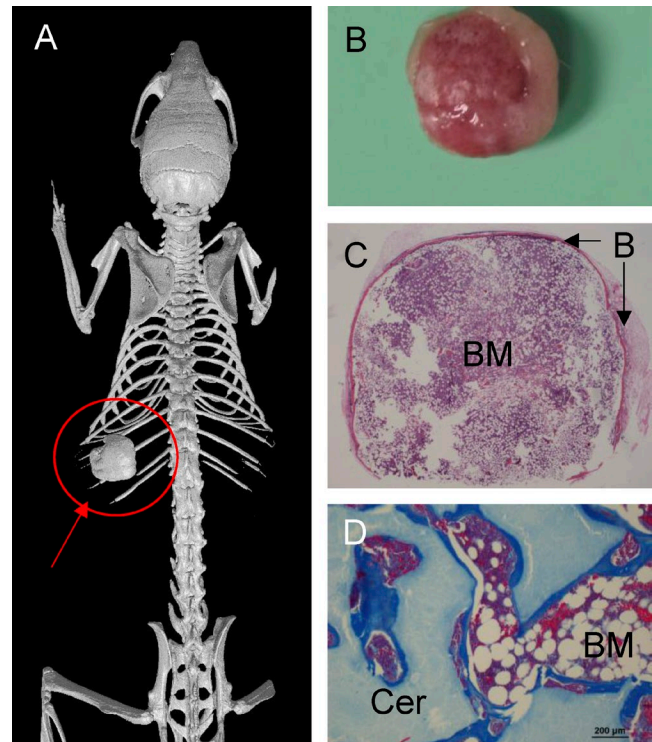


Figure 2. Ectopic bone “ossicle.” (A) Whole body micro-computerized tomography image showing bone tissue in a mouse. The red circle and the arrow show the location of a subcutaneous ossicle structure. (B) Gross morphology of a mouse-harvested ossicle. (C) Hematoxylin/eosin histological staining of an ossicle based on an implant of hMSC carrier gelatin sponge with BMP-2. Note bone tissue (B and black arrows) forming a ring on the surface of the ossicle and a core resembling adult BM tissue with trabecular bone, hematopoietic cells, adipocytes, and vascular structures. (D) Masson’s trichrome histological staining of an ossicle based on hMSC carrier ceramic implant. Note the remaining ceramic material in pale blue (Cer), newly formed bone in the surface of the ceramics in dark blue, and mature BM tissue with hematopoietic cells, adipocytes, and vascular structures with erythrocytes in red.

the “niche-like units” for other cell types to reside in. Once the osteogenic ability of implanted cells was defined, the aim of these studies shifted to understanding the multipotency of hMSCs and their effect on the niche. The resulting human MSC carrier implants yielded human–mouse chimeric tissues colonized by mouse hematopoietic cells, suggesting this approach generates “humanized” microenvironments with niche properties, useful for hematopoietic studies and metastatic processes of various cancers (Moreau et al., 2007; Lee et al., 2012; Bersani et al., 2014; Holzapfel et al., 2014; Čulen et al., 2015; Francis et al., 2016; Nelson and Roy, 2016; Theocharides et al., 2016; Aguado et al., 2017; Martine et al., 2017).

Among the various available materials, osteoinductive ceramic scaffolds have also been used in combination with hMSCs in mouse subcutaneous implantation approaches and gave rise to the formation of ossicles of human–mouse chimeric bone with mature mouse BM tissues (Krebsbach et al., 1997; Chai et al., 2012). Using osteoinductive ceramics and hMSCs as implantable materials, a MCAM⁺/CD146⁺ subpopulation of human BM stroma cells associated with hematopoietic niche formation was identified (Sacchetti et al., 2007). The implanted

human stromal cells induced the formation of sinusoids inside the implants, and these cells produced angiopoietin-1, a ligand for the Tie-2 cell surface receptor, in the HSC niche (Sacchetti et al., 2007), providing evidence for a mechanism by which HSCs can home to the new humanized niche.

To challenge the *in vivo* multipotent osteogenic properties of implanted human cells, various studies have used BMPs in combination with different implantable materials and hMSCs (Park et al., 2009; Burastero et al., 2010). For example, BMP-2 carrier ceramic scaffolds have been used to define the *in vivo* differentiation multipotency of hMSCs (Abarrategi et al., 2013). Furthermore, hMSCs have also been genetically modified to express BMP-2 or BMP-7, and these cells can generate subcutaneous ossicles in mice (Turgeman et al., 2001; Dragoo et al., 2005; Kang et al., 2007). Interestingly, the use of human bone-forming growth factors can generate mature BM tissue with hematopoiesis, implying the presence of a human niche in these xenograft structures. In a related study, this model system was also successfully used to study human breast cancer metastasis (Moreau et al., 2007). Therefore, such models form humanized hematopoietic microenvironments and are applicable for understanding the role of the BM microenvironment and stem cells in tumor metastasis.

Another approach to generate humanized niches is to *in vitro* culture cell-seeded materials before implantation, an approach used in various other studies to further our understanding of material niche formation. *In vivo*, endochondral and intramembranous ossification processes are associated with hematopoietic niche formation (Reinisch et al., 2015), and therefore, the *in vitro* endochondral priming procedure has been tested to induce the formation of humanized bone and BM tissues *in vivo*. Interestingly, using this procedure, hMSCs have been associated with cartilage tissue remodeling, as well as vascularization of the newly formed bone tissues, perhaps because of the angiogenic factors produced by chondrocytes, which naturally stimulate angiogenesis *in vivo* (Peltari et al., 2006; Farrell et al., 2008, 2011; Scotti et al., 2010, 2013; Sheehy et al., 2015; Thompson et al., 2015; Visser et al., 2015; Yang et al., 2015; Freeman and McNamara, 2017). In another study, ceramic-coated materials seeded with hMSCs were cultured initially *in vitro* with the addition of BMP-7 and subsequently implanted in mice, generating a chimeric bone construct with metabolically active tissue producing extracellular matrix components. This tissue-engineered chimeric niche was demonstrated to serve as a reliable platform to study prostate cancer bone metastases (Holzapfel et al., 2014).

Human vasculature structure, and therefore the human perivascular niche for hematopoietic cells, can also be generated in structures implanted in mice. HUVECs subcutaneously implanted in Matrigel or other carrier materials can form a human vascular network (Schechner et al., 2000; Skovseth et al., 2007; Cooper and Sefton, 2011). Moreover, the exogenous overexpression of the *E4ORF1* gene in HUVECs improves their survival *in vivo* and their ability to form a humanized vasculature network (Seandel et al., 2008). As mesenchymal and ECs closely interact during neovascularization, MSCs have been co-implanted with human ECs, improving the vascular tissue formation as a result of the perivascular function of implanted MSCs (Koike et al., 2004;

Scherberich et al., 2007; Ghanaati et al., 2011; McFadden et al., 2013; Pedersen et al., 2013; Lin et al., 2017).

It is important to note that some studies have opted to use similar tools to those described here to develop *in vitro* methods, aiming to study human hematopoietic cell behavior under *ex vivo* conditions. Based on co-culture studies, three-dimensional (3D) models have been developed using various types of materials with different human stromal cells along with human hematopoietic cells (Jing et al., 2010; Ferreira et al., 2012; Leisten et al., 2012; Mortera-Blanco et al., 2012; Sharma et al., 2012; Cuddihy et al., 2013; Raic et al., 2014; Bara et al., 2015; Choi et al., 2015; Miyoshi et al., 2015; Dhama et al., 2016; Dong et al., 2016). A novel BM-on-a-chip system, where human BM tissue is generated in a polydimethylsiloxane device *in vivo* and then cultured in a microfluidic system, has been recently developed (Torisawa et al., 2014). Using a similar concept, a 3D co-culture system based on a hydroxyapatite coated zirconium oxide scaffold seeded with hMSCs and HSPCs has also been developed (Sieber et al., 2017). Both these studies demonstrated that the engineered BM retains HSPCs within the formed “hematopoietic-like niche,” in proportions comparable with *in vivo* studies, for up to 28 d in culture (Torisawa et al., 2014; Sieber et al., 2017). These *in vitro* models provide an interesting alternative, with the ability to genetically or pharmacologically manipulate individual hematopoietic cell populations or to add other stroma components (such as ECs), as well as cytokines, in a step-wise manner *in vitro* and then analyze the response of the engineered BM.

Although most of the studies reporting *in vivo* bone forming assays and *in vitro* BM modeling used as stroma primary BM-derived cells, some of these used primary stroma niche cells from other tissues (Dragoo et al., 2005; Kang et al., 2007; Ferreira et al., 2012; Leisten et al., 2012; Mortera-Blanco et al., 2012; Sharma et al., 2012; Raic et al., 2014; Reinisch et al., 2015), transfected or transduced cells (Turgeman et al., 2001; Dragoo et al., 2005; Kang et al., 2007; Seandel et al., 2008), or even stroma cell lines (Miyoshi et al., 2015). It is worth noting that the source and status of stroma compartment used should be taken into account, as it may influence the cellular cross talk and niche function of stroma cells.

Subcutaneous humanized niche to study human healthy HSPCs

The recent technological advances in generating material-based humanized hematopoietic niches have not only been examined for mouse hematopoietic cell colonization, but also in human hematopoietic transplantation studies (Fig. 3).

Polyacrylamide hydrogel-based scaffolds seeded with human stromal cells and implanted in immunodeficient mice, followed by injection of BM CD34⁺ cells, provided evidence of colonization by human hematopoietic cells (Lee et al., 2012, 2016). Notably, there was an increased secretion of cytokines such as IL-6 and VEGF inside the implanted scaffolds, originating from the hMSCs, enhancing angiogenesis as well as homing of hHSCs (Lee et al., 2012). After 16 wk, human CD45⁺ cell engraftment was detected in the scaffolds, as well as the native mouse BM (Lee et al., 2012). These scaffolds were further developed by genetically engineering the human stroma cells before their implantation in scaffolds, aiming to generate an array of humanized

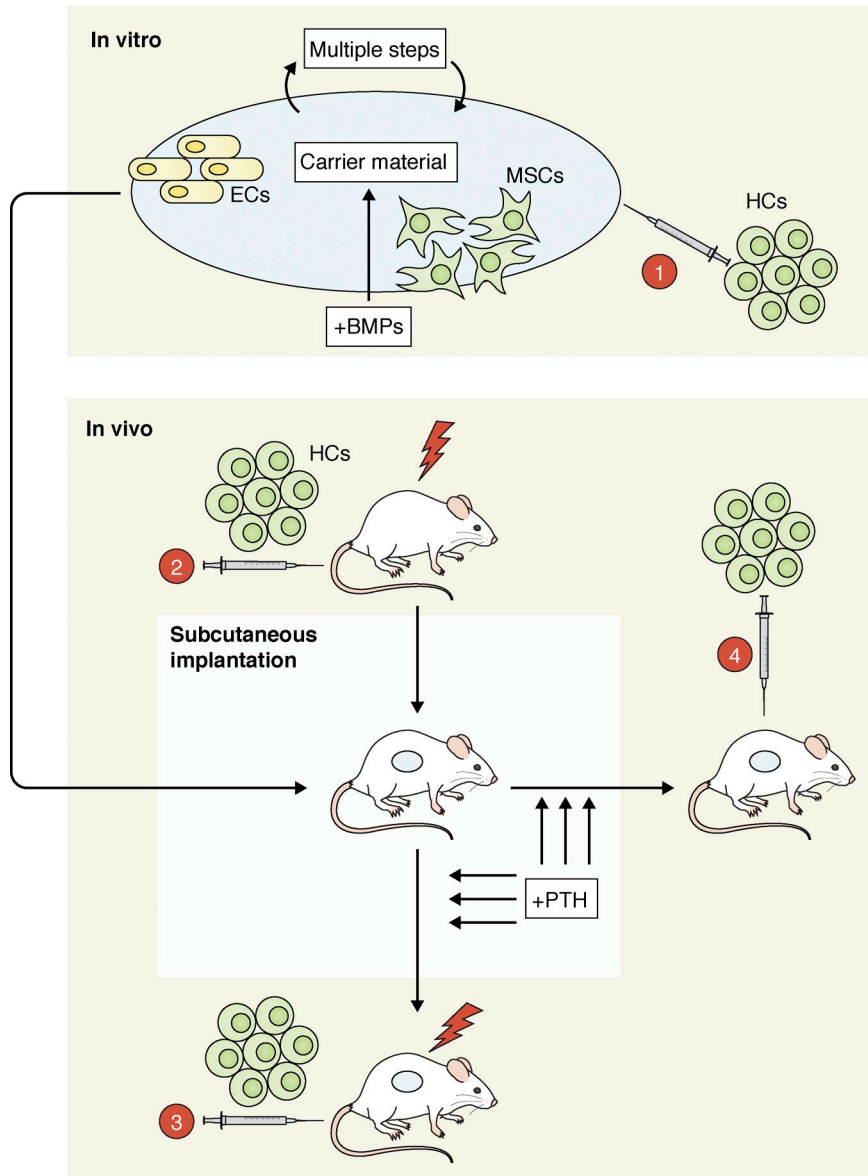


Figure 3. Different approaches to bioengineer humanized hematopoietic niche. All approaches are based on an in vitro step to prepare an implantable structure with hMSCs and a cell carrier material. Some approaches include an in vitro cell differentiation step, the co-seeding of hECs, or the addition of osteogenic factors such as BMPs before implantation in mice. Following these first step, the human cell carrier devices are then implanted in mice aiming to generate subcutaneous humanized niches in vivo. Human hematopoietic cells can be integrated in the system at different steps. They can be seeded in vitro **(1)**, before the in vivo implantation of the device. They can be i.v. injected in the mouse before **(2)** or after the implantation of the device **(3)**, and they can also be injected directly inside the device after implantation **(4)**. Bone formation in vivo may be promoted via systemic PTH injection. Red symbols on top of the mice represent sublethal irradiation. HCs, human BM cells.

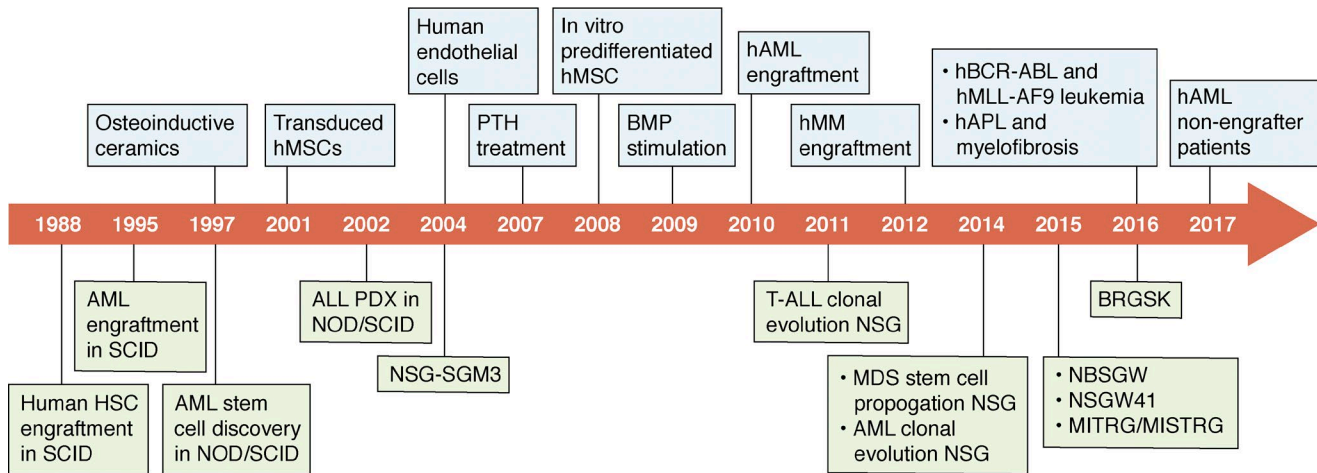
niches within the same scaffold (Lee et al., 2016). This enabled the authors to study the effect of expressing different cytokines in the scaffolds on human HSC recruitment, engraftment, and differentiation potential (Lee et al., 2016).

In a separate study, hMSCs and ECs, supplemented with Matrigel, were directly injected in NSG mice, followed by intravenous injection of human cord blood cells (Chen et al., 2012). This approach enabled the formation of extramedullary bone, with BM cavities exhibiting hypoxic environments in discrete areas, mirroring the human BM niche. Moreover, these extramedullary humanized niches supported both human as well as murine hematopoietic cells, with engraftment levels of human myelolympho lineages comparable with those in host mouse BM tissues (Chen et al., 2012). The use of injectable Matrigel as cell carrier material has been further investigated by Reinisch et al. (2015), who demonstrated that, among hMSCs from different sources, only BM-derived MSCs were able to form a BM cavity, through a vascularized cartilage intermediate: a process the authors named “endochondral signature niche formation.” In

this case, PTH was used to induce in vivo human stromal cell differentiation and ossicle formation (Reinisch et al., 2017). These in vivo humanized structures supported human hematopoiesis, with functional human HSCs able to successfully engraft secondary recipient mice (Reinisch et al., 2015).

A further subcutaneous scaffold structure, using biodegradable polycaprolactone seeded with hMSCs and cultured in vitro with osteogenic differentiation media before implantation demonstrated that it is feasible to create humanized bone constructs in NSG mice with humanized niche formation (Holzapfel et al., 2015a). When human CD34⁺ cells were injected by retro-orbital intravenous injection, these humanized structures were capable of recapitulating both the morphological features as well as biological functions of the human niche, with a higher engraftment of hCD45⁺ cells in the humanized scaffolds compared with the host mouse bones. This led authors to speculate that species-specific HSC microenvironment interactions may be vital for human hematopoietic xenograft studies (Holzapfel et al., 2015a).

Human MSC-based implantable hematopoietic niche



Human hematopoietic PDX in mouse BM niche

Figure 4. **Timeline of PDX in human hematopoietic context. (Bottom)** Milestones in human hematopoietic PDX approaches are reported, based on engraftment in host mouse BM niche. **(Top)** Milestones in human hematopoietic PDX approaches are reported, based on the generation of implantable humanized microenvironment with hMSCs. Refer to [Table 1](#) and text for more details and references.

The aforementioned scaffold systems vary in materials and methodology, but all rely on bone formation for MSCs as a key step in *in vivo* scaffold development. Interestingly, an alternative, non-bone-forming, implantable approach has been reported to also maintain and expand human HSPCs. In this case, cord blood-derived human CD34⁺ cells were included with hMSCs and ECs in carrier scaffolds before implantation, aiming to simultaneously provide human HSPCs and human niche components together in the scaffolds to integrate signals from the three key components. Interestingly, these scaffolds allow the long-term engraftment of human HSPCs, with improved myeloid development compared with lymphoid-biased engraftment usually seen in the BM of intravenous/intrabone injected NSG mice ([Abarrategi et al., 2017](#); [Passaro et al., 2017a](#)).

Subcutaneous humanized niche to study human malignant hematopoietic cells

The approaches describing implanting scaffolds with a humanized microenvironment for investigating normal hematopoietic engraftment have proven equally valuable in studying hematopoietic malignancies. Indeed, recently there has been great success using scaffold approaches to model hematopoietic malignancies *in vivo*, using primary patient cells that are poor “engrafters” in host mouse BM tissue ([Fig. 4](#); [Čulen et al., 2015](#); [Ho et al., 2015](#); [Holzapfel et al., 2015b](#); [Flores-Figueroa and Gratzinger, 2016](#); [Nelson and Roy, 2016](#); [Theocharides et al., 2016](#)).

The first study of successful AML engraftment in humanized microenvironment came from [Vaiselbuh et al. \(2010\)](#), who developed an ectopic niche by coating polyurethane scaffolds with human BM-MSCs. After the implantation of these scaffolds into NOD-SCID mice, *de novo* vascularization and osteoclast as well as adipocyte development demonstrated an organized human BM microenvironment 8 wk after implantation. Primary AML cells injected directly in preimplanted scaffolds, or

injected retroorbitally, engrafted in the scaffolds and were able to proliferate in a process the authors postulated is reliant on the CXCL12–CXCR4 axis. Notably, human leukemic cells migrated out of the scaffolds and colonized the murine BM, liver, and spleen during later stages, raising the possibility that there might be a requirement for human-specific stroma during the initial stages of leukemia establishment, but this may not be required for relocation at later stages of leukemic development. Furthermore, CD34⁺ LSCs were observed in scaffolds 5 mo after implantation, and these LSCs were found in direct contact with the human stroma, suggesting it as a preferable niche for this subset of leukemic cells ([Vaiselbuh et al., 2010](#)).

In addition to AML, [Groen et al. \(2012\)](#) applied the use of humanized scaffolds to engraft primary multiple myeloma patient samples, which are known to be highly dependent on the human BM microenvironment for their survival and growth. Biphasic calcium phosphate-based osteoinductive ceramic particles were loaded with hMSCs, cultured in osteogenic differentiation media for 7 d, and then implanted subcutaneously in RAG2^{-/-}γc^{-/-} mice ([Siddappa et al., 2007, 2008](#); [Prins et al., 2009](#); [Groen et al., 2012](#)). After successful implantation of scaffolds, primary multiple myeloma patient samples were injected either directly into the humanized ossicles or, alternatively, via an intracardiac route ([Groen et al., 2012](#)). All patient samples in this study were able to successfully engraft in the humanized ossicles, whereas only one patient sample was able to engraft in the host mouse BM. Using the same technical approach, MLL-AF9-transduced cord blood CD34⁺ cells and CD34⁺ cells from CML patients were implanted in humanized scaffold in NSG mice. Transcriptome analysis of the human leukemic cells recovered demonstrated that “stemness,” as well as the disease phenotype, was better preserved in the humanized niche compared with the murine BM ([Sontakke et al., 2016](#)). Moreover, similar scaffolds were formed with genetically engineered hMSCs, which secrete

human IL-3 and thrombopoietin (TPO), with better preservation of the myeloid compartment (Carretta et al., 2017).

A later study demonstrated the great promise for modeling previously nonengrafting AML in humanized microenvironments. Using ceramic scaffolds coated with hMSCs, Antonelli et al. (2016) showed positive engraftment (29/39 patients engrafted) from a large cohort of AML patients. Within 12 wk, subcutaneously implanted scaffolds formed structures mimicking the human BM niche, including bone formation with embedded mouse vasculature. Interestingly, favorable-risk AML patients with an inversion of chromosome (16) aberration, which usually do not engraft in the murine BM, were able to engraft in humanized scaffolds in NSG mice. BM cells from intermediate-risk AML patients engrafted in scaffolds in NSG and Rag2^{-/-}γc^{-/-} mouse models, and these patient cells also colonized the host murine organs, including BM, spleen, and liver. More importantly, ossicles enabled maintenance of the clonal heterogeneity in xenografted cells recovered from primary transplants, and their LSC self-renewal capacity was retained, as demonstrated by serial transplantation assays (Antonelli et al., 2016).

In a similar study using a different technical approach, Reinisch et al. (2016) demonstrated a similar retention of subclonal architecture in AML patient samples. Humanized ossicles were obtained by PTH treatment in hMSC carriers and then subcutaneously injected into mice, and normal, as well as malignant human, hematopoietic cells were transplanted in NSG mice 8 wk after the preimplantation of hMSC carrier. With a total in vivo experiment time of 34 wk, results showed that human AML cells inside humanized ossicles recapitulated the original subclonal architecture of AML patient samples with a higher frequency of AML LSCs compared with traditional injection methods. Furthermore, this model also enabled the successful engraftment of acute promyelocytic leukemia patient cells and myelofibrosis patient samples (harboring *JAK2* or calreticulin mutation), which have previously failed to engraft in NSG mouse BM (Reinisch et al., 2016).

In two recent studies, hMSCs seeded in gelatin-based carrier scaffolds were used to study leukemic cells from AML patients in vivo. These studies used both non-bone-forming as well as BMP-2 bone-forming scaffolds and reported the engraftment kinetics of AML patient-derived cells. It is important to note that these patient cells were not able to engraft in the BM of the mouse, therefore highlighting the need for the humanized niche for human LSC maintenance. This humanized scaffold approach can be used not only with BM-derived hMSCs, but also with human ECs, providing the opportunity to mimic multiple different human hematopoietic niches in the implanted scaffolds (Abarrategi et al., 2017; Passaro et al., 2017a). These studies suggested that donor MSCs can act as a cell source for neotissue formation after in vivo implantation of the scaffolds, while also enabling the recruitment of host cells that can subsequently participate in this neovascularization.

Finally, Battula et al. (2017) developed a different approach for studying human AML in mice, a process they termed “human bone implant” in mice. This approach uses freshly collected human BM biopsies from hip replacement patients and directly transplants it subcutaneously into NSG mice, using Matrigel as a

carrier. The mouse-implanted human BM tissue undergoes vascularization and bone restoration, providing a functional human BM microenvironment capable of supporting the engraftment with human leukemia, a process the authors related to increased osteogenic activity in human bone implant (Battula et al., 2017).

In summary, the evolution of strategies used over the years to develop preclinical models to study human normal and malignant hematopoiesis have tried to answer a multitude of questions using various tissue engineering approaches. Some of these studies have developed various protocols where carrier material scaffolds require various periods of in vitro culturing prior to in vivo implantation (Martine et al., 2017), whereas others use in vivo models directly (Abarrategi et al., 2017; Passaro et al., 2017a), with varying experimental time frames ranging from 12 wk (Abarrategi et al., 2017) to 34 wk (Reinisch et al., 2016). Another important aspect to note is the use of stromal cell types for generating the humanized niche. Most studies have only used human mesenchymal cells, whereas others have co-injected human ECs (or total human BM cells) to generate vascular structures, thereby increasing the humanized nature of the implants (Chen et al., 2012; Battula et al., 2017; Passaro et al., 2017a). Lastly, the use of conditioning regimens in mice (such as irradiation) after the implantation of the cell-seeded biomaterial (Holzapfel et al., 2015a; Reinisch et al., 2016, 2017) is somewhat controversial, as it may adversely affect the newly formed hematopoietic niche and regress the vasculatures that are derived from hMSCs and ECs.

It is worth noting that each of these approaches have specific and sometimes unique scientific values, and all will undeniably play a significant role in various aspects of future preclinical studies.

Conclusions and future directions

Modeling human hematopoiesis in mice is a thoroughly developed approach, with applications in stem cell studies, clonal evolution, and drug screening, to name a few. Continuous efforts have been made to develop new mouse models with a more humanized microenvironment, which would be more permissive for human studies. However, despite great improvements in human hematopoietic cell engraftment and the success in recapitulating disease phenotypes, some malignancies are still difficult to model in xenotransplantation. Moreover, current PDX models present some limitations related to lacking/insufficient immune systems or interspecies differences of growth factors and receptors, among others.

Advancements in bioengineering and carrier materials have provided an improved model system that can generate humanized microenvironments, which can be used as an alternative to the traditional xenotransplantation approach. Although traditional in vivo assays have proven useful in understanding human hematopoiesis in the mouse microenvironment, implantable scaffold methods are able to incorporate assessment of multicellular interactions between human stromal cells and HSCs. However, questions remain regarding the specific role of the mouse vasculature, as well as the cytokines supplied by the mouse system to these humanized scaffolds. Other important aspects that need to be examined are the optimal human stromal cell types, carrier material, and in vitro culture conditions that would allow

the development of a robust human niche *in vivo*. It is important to engineer scaffolds that can closely mimic the multicellular aspects of the human bone, but considering the complexity of the human BM tissue, even in the best scenario, these engineered scaffolds will still represent a model with their own specific limitations. Despite this, this new approach can provide an important tool to potentially generate patient-specific human microenvironments in mice that can be used to unravel the role of human tumor microenvironments, disease pathology, and physiological response to drugs. Ultimately, the use of many of these bioengineered models and continuous efforts to improve their effectiveness, as well as physiological relevance, will propel preclinical studies to a new era of targeted therapeutic development. This represents an exciting period, wherein these preclinical mouse models will not only serve simply to confirm clinical outcomes, but also have the potential to routinely enhance clinical success.

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