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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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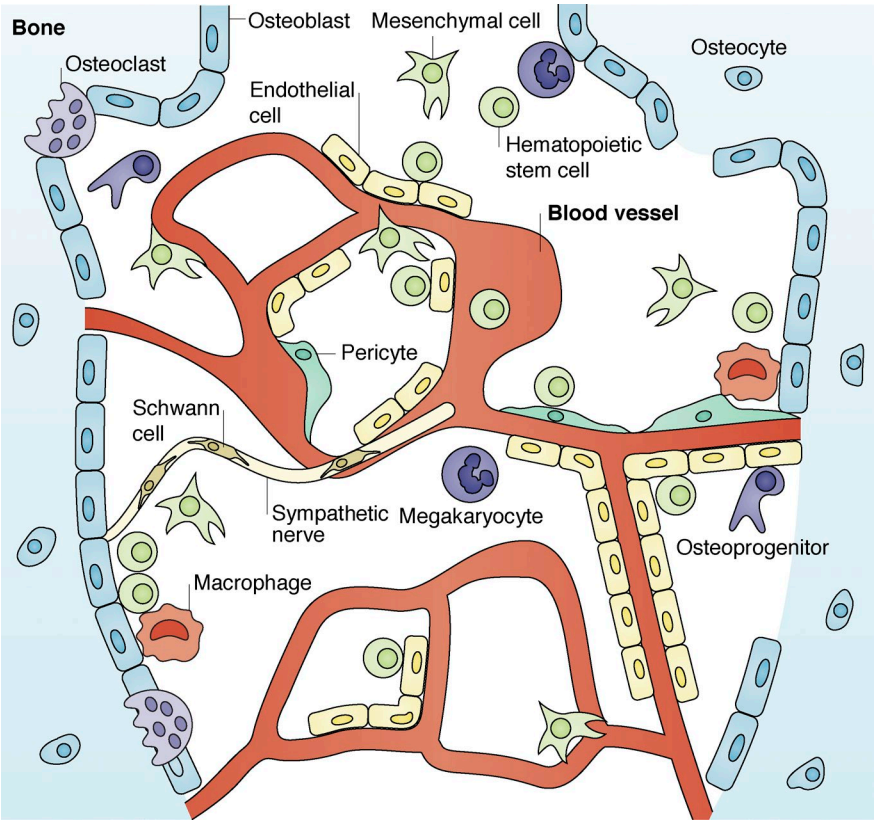


Figure 1. **The hematopoietic BM niche.** The BM is a heterogeneous environment composed of different types of cells. The two main architectural scaffolds of the tissue are the bone and the vessels, integrated in a complex network connected to nerve fibers. Associated with these structures are different 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-2Rγ$^{null}$ | *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$^{tm1.Flv}$Csf1$^{tm1(CSF1)Flv}$Csf2Il3$^{tm1.1(CSF2,IL3)Flv}$Thpo$^{tm1.1(TPO)Flv}$Il2rg$^{tm1.1Flv}$/J | MITRG | Rag2$^{tm1.Flv}$; Csf1$^{tm1(CSF1)Flv}$; Csf2Il3$^{tm1.1(CSF2,IL3)Flv}$;Thpo$^{tm1.1(TPO)Flv}$; 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$^{tm1.1(CSF2,IL3)Flv}$ Thpo$^{tm1.1(TPO)Flv}$ Il2rg$^{tm1.1Flv}$ Tg(SIRPA)1Flv/J | MISTRG | Rag2$^{tm1.1Flv}$ Csf1$^{tm1(CSF1)Flv}$ Csf2/Il3$^{tm1.1(CSF2,IL3)Flv}$ Thpo$^{tm1.1(TPO)Flv}$ 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; Traggiai 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; Arinzeh 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

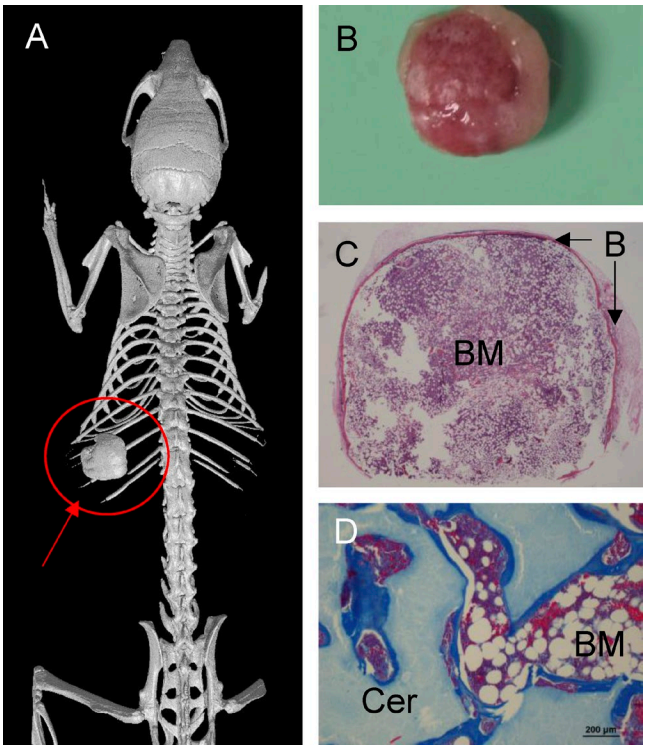


Figure 2. **Ectopic bone "ossicle." (A)** Whole body micro–computerized tomography image showing bone tissue in a mouse. The red circle and the arrow show the location of a subcutaneous ossicle structure. **(B)** Gross morphology of a mouse-harvested ossicle. **(C)** Hematoxylin/eosin histological staining 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 (Pelttari 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; Dhami 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

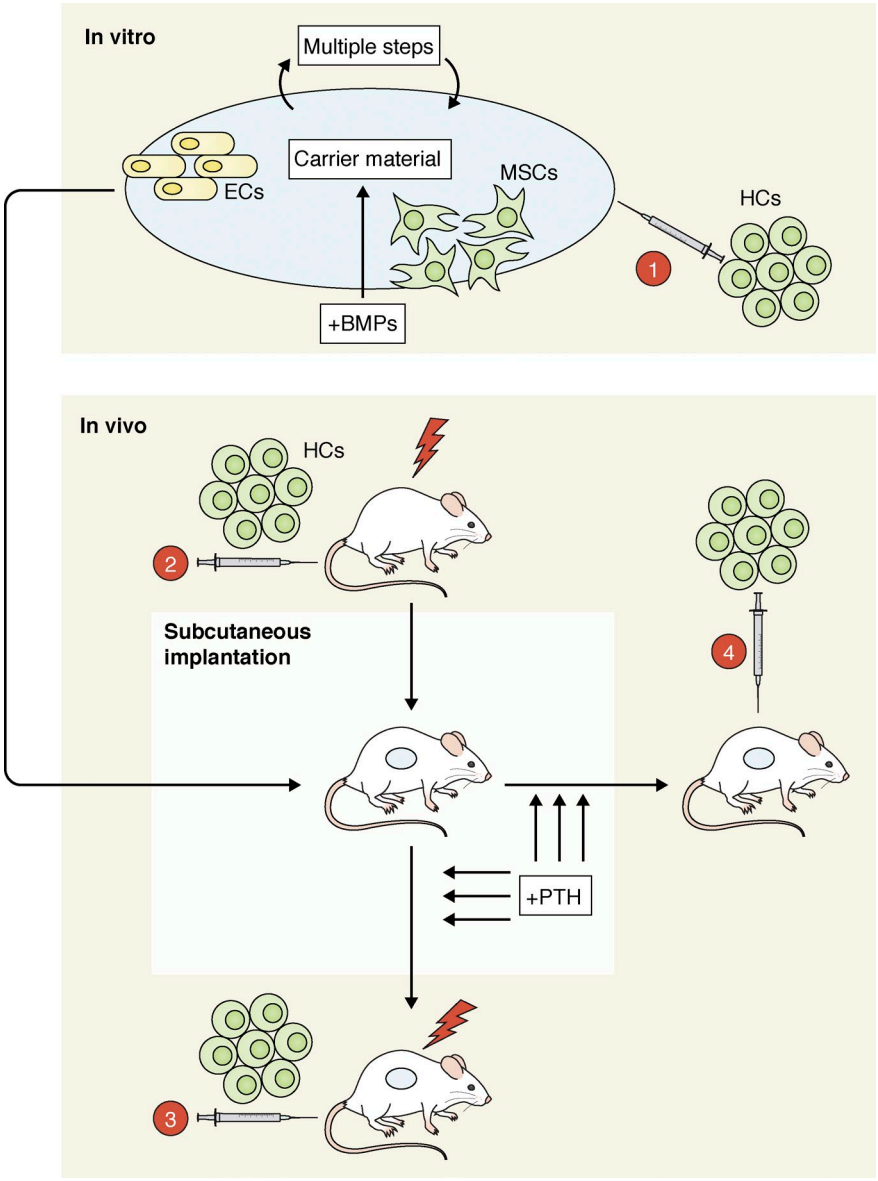


Figure 3. **Different approaches to bioengineer humanized hematopoietic niche.** All approaches are based on an in vitro step to prepare an implantable structure with hMSCs and a cell carrier material. Some approaches include an in vitro cell differentiation step, the co-seeding of hECs, or the addition 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).

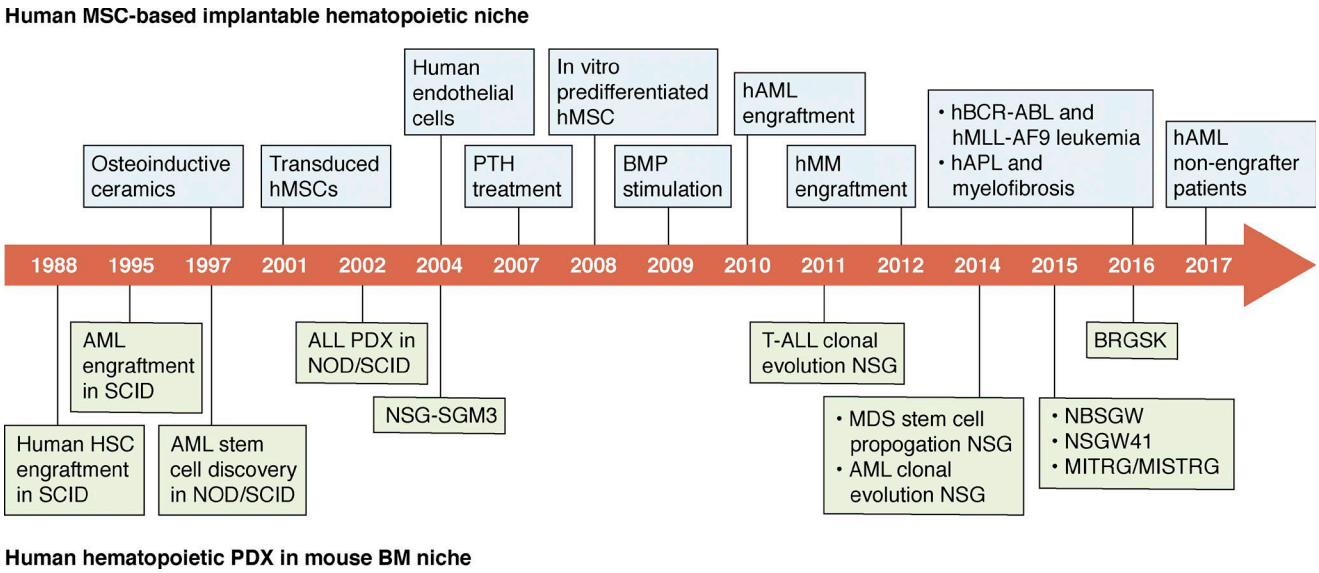


Figure 4. **Timeline of PDX in human hematopoietic context. (Bottom)** Milestones in human hematopoietic PDX approaches are reported, based on engraftment in host mouse BM niche. **(Top)** Milestones in human hematopoietic PDX approaches are reported, based on the generation of implantable humanized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

The aformentioned 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$^{-/-}\gamma_c^{-/-}$ 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$^{-/-}\gamma c^{-/-}$ 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 regiments 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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## References

Abarrategi, A., A. Civantos, V. Ramos, J.V. Sanz Casado, and J.L. López-Lacomba. 2008. Chitosan film as rhBMP2 carrier: delivery properties for bone tissue application. *Biomacromolecules*. 9:711–718. https://doi.org/10.1021/bm701049g

Abarrategi, A., J. García-Cantalejo, C. Moreno-Vicente, A. Civantos, V. Ramos, J.V. Casado, S. Pérez-Rial, R. Martínez-Corriá, and J.L. López-Lacomba. 2009. Gene expression profile on chitosan/rhBMP-2 films: A novel osteoinductive coating for implantable materials. *Acta Biomater.* 5:2633–2646. https://doi.org/10.1016/j.actbio.2009.02.031

Abarrategi, A., R. Perez-Tavarez, M.A. Rodriguez-Milla, I. Cubillo, F. Mulero, A. Alfranca, J.L. Lopez-Lacomba, and J. García-Castro. 2013. In vivo ectopic implantation model to assess human mesenchymal progenitor cell potential. *Stem Cell Rev.* 9:833–846. https://doi.org/10.1007/s12015-013-9464-1

Abarrategi, A., K. Foster, A. Hamilton, S.A. Mian, D. Passaro, J. Gribben, G. Mufti, and D. Bonnet. 2017. Versatile humanized niche model enables study of normal and malignant human hematopoiesis. *J. Clin. Invest.* 127:543–548. https://doi.org/10.1172/JCI89364

Adams, G.B., and D.T. Scadden. 2008. A niche opportunity for stem cell therapeutics. *Gene Ther.* 15:96–99. https://doi.org/10.1038/sj.gt.3303063

Adams, G.B., R.P. Martin, I.R. Alley, K.T. Chabner, K.S. Cohen, L.M. Calvi, H.M. Kronenberg, and D.T. Scadden. 2007. Therapeutic targeting of a stem cell niche. *Nat. Biotechnol.* 25:238–243. https://doi.org/10.1038/nbt1281

Aguado, B.A., G.G. Bushnell, S.S. Rao, J.S. Jeruss, and L.D. Shea. 2017. Engineering the pre-metastatic niche. *Nat. Biomed. Eng.* 1:0077. https://doi.org/10.1038/s41551-017-0077

Antonelli, A., W.A. Noort, J. Jaques, B. de Boer, R. de Jong-Korlaar, A.Z. Brouwers-Vos, L. Lubbers-Aalders, J.F. van Velzen, A.C. Bloem, H. Yuan, et al. 2016. Establishing human leukemia xenograft mouse models by implanting human bone marrow-like scaffold-based niches. *Blood.* 128:2949–2959. https://doi.org/10.1182/blood-2016-05-719021

Arinzeh, T.L., T. Tran, J. Mcalary, and G. Daculsi. 2005. A comparative study of biphasic calcium phosphate ceramics for human mesenchymal stem-cell-induced bone formation. *Biomaterials.* 26:3631–3638. https://doi.org/10.1016/j.biomaterials.2004.09.035

Baersch, G., T. Möllers, A. Hötte, B. Dockhorn-Dworniczak, C. Rübe, J. Ritter, H. Jürgens, and J. Vormoor. 1997. Good engraftment of B-cell precursor ALL in NOD-SCID mice. *Klin. Padiatr.* 209:178–185. https://doi.org/10.1055/s-2008-1043947

Bara, J.J., M. Herrmann, U. Menzel, L. Benneker, M. Alini, and M.J. Stoddart. 2015. Three-dimensional culture and characterization of mononuclear cells from human bone marrow. *Cytotherapy.* 17:458–472. https://doi.org/10.1016/j.jcyt.2014.12.011

Barradas, A.M., H. Yuan, C.A. van Blitterswijk, and P. Habibovic. 2011. Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. *Eur. Cell. Mater.* 21:407–429. https://doi.org/10.22203/eCM.v021a31

Battula, V.L., P.M. Le, J.C. Sun, K. Nguyen, B. Yuan, X. Zhou, S. Sonnylal, T. McQueen, V. Ruvolo, K.A. Michel, et al. 2017. AML-induced osteogenic differentiation in mesenchymal stromal cells supports leukemia growth. *JCI Insight.* 2:e90036. https://doi.org/10.1172/jci.insight.90036

Bersani, F., J. Lee, M. Yu, R. Morris, R. Desai, S. Ramaswamy, M. Toner, D.A. Haber, and B. Parekkadan. 2014. Bioengineered implantable scaffolds as a tool to study stromal-derived factors in metastatic cancer models. *Cancer Res.* 74:7229–7238. https://doi.org/10.1158/0008-5472.CAN-14-1809

Bianco, P., and P.G. Robey. 2015. Skeletal stem cells. *Development.* 142:1023–1027. https://doi.org/10.1242/dev.102210

Bianco, P., X. Cao, P.S. Frenette, J.J. Mao, P.G. Robey, P.J. Simmons, and C.-Y. Wang. 2013. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nat. Med.* 19:35–42. https://doi.org/10.1038/nm.3028

Bonnet, D., and J.E. Dick. 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* 3:730–737. https://doi.org/10.1038/nm0797-730

Borgmann, A., C. Baldy, A. von Stackelberg, B. Beyermann, I. Fichtner, P. Nürnberg, and G. Henze. 2000. Childhood all blasts retain phenotypic and genotypic characteristics upon long-term serial passage in NOD/SCID mice. *Pediatr. Hematol. Oncol.* 17:635–650. https://doi.org/10.1080/08880010050211349

Bosma, G.C., R.P. Custer, and M.J. Bosma. 1983. A severe combined immunodeficiency mutation in the mouse. *Nature.* 301:527–530. https://doi.org/10.1038/301527a0

Brehm, M.A., A. Cuthbert, C. Yang, D.M. Miller, P. DiIorio, J. Laning, L. Burzenski, B. Gott, O. Foreman, A. Kavirayani, et al. 2010. Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation. *Clin. Immunol.* 135:84–98. https://doi.org/10.1016/j.clim.2009.12.008

Bruns, I., D. Lucas, S. Pinho, J. Ahmed, M.P. Lambert, Y. Kunisaki, C. Scheiermann, L. Schiff, M. Poncz, A. Bergman, and P.S. Frenette. 2014. Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. *Nat. Med.* 20:1315–1320. https://doi.org/10.1038/nm.3707

Burastero, G., S. Scarfì, C. Ferraris, C. Fresia, N. Sessarego, F. Fruscione, F. Monetti, F. Scarfò, P. Schupbach, M. Podestà, et al. 2010. The association of human mesenchymal stem cells with BMP-7 improves bone regeneration of critical-size segmental bone defects in athymic rats. *Bone.* 47:117–126. https://doi.org/10.1016/j.bone.2010.03.023

Cao, X., E.W. Shores, J. Hu-Li, M.R. Anver, B.L. Kelsall, S.M. Russell, J. Drago, M. Noguchi, A. Grinberg, E.T. Bloom, et al. 1995. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity.* 2:223–238. https://doi.org/10.1016/1074-7613(95)90047-0

Carretta, M., B. de Boer, J. Jaques, A. Antonelli, S.J. Horton, H. Yuan, J.D. de Bruijn, R.W.J. Groen, E. Vellenga, and J.J. Schuringa. 2017. Genetically engineered mesenchymal stromal cells produce IL-3 and TPO to further improve human scaffold-based xenograft models. *Exp. Hematol.* 51:36–46. https://doi.org/10.1016/j.exphem.2017.04.008

Chai, Y.C., S.J. Roberts, E. Desmet, G. Kerckhofs, N. van Gastel, L. Geris, G. Carmeliet, J. Schrooten, and F.P. Luyten. 2012. Mechanisms of ectopic bone formation by human osteoprogenitor cells on CaP biomaterial carriers. *Biomaterials.* 33:3127–3142. https://doi.org/10.1016/j.biomaterials.2012.01.015

Chan, C.K., E.Y. Seo, J.Y. Chen, D. Lo, A. McArdle, R. Sinha, R. Tevlin, J. Seita, J. Vincent-Tompkins, T. Wearda, et al. 2015. Identification and specification of the mouse skeletal stem cell. *Cell.* 160:285–298. https://doi.org/10.1016/j.cell.2014.12.002

Chattopadhyay, S., A.L. Stewart, S. Mukherjee, C. Huang, K.A. Hartwell, P.G. Miller, R. Subramanian, L.C. Carmody, R.Z. Yusuf, D.B. Sykes, et al. 2015.

Niche-Based Screening in Multiple Myeloma Identifies a Kinesin-5 Inhibitor with Improved Selectivity over Hematopoietic Progenitors. *Cell Reports.* 10:755–770. https://doi.org/10.1016/j.celrep.2015.01.017

Chelstrom, L.M., R. Gunther, J. Simon, S.C. Raimondi, R. Krance, W.M. Crist, and F.M. Uckun. 1994. Childhood acute myeloid leukemia in mice with severe combined immunodeficiency. *Blood.* 84:20–26.

Chen, Y., R. Jacamo, Y.X. Shi, R.Y. Wang, V.L. Battula, S. Konoplev, D. Strunk, N.A. Hofmann, A. Reinisch, M. Konopleva, and M. Andreeff. 2012. Human extramedullary bone marrow in mice: a novel in vivo model of genetically controlled hematopoietic microenvironment. *Blood.* 119:4971–4980. https://doi.org/10.1182/blood-2011-11-389957

Choi, J.S., B.P. Mahadik, and B.A. Harley. 2015. Engineering the hematopoietic stem cell niche: Frontiers in biomaterial science. *Biotechnol. J.* 10:1529–1545. https://doi.org/10.1002/biot.201400758

Chow, A., D. Lucas, A. Hidalgo, S. Méndez-Ferrer, D. Hashimoto, C. Scheiermann, M. Battista, M. Leboeuf, C. Prophete, N. van Rooijen, et al. 2011. Bone marrow CD169$^+$ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J. Exp. Med.* 208:261–271. https://doi.org/10.1084/jem.20101688

Civantos, A., E. Martínez-Campos, V. Ramos, C. Elvira, A. Gallardo, and A. Abarrategi. 2017. Titanium Coatings and Surface Modifications: Toward Clinically Useful Bioactive Implants. *ACS Biomater. Sci. Eng.* 3:1245–1261. https://doi.org/10.1021/acsbiomaterials.6b00604

Clappier, E., B. Gerby, F. Sigaux, M. Delord, F. Touzri, L. Hernandez, P. Ballerini, A. Baruchel, F. Pflumio, and J. Soulier. 2011. Clonal selection in xenografted human T cell acute lymphoblastic leukemia recapitulates gain of malignancy at relapse. *J. Exp. Med.* 208:653–661. https://doi.org/10.1084/jem.20110105

Clarke, C.J., and T.L. Holyoake. 2017. Preclinical approaches in chronic myeloid leukemia: from cells to systems. *Exp. Hematol.* 47:13–23. https://doi.org/10.1016/j.exphem.2016.11.005

Cooper, T.P., and M.V. Sefton. 2011. Fibronectin coating of collagen modules increases in vivo HUVEC survival and vessel formation in SCID mice. *Acta Biomater.* 7:1072–1083. https://doi.org/10.1016/j.actbio.2010.11.008

Cosgun, K.N., S. Rahmig, N. Mende, S. Reinke, I. Hauber, C. Schäfer, A. Petzold, H. Weisbach, G. Heidkamp, A. Purbojo, et al. 2014. Kit regulates HSC engraftment across the human-mouse species barrier. *Cell Stem Cell.* 15:227–238. https://doi.org/10.1016/j.stem.2014.06.001

Cuddihy, M.J., Y. Wang, C. Machi, J.H. Bahng, and N.A. Kotov. 2013. Replication of bone marrow differentiation niche: comparative evaluation of different three-dimensional matrices. *Small.* 9:1008–1015. https://doi.org/10.1002/smll.201202133

Čulen, M., D. Dvořáková, L. Semerád, Z. Šustková, J. Bouchnerová, M. Palacková, J. Mayer, and Z. Ráčil. 2015. Incorporation of humanized niche as a strategy for improving leukemic engraftment in immunodeficient mice. *Transfuze a Hematologie Dnes [Transfusion and Haematology Today].* 21:36–41.

Dao, M.A., K.A. Pepper, and J.A. Nolta. 1997. Long-term cytokine production from engineered primary human stromal cells influences human hematopoiesis in an in vivo xenograft model. *Stem Cells.* 15:443–454. https://doi.org/10.1002/stem.150443

Das, R., T. Strowig, R. Verma, S. Koduru, A. Hafemann, S. Hopf, M.H. Kocoglu, C. Borsotti, L. Zhang, A. Branagan, et al. 2016. Microenvironment-dependent growth of preneoplastic and malignant plasma cells in humanized mice. *Nat. Med.* 22:1351–1357. https://doi.org/10.1038/nm.4202

Dazzi, F., D. Capelli, R. Hasserjian, F. Cotter, M. Corbo, A. Poletti, W. Chinswangwatanakul, J.M. Goldman, and M.Y. Gordon. 1998. The kinetics and extent of engraftment of chronic myelogenous leukemia cells in non-obese diabetic/severe combined immunodeficiency mice reflect the phase of the donor's disease: an in vivo model of chronic myelogenous leukemia biology. *Blood.* 92:1390–1396.

Dhami, S.P.S., S.S. Kappala, A. Thompson, and E. Szegezdi. 2016. Three-dimensional ex vivo co-culture models of the leukaemic bone marrow niche for functional drug testing. *Drug Discov. Today.* 21:1464–1471. https://doi.org/10.1016/j.drudis.2016.04.019

Dong, L., W.M. Yu, H. Zheng, M.L. Loh, S.T. Bunting, M. Pauly, G. Huang, M. Zhou, H.E. Broxmeyer, D.T. Scadden, and C.K. Qu. 2016. Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. *Nature.* 539:304–308. https://doi.org/10.1038/nature20131

Dragoo, J.L., J.R. Lieberman, R.S. Lee, D.A. Deugarte, Y. Lee, P.A. Zuk, M.H. Hedrick, and P. Benhaim. 2005. Tissue-engineered bone from BMP-2-transduced stem cells derived from human fat. *Plast. Reconstr. Surg.* 115:1665–1673. https://doi.org/10.1097/01.PRS.0000161459.90856.AB

Ebinger, S., E.Z. Özdemir, C. Ziegenhain, S. Tiedt, C. Castro Alves, M. Grunert, M. Dworzak, C. Lutz, V.A. Turati, T. Enver, et al. 2016. Characterization of rare, dormant, and therapy-resistant cells in acute lymphoblastic leukemia. *Cancer Cell.* 30:849–862. https://doi.org/10.1016/j.ccell.2016.11.002

Eisterer, W., X. Jiang, O. Christ, H. Glimm, K.H. Lee, E. Pang, K. Lambie, G. Shaw, T.L. Holyoake, A.L. Petzer, et al. 2005. Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease. *Leukemia.* 19:435–441. https://doi.org/10.1038/sj.leu.2403649

El Bialy, I., W. Jiskoot, and M. Reza Nejadnik. 2017. Formulation, Delivery and Stability of Bone Morphogenetic Proteins for Effective Bone Regeneration. *Pharm. Res.* 34:1152–1170. https://doi.org/10.1007/s11095-017-2147-x

Elder, A., S. Bomken, I. Wilson, H.J. Blair, S. Cockell, F. Ponthan, K. Dormon, D. Pal, O. Heidenreich, and J. Vormoor. 2017. Abundant and equipotent founder cells establish and maintain acute lymphoblastic leukaemia. *Leukemia.* 31:2577–2586. https://doi.org/10.1038/leu.2017.140

Ellegast, J.M., P.J. Rauch, L.V. Kovtonyuk, R. Müller, U. Wagner, Y. Saito, N. Wildner-Verhey van Wijk, C. Fritz, A. Rafiei, V. Lysenko, et al. 2016. inv(16) and NPM1mut AMLs engraft human cytokine knock-in mice. *Blood.* 128:2130–2134. https://doi.org/10.1182/blood-2015-12-689356

Farrell, E., O.P. van der Jagt, W. Koevoet, N. Kops, C.J. van Manen, C.A. Hellingman, H. Jahr, F.J. O'Brien, J.A. Verhaar, H. Weinans, and G.J. van Osch. 2008. Chondrogenic priming of human bone marrow stromal cells: a better route to bone repair? *Tissue Eng. Part C Methods.* 15:285–295. https://doi.org/10.1089/ten.tec.2008.0297

Farrell, E., S.K. Both, K.I. Odörfer, W. Koevoet, N. Kops, F.J. O'Brien, R.J. Baatenburg de Jong, J.A. Verhaar, V. Cuijpers, J. Jansen, et al. 2011. In-vivo generation of bone via endochondral ossification by in-vitro chondrogenic priming of adult human and rat mesenchymal stem cells. *BMC Musculoskelet. Disord.* 12:31. https://doi.org/10.1186/1471-2474-12-31

Ferreira, M.S.V., W. Jahnen-Dechent, N. Labude, M. Bovi, T. Hieronymus, M. Zenke, R.K. Schneider, and S. Neuss. 2012. Cord blood-hematopoietic stem cell expansion in 3D fibrin scaffolds with stromal support. *Biomaterials.* 33:6987–6997. https://doi.org/10.1016/j.biomaterials.2012.06.029

Feuring-Buske, M., B. Gerhard, J. Cashman, R.K. Humphries, C.J. Eaves, and D.E. Hogge. 2003. Improved engraftment of human acute myeloid leukemia progenitor cells in beta 2-microglobulin-deficient NOD/SCID mice and in NOD/SCID mice transgenic for human growth factors. *Leukemia.* 17:760–763. https://doi.org/10.1038/sj.leu.2402882

Flores-Figueroa, E., and D. Gratzinger. 2016. Beyond the niche: myelodysplastic syndrome topobiology in the laboratory and in the clinic. *Int. J. Mol. Sci.* 17:553. https://doi.org/10.3390/ijms17040553

Francis, O.L., T.-A.M. Milford, C. Beldiman, and K.J. Payne. 2016. Fine-tuning patient-derived xenograft models for precision medicine approaches in leukemia. *J. Investig. Med.* 64:740–744. https://doi.org/10.1136/jim-2016-000076

Freeman, F.E., and L.M. McNamara. 2017. Endochondral priming: a developmental engineering strategy for bone tissue regeneration. *Tissue Eng. Part B Rev.* 23:128–141. https://doi.org/10.1089/ten.teb.2016.0197

Frisch, B.J., J.M. Ashton, L. Xing, M.W. Becker, C.T. Jordan, and L.M. Calvi. 2012. Functional inhibition of osteoblastic cells in an in vivo mouse model of myeloid leukemia. *Blood.* 119:540–550. https://doi.org/10.1182/blood-2011-04-348151

Fulop, G.M., and R.A. Phillips. 1990. The scid mutation in mice causes a general defect in DNA repair. *Nature.* 347:479–482. https://doi.org/10.1038/347479a0

Ghanaati, S., S. Fuchs, M.J. Webber, C. Orth, M. Barbeck, M.E. Gomes, R.L. Reis, and C.J. Kirkpatrick. 2011. Rapid vascularization of starch-poly(-caprolactone) in vivo by outgrowth endothelial cells in co-culture with primary osteoblasts. *J. Tissue Eng. Regen. Med.* 5:e136–e143. https://doi.org/10.1002/term.373

Giustacchini, A., S. Thongjuea, N. Barkas, P.S. Woll, B.J. Povinelli, C.A.G. Booth, P. Sopp, R. Norfo, A. Rodriguez-Meira, N. Ashley, et al. 2017. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. *Nat. Med.* 23:692–702. https://doi.org/10.1038/nm.4336

Goardon, N., E. Marchi, A. Atzberger, L. Quek, A. Schuh, S. Soneji, P. Woll, A. Mead, K.A. Alford, R. Rout, et al. 2011. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. *Cancer Cell.* 19:138–152. https://doi.org/10.1016/j.ccr.2010.12.012

Greenwald, A.S., S.D. Boden, V.M. Goldberg, Y. Khan, C.T. Laurencin, and R.N. Rosier. American Academy of Orthopaedic Surgeons. The Committee on Biological Implants. 2001. Bone-graft substitutes: facts, fictions, and applications. *J. Bone Joint Surg. Am.* 83-A(Suppl 2 Pt 2):98–103. https://doi.org/10.2106/00004623-200100022-00007

Greiner, D.L., R.A. Hesselton, and L.D. Shultz. 1998. SCID mouse models of human stem cell engraftment. *Stem Cells.* 16:166–177. https://doi.org/10.1002/stem.160166

Groen, R.W., W.A. Noort, R.A. Raymakers, H.J. Prins, L. Aalders, F.M. Hofhuis, P. Moerer, J.F. van Velzen, A.C. Bloem, B. van Kessel, et al. 2012. Reconstructing the human hematopoietic niche in immunodeficient mice: opportunities for studying primary multiple myeloma. *Blood.* 120:e9–e16. https://doi.org/10.1182/blood-2012-03-414920

Hartwell, K.A., P.G. Miller, S. Mukherjee, A.R. Kahn, A.L. Stewart, D.J. Logan, J.M. Negri, M. Duvet, M. Järås, R. Puram, et al. 2013. Niche-based screening identifies small-molecule inhibitors of leukemia stem cells. *Nat. Chem. Biol.* 9:840–848. https://doi.org/10.1038/nchembio.1367

He, J., O. Abdel-Wahab, M.K. Nahas, K. Wang, R.K. Rampal, A.M. Intlekofer, J. Patel, A. Krivstov, G.M. Frampton, L.E. Young, et al. 2016. Integrated genomic DNA/RNA profiling of hematologic malignancies in the clinical setting. *Blood.* 127:3004–3014. https://doi.org/10.1182/blood-2015-08-664649

Heisterkamp, N., J.R. Stephenson, J. Groffen, P.F. Hansen, A. de Klein, C.R. Bartram, and G. Grosveld. 1983. Localization of the c-ab1 oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature.* 306:239–242. https://doi.org/10.1038/306239a0

Hesselton, R.M., D.L. Greiner, J.P. Mordes, T.V. Rajan, J.L. Sullivan, and L.D. Shultz. 1995. High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to human immunodeficiency virus type 1 infection in NOD/LtSz-scid/scid mice. *J. Infect. Dis.* 172:974–982. https://doi.org/10.1093/infdis/172.4.974

Ho, M.S., R.L. Medcalf, S.A. Livesey, and K. Traianedes. 2015. The dynamics of adult haematopoiesis in the bone and bone marrow environment. *Br. J. Haematol.* 170:472–486. https://doi.org/10.1111/bjh.13445

Hogan, C.J., E.J. Shpall, O. McNulty, I. McNiece, J.E. Dick, L.D. Shultz, and G. Keller. 1997. Engraftment and development of human CD34(+)-enriched cells from umbilical cord blood in NOD/LtSz-scid/scid mice. *Blood.* 90:85–96.

Hoggatt, J., Y. Kfoury, and D.T. Scadden. 2016. Hematopoietic stem cell niche in health and disease. *Annu. Rev. Pathol.* 11:555–581. https://doi.org/10.1146/annurev-pathol-012615-044414

Holyoake, T., X. Jiang, C. Eaves, and A. Eaves. 1999. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. *Blood.* 94:2056–2064.

Holzapfel, B.M., F. Wagner, D. Loessner, N.P. Holzapfel, L. Thibaudeau, R. Crawford, M.-T. Ling, J.A. Clements, P.J. Russell, and D.W. Hutmacher. 2014. Species-specific homing mechanisms of human prostate cancer metastasis in tissue engineered bone. *Biomaterials.* 35:4108–4115. https://doi.org/10.1016/j.biomaterials.2014.01.062

Holzapfel, B.M., D.W. Hutmacher, B. Nowlan, V. Barbier, L. Thibaudeau, C. Theodoropoulos, J.D. Hooper, D. Loessner, J.A. Clements, P.J. Russell, et al. 2015a. Tissue engineered humanized bone supports human hematopoiesis in vivo. *Biomaterials.* 61:103–114. https://doi.org/10.1016/j.biomaterials.2015.04.057

Holzapfel, B.M., F. Wagner, L. Thibaudeau, J.-P. Levesque, and D.W. Hutmacher. 2015b. Concise review: humanized models of tumor immunology in the 21st century: convergence of cancer research and tissue engineering. *Stem Cells.* 33:1696–1704. https://doi.org/10.1002/stem.1978

Ishikawa, F., M. Yasukawa, B. Lyons, S. Yoshida, T. Miyamoto, G. Yoshimoto, T. Watanabe, K. Akashi, L.D. Shultz, and M. Harada. 2005. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor gamma chain$^{null}$ mice. *Blood.* 106:1565–1573. https://doi.org/10.1182/blood-2005-02-0516

Ito, M., H. Hiramatsu, K. Kobayashi, K. Suzue, M. Kawahata, K. Hioki, Y. Ueyama, Y. Koyanagi, K. Sugamura, K. Tsuji, et al. 2002. NOD/SCID/γ(c) (null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood.* 100:3175–3182. https://doi.org/10.1182/blood-2001-12-0207

Jing, D., A.-V. Fonseca, N. Alakel, F.A. Fierro, K. Muller, M. Bornhauser, G. Ehninger, D. Corbeil, and R. Ordemann. 2010. Hematopoietic stem cells in co-culture with mesenchymal stromal cells--modeling the niche compartments in vitro. *Haematologica.* 95:542–550. https://doi.org/10.3324/haematol.2009.010736

Kamel-Reid, S., and J.E. Dick. 1988. Engraftment of immune-deficient mice with human hematopoietic stem cells. *Science.* 242:1706–1709. https://doi.org/10.1126/science.2904703

Kang, Y., W.M. Liao, Z.H. Yuan, P.Y. Sheng, L.J. Zhang, X.W. Yuan, and L. Lei. 2007. In vitro and in vivo induction of bone formation based on adeno-associated virus-mediated BMP-7 gene therapy using human adipose-derived mesenchymal stem cells. *Acta Pharmacol. Sin.* 28:839–849. https://doi.org/10.1111/j.1745-7254.2007.00583.x

Klco, J.M., D.H. Spencer, C.A. Miller, M. Griffith, T.L. Lamprecht, M. O'Laughlin, C. Fronick, V. Magrini, R.T. Demeter, R.S. Fulton, et al. 2014. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell.* 25:379–392. https://doi.org/10.1016/j.ccr.2014.01.031

Kode, A., J.S. Manavalan, I. Mosialou, G. Bhagat, C.V. Rathinam, N. Luo, H. Khiabanian, A. Lee, V.V. Murty, R. Friedman, et al. 2014. Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. *Nature.* 506:240–244. https://doi.org/10.1038/nature12883

Koike, N., D. Fukumura, O. Gralla, P. Au, J.S. Schechner, and R.K. Jain. 2004. Tissue engineering: Creation of long-lasting blood vessels. *Nature.* 428:138–139. https://doi.org/10.1038/428138a

Koller, B.H., P. Marrack, J.W. Kappler, and O. Smithies. 1990. Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8+ T cells. *Science.* 248:1227–1230. https://doi.org/10.1126/science.2112266

Krause, D.S., K. Fulzele, A. Catic, C.C. Sun, D. Dombkowski, M.P. Hurley, S. Lezeau, E. Attar, J.Y. Wu, H.Y. Lin, et al. 2013. Differential regulation of myeloid leukemias by the bone marrow microenvironment. *Nat. Med.* 19:1513–1517. https://doi.org/10.1038/nm.3364

Krebsbach, P.H., S.A. Kuznetsov, K. Satomura, R.V. Emmons, D.W. Rowe, and P.G. Robey. 1997. Bone formation in vivo: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. *Transplantation.* 63:1059–1069. https://doi.org/10.1097/00007890-199704270-00003

Lapidot, T., F. Pflumio, M. Doedens, B. Murdoch, D.E. Williams, and J.E. Dick. 1992. Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. *Science.* 255:1137–1141. https://doi.org/10.1126/science.1372131

Lapidot, T., C. Sirard, J. Vormoor, B. Murdoch, T. Hoang, J. Caceres-Cortes, M. Minden, B. Paterson, M.A. Caligiuri, and J.E. Dick. 1994. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature.* 367:645–648. https://doi.org/10.1038/367645a0

Lapidot, T., Y. Fajerman, and O. Kollet. 1997. Immune-deficient SCID and NOD/SCID mice models as functional assays for studying normal and malignant human hematopoiesis. *J. Mol. Med. (Berl.).* 75:664–673. https://doi.org/10.1007/s001090050150

Lee, J., M. Li, J. Milwid, J. Dunham, C. Vinegoni, R. Gorbatov, Y. Iwamoto, F. Wang, K. Shen, K. Hatfield, et al. 2012. Implantable microenvironments to attract hematopoietic stem/cancer cells. *Proc. Natl. Acad. Sci. USA.* 109:19638–19643. https://doi.org/10.1073/pnas.1208384109

Lee, J., D. Heckl, and B. Parekkadan. 2016. Multiple genetically engineered humanized microenvironments in a single mouse. *Biomater. Res.* 20:19. https://doi.org/10.1186/s40824-016-0066-2

Leisten, I., R. Kramann, M.S. Ventura Ferreira, M. Bovi, S. Neuss, P. Ziegler, W. Wagner, R. Knüchel, and R.K. Schneider. 2012. 3D co-culture of hematopoietic stem and progenitor cells and mesenchymal stem cells in collagen scaffolds as a model of the hematopoietic niche. *Biomaterials.* 33:1736–1747. https://doi.org/10.1016/j.biomaterials.2011.11.034

le Viseur, C., M. Hotfilder, S. Bomken, K. Wilson, S. Röttgers, A. Schrauder, A. Rosemann, J. Irving, R.W. Stam, L.D. Shultz, et al. 2008. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. *Cancer Cell.* 14:47–58. https://doi.org/10.1016/j.ccr.2008.05.015

Lin, C.H.S., K. Kaushansky, and H. Zhan. 2016. JAK2V617F-mutant vascular niche contributes to JAK2V617F clonal expansion in myeloproliferative neoplasms. *Blood Cells Mol. Dis.* 62:42–48. https://doi.org/10.1016/j.bcmd.2016.09.004

Lin, R.-Z., C.N. Lee, R. Moreno-Luna, J. Neumeyer, B. Piekarski, P. Zhou, M.A. Moses, M. Sachdev, W.T. Pu, S. Emani, and J.M. Melero-Martin. 2017. Host non-inflammatory neutrophils mediate the engraftment of bioengineered vascular networks. *Nat. Biomed. Eng.* 1:0081. https://doi.org/10.1038/s41551-017-0081

Lowry, P.A., L.D. Shultz, D.L. Greiner, R.M. Hesselton, E.L. Kittler, C.Y. Tiarks, S.S. Rao, J. Reilly, J.H. Leif, H. Ramshaw, et al. 1996. Improved engraftment of human cord blood stem cells in NOD/LtSz-scid/scid mice after irradiation or multiple-day injections into unirradiated recipients. *Biol. Blood Marrow Transplant.* 2:15–23.

Mankani, M.H., S.A. Kuznetsov, B. Fowler, A. Kingman, and P.G. Robey. 2001. In vivo bone formation by human bone marrow stromal cells: effect of carrier particle size and shape. *Biotechnol. Bioeng.* 72:96–107.

Mankani, M.H., S.A. Kuznetsov, N.A. Avila, A. Kingman, and P.G. Robey. 2004. Bone formation in transplants of human bone marrow stromal cells and hydroxyapatite-tricalcium phosphate: prediction with quantitative

CT in mice. *Radiology.* 230:369–376. https://doi.org/10.1148/radiol.2302011529

Martine, L.C., B.M. Holzapfel, J.A. McGovern, F. Wagner, V.M. Quent, P. Hesami, F.M. Wunner, C. Vaquette, E.M. De-Juan-Pardo, T.D. Brown, et al. 2017. Engineering a humanized bone organ model in mice to study bone metastases. *Nat. Protoc.* 12:639–663. https://doi.org/10.1038/nprot.2017.002

Matsuoka, Y., R. Nakatsuka, K. Sumide, H. Kawamura, M. Takahashi, T. Fujioka, Y. Uemura, H. Asano, Y. Sasaki, M. Inoue, et al. 2015. Prospectively Isolated Human Bone Marrow Cell-Derived MSCs Support Primitive Human CD34-Negative Hematopoietic Stem Cells. *Stem Cells.* 33:1554–1565. https://doi.org/10.1002/stem.1941

Maykel, J., J.H. Liu, H. Li, L.D. Shultz, D.L. Greiner, and J. Houghton. 2014. NOD-scidIl2rg (tm1Wjl) and NOD-Rag1 (null) Il2rg (tm1Wjl): a model for stromal cell-tumor cell interaction for human colon cancer. *Dig. Dis. Sci.* 59:1169–1179. https://doi.org/10.1007/s10620-014-3168-5

McCune, J.M., R. Namikawa, H. Kaneshima, L.D. Shultz, M. Lieberman, and I.L. Weissman. 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science.* 241:1632–1639. https://doi.org/10.1126/science.2971269

McFadden, T.M., G.P. Duffy, A.B. Allen, H.Y. Stevens, S.M. Schwarzmaier, N. Plesnila, J.M. Murphy, F.P. Barry, R.E. Guldberg, and F.J. O'Brien. 2013. The delayed addition of human mesenchymal stem cells to pre-formed endothelial cell networks results in functional vascularization of a collagen-glycosaminoglycan scaffold in vivo. *Acta Biomater.* 9:9303–9316. https://doi.org/10.1016/j.actbio.2013.08.014

McIntosh, B.E., and M.E. Brown. 2015. No irradiation required: The future of humanized immune system modeling in murine hosts. *Chimerism.* 6:40–45. https://doi.org/10.1080/19381956.2016.1162360

Medyouf, H. 2017. The microenvironment in human myeloid malignancies: emerging concepts and therapeutic implications. *Blood.* 129:1617–1626. https://doi.org/10.1182/blood-2016-11-696070

Medyouf, H., M. Mossner, J.C. Jann, F. Nolte, S. Raffel, C. Herrmann, A. Lier, C. Eisen, V. Nowak, B. Zens, et al. 2014. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell Stem Cell.* 14:824–837. https://doi.org/10.1016/j.stem.2014.02.014

Méndez-Ferrer, S., D. Lucas, M. Battista, and P.S. Frenette. 2008. Haematopoietic stem cell release is regulated by circadian oscillations. *Nature.* 452:442–447. https://doi.org/10.1038/nature06685

Mian, S.A., K. Rouault-Pierre, A.E. Smith, T. Seidl, I. Pizzitola, A. Kizilors, A.G. Kulasekararaj, D. Bonnet, and G.J. Mufti. 2015. SF3B1 mutant MDS-initiating cells may arise from the haematopoietic stem cell compartment. *Nat. Commun.* 6:10004. https://doi.org/10.1038/ncomms10004

Miyoshi, H., M. Morita, N. Ohshima, and C. Sato. 2015. Expansion of mouse hematopoietic progenitor cells in three-dimensional cocultures on frozen-thawed stromal cell layers formed within porous scaffolds. *Exp. Hematol.* 43:115–124. https://doi.org/10.1016/j.exphem.2014.08.008

Mombaerts, P., J. Iacomini, R.S. Johnson, K. Herrup, S. Tonegawa, and V.E. Papaioannou. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell.* 68:869–877. https://doi.org/10.1016/0092-8674(92)90030-G

Moreau, J.E., K. Anderson, J.R. Mauney, T. Nguyen, D.L. Kaplan, and M. Rosenblatt. 2007. Tissue-engineered bone serves as a target for metastasis of human breast cancer in a mouse model. *Cancer Res.* 67:10304–10308. https://doi.org/10.1158/0008-5472.CAN-07-2483

Mortera-Blanco, T., M. Rende, H. Macedo, S. Farah, A. Bismarck, A. Mantalaris, and N. Panoskaltsis. 2012. Ex vivo mimicry of normal and abnormal human hematopoiesis. *J. Vis. Exp.* 3654. https://doi.org/10.3791/3654

Mosier, D.E., R.J. Gulizia, S.M. Baird, and D.B. Wilson. 1988. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature.* 335:256–259. https://doi.org/10.1038/335256a0

Nelson, M.R., and K. Roy. 2016. Bone-marrow mimicking biomaterial niches for studying hematopoietic stem and progenitor cells. *J. Mater. Chem. B Mater. Biol. Med.* 4:3490–3503. https://doi.org/10.1039/C5TB02644J

Nicolini, F.E., J.D. Cashman, D.E. Hogge, R.K. Humphries, and C.J. Eaves. 2004. NOD/SCID mice engineered to express human IL-3, GM-CSF and Steel factor constitutively mobilize engrafted human progenitors and compromise human stem cell regeneration. *Leukemia.* 18:341–347. https://doi.org/10.1038/sj.leu.2403222

Nijmeijer, B.A., P. Mollevanger, S.L. van Zelderen-Bhola, H.C. Kluin-Nelemans, R. Willemze, and J.H. Falkenburg. 2001. Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. *Exp. Hematol.* 29:322–329. https://doi.org/10.1016/S0301-472X(00)00669-X

Notta, F., C.G. Mullighan, J.C. Wang, A. Poeppl, S. Doulatov, L.A. Phillips, J. Ma, M.D. Minden, J.R. Downing, and J.E. Dick. 2011. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. *Nature.* 469:362–367. https://doi.org/10.1038/nature09733

Nowell, P.C., and D.A. Hungerford. 1961. Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J. Natl. Cancer Inst.* 27:1013–1035.

Nusspaumer, G., S. Jaiswal, A. Barbero, R. Reinhardt, D. Ishay Ronen, A. Haumer, T. Lufkin, I. Martin, and R. Zeller. 2017. Ontogenic Identification and Analysis of Mesenchymal Stromal Cell Populations during Mouse Limb and Long Bone Development. *Stem Cell Reports.* 9:1124–1138. https://doi.org/10.1016/j.stemcr.2017.08.007

Paczulla, A.M., S. Dirnhofer, M. Konantz, M. Medinger, H.R. Salih, K. Rothfelder, D.A. Tsakiris, J.R. Passweg, P. Lundberg, and C. Lengerke. 2017. Long-term observation reveals high-frequency engraftment of human acute myeloid leukemia in immunodeficient mice. *Haematologica.* 102:854–864. https://doi.org/10.3324/haematol.2016.153528

Papaemmanuil, E., M. Gerstung, L. Bullinger, V.I. Gaidzik, P. Paschka, N.D. Roberts, N.E. Potter, M. Heuser, F. Thol, N. Bolli, et al. 2016. Genomic classification and prognosis in acute myeloid leukemia. *N. Engl. J. Med.* 374:2209–2221. https://doi.org/10.1056/NEJMoa1516192

Park, K.-H., H. Kim, S. Moon, and K. Na. 2009. Bone morphogenic protein-2 (BMP-2) loaded nanoparticles mixed with human mesenchymal stem cell in fibrin hydrogel for bone tissue engineering. *J. Biosci. Bioeng.* 108:530–537. https://doi.org/10.1016/j.jbiosc.2009.05.021

Passaro, D., C.T. Quang, and J. Ghysdael. 2016. Microenvironmental cues for T-cell acute lymphoblastic leukemia development. *Immunol. Rev.* 271:156–172. https://doi.org/10.1111/imr.12402

Passaro, D., A. Abarrategi, K. Foster, L. Ariza-McNaughton, and D. Bonnet. 2017a. Bioengineering of Humanized Bone Marrow Microenvironments in Mouse and Their Visualization by Live Imaging. *J. Vis. Exp.* 126:55914. https://doi.org/10.3791/55914

Passaro, D., A. Di Tullio, A. Abarrategi, K. Rouault-Pierre, K. Foster, L. Ariza-McNaughton, B. Montaner, P. Chakravarty, L. Bhaw, G. Diana, et al. 2017b. Increased Vascular Permeability in the Bone Marrow Microenvironment Contributes to Disease Progression and Drug Response in Acute Myeloid Leukemia. *Cancer Cell.* 32:324–341.e6. https://doi.org/10.1016/j.ccell.2017.08.001

Pearce, D.J., D. Taussig, K. Zibara, L.L. Smith, C.M. Ridler, C. Preudhomme, B.D. Young, A.Z. Rohatiner, T.A. Lister, and D. Bonnet. 2006. AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. *Blood.* 107:1166–1173. https://doi.org/10.1182/blood-2005-06-2325

Pearson, T., L.D. Shultz, D. Miller, M. King, J. Laning, W. Fodor, A. Cuthbert, L. Burzenski, B. Gott, B. Lyons, et al. 2008. Non-obese diabetic-recombination activating gene-1 (NOD-Rag1 null) interleukin (IL)-2 receptor common gamma chain (IL2r gamma null) null mice: a radioresistant model for human lymphohaematopoietic engraftment. *Clin. Exp. Immunol.* 154:270–284. https://doi.org/10.1111/j.1365-2249.2008.03753.x

Pedersen, T.O., A.L. Blois, Z. Xing, Y. Xue, Y. Sun, A. Finne-Wistrand, L.A. Akslen, J.B. Lorens, K.N. Leknes, I. Fristad, and K. Mustafa. 2013. Endothelial microvascular networks affect gene-expression profiles and osteogenic potential of tissue-engineered constructs. *Stem Cell Res. Ther.* 4:52. https://doi.org/10.1186/scrt202

Pelttari, K., A. Winter, E. Steck, K. Goetzke, T. Hennig, B.G. Ochs, T. Aigner, and W. Richter. 2006. Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. *Arthritis Rheum.* 54:3254–3266. https://doi.org/10.1002/art.22136

Pflumio, F., B. Izac, A. Katz, L.D. Shultz, W. Vainchenker, and L. Coulombel. 1996. Phenotype and function of human hematopoietic cells engrafting immune-deficient CB17-severe combined immunodeficiency mice and nonobese diabetic-severe combined immunodeficiency mice after transplantation of human cord blood mononuclear cells. *Blood.* 88:3731–3740.

Prins, H.-J., H. Rozemuller, S. Vonk-Griffioen, V.G. Verweij, W.J. Dhert, I.C. Slaper-Cortenbach, and A.C. Martens. 2009. Bone-forming capacity of mesenchymal stromal cells when cultured in the presence of human platelet lysate as substitute for fetal bovine serum. *Tissue Eng. Part A.* 15:3741–3751. https://doi.org/10.1089/ten.tea.2008.0666

Quek, L., G.W. Otto, C. Garnett, L. Lhermitte, D. Karamitros, B. Stoilova, I.J. Lau, J. Doondeea, B. Usukhbayar, A. Kennedy, et al. 2016. Genetically distinct leukemic stem cells in human CD34$^-$ acute myeloid leukemia are arrested at a hemopoietic precursor-like stage. *J. Exp. Med.* 213:1513–1535. https://doi.org/10.1084/jem.20151775

Raaijmakers, M.H., S. Mukherjee, S. Guo, S. Zhang, T. Kobayashi, J.A. Schoonmaker, B.L. Ebert, F. Al-Shahrour, R.P. Hasserjian, E.O. Scadden, et al. 2010. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature*. 464:852–857. https://doi.org/10.1038/nature08851

Raic, A., L. Rödling, H. Kalbacher, and C. Lee-Thedieck. 2014. Biomimetic macroporous PEG hydrogels as 3D scaffolds for the multiplication of human hematopoietic stem and progenitor cells. *Biomaterials*. 35:929–940. https://doi.org/10.1016/j.biomaterials.2013.10.038

Ramasamy, S.K., A.P. Kusumbe, T. Itkin, S. Gur-Cohen, T. Lapidot, and R.H. Adams. 2016. Regulation of Hematopoiesis and Osteogenesis by Blood Vessel-Derived Signals. *Annu. Rev. Cell Dev. Biol.* 32:649–675. https://doi.org/10.1146/annurev-cellbio-111315-124936

Raynaud, C.M., J.M. Butler, N.M. Halabi, F.S. Ahmad, B. Ahmed, S. Rafii, and A. Rafii. 2013. Endothelial cells provide a niche for placental hematopoietic stem/progenitor cell expansion through broad transcriptomic modification. *Stem Cell Res. (Amst.)*. 11:1074–1090. https://doi.org/10.1016/j.scr.2013.07.010

Rehe, K., K. Wilson, S. Bomken, D. Williamson, J. Irving, M.L. den Boer, M. Stanulla, M. Schrappe, A.G. Hall, O. Heidenreich, and J. Vormoor. 2013. Acute B lymphoblastic leukaemia-propagating cells are present at high frequency in diverse lymphoblast populations. *EMBO Mol. Med.* 5:38–51. https://doi.org/10.1002/emmm.201201703

Reinisch, A., N. Etchart, D. Thomas, N.A. Hofmann, M. Fruehwirth, S. Sinha, C.K. Chan, K. Senarath-Yapa, E.-Y. Seo, T. Wearda, et al. 2015. Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. *Blood*. 125:249–260. https://doi.org/10.1182/blood-2014-04-572255

Reinisch, A., D. Thomas, M.R. Corces, X. Zhang, D. Gratzinger, W.J. Hong, K. Schallmoser, D. Strunk, and R. Majeti. 2016. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. *Nat. Med.* 22:812–821. https://doi.org/10.1038/nm.4103

Reinisch, A., D.C. Hernandez, K. Schallmoser, and R. Majeti. 2017. Generation and use of a humanized bone-marrow-ossicle niche for hematopoietic xenotransplantation into mice. *Nat. Protoc.* 12:2169–2188. https://doi.org/10.1038/nprot.2017.088

Rombouts, W.J., A.C. Martens, and R.E. Ploemacher. 2000. Identification of variables determining the engraftment potential of human acute myeloid leukemia in the immunodeficient NOD/SCID human chimera model. *Leukemia*. 14:889–897. https://doi.org/10.1038/sj.leu.2401777

Rongvaux, A., T. Willinger, J. Martinek, T. Strowig, S.V. Gearty, L.L. Teichmann, Y. Saito, F. Marches, S. Halene, A.K. Palucka, et al. 2014. Development and function of human innate immune cells in a humanized mouse model. *Nat. Biotechnol.* 32:364–372. https://doi.org/10.1038/nbt.2858

Rouault-Pierre, K., S.A. Mian, M. Goulard, A. Abarrategi, A. Di Tulio, A.E. Smith, A. Mohamedali, S. Best, A.M. Nloga, A.G. Kulasekararaj, et al. 2017a. Preclinical modeling of myelodysplastic syndromes. *Leukemia*. 31:2702–2708. https://doi.org/10.1038/leu.2017.172

Rouault-Pierre, K., A.E. Smith, S.A. Mian, I. Pizzitola, A.G. Kulasekararaj, G.J. Mufti, and D. Bonnet. 2017b. Myelodysplastic syndrome can propagate from the multipotent progenitor compartment. *Haematologica*. 102:e7–e10. https://doi.org/10.3324/haematol.2016.152520

Rowley, J.D. 1973. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 243:290–293. https://doi.org/10.1038/243290a0

Sacchetti, B., A. Funari, S. Michienzi, S. Di Cesare, S. Piersanti, I. Saggio, E. Tagliafico, S. Ferrari, P.G. Robey, M. Riminucci, and P. Bianco. 2007. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell*. 131:324–336. https://doi.org/10.1016/j.cell.2007.08.025

Saito, Y., J.M. Ellegast, A. Rafiei, Y. Song, D. Kull, M. Heikenwalder, A. Rongvaux, S. Halene, R.A. Flavell, and M.G. Manz. 2016. Peripheral blood CD34+ cells efficiently engraft human cytokine knock-in mice. *Blood*. 128:1829–1833. https://doi.org/10.1182/blood-2015-10-676452

Sánchez-Aguilera, A., and S. Méndez-Ferrer. 2017. The hematopoietic stem-cell niche in health and leukemia. *Cell. Mol. Life Sci.* 74:579–590. https://doi.org/10.1007/s00018-016-2306-y

Schajnovitz, A., and D.T. Scadden. 2014. Bone's dark side: mutated osteoblasts implicated in leukemia. *Cell Res.* 24:383–384. https://doi.org/10.1038/cr.2014.26

Schechner, J.S., A.K. Nath, L. Zheng, M.S. Kluger, C.C. Hughes, M.R. Sierra-Honigmann, M.I. Lorber, G. Tellides, M. Kashgarian, A.L. Bothwell, and J.S. Pober. 2000. In vivo formation of complex microvessels lined by human endothelial cells in an immunodeficient mouse. *Proc. Natl. Acad. Sci. USA*. 97:9191–9196. https://doi.org/10.1073/pnas.150242297

Schepers, K., E.M. Pietras, D. Reynaud, J. Flach, M. Binnewies, T. Garg, A.J. Wagers, E.C. Hsiao, and E. Passegué. 2013. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell Stem Cell*. 13:285–299. https://doi.org/10.1016/j.stem.2013.06.009

Scherberich, A., R. Galli, C. Jaquiery, J. Farhadi, and I. Martin. 2007. Three-dimensional perfusion culture of human adipose tissue-derived endothelial and osteoblastic progenitors generates osteogenic constructs with intrinsic vascularization capacity. *Stem Cells*. 25:1823–1829. https://doi.org/10.1634/stemcells.2007-0124

Schneider, A., J.M. Taboas, L.K. McCauley, and P.H. Krebsbach. 2003. Skeletal homeostasis in tissue-engineered bone. *J. Orthop. Res.* 21:859–864. https://doi.org/10.1016/S0736-0266(03)00042-1

Scott, M.A., B. Levi, A. Askarinam, A. Nguyen, T. Rackohn, K. Ting, C. Soo, and A.W. James. 2012. Brief review of models of ectopic bone formation. *Stem Cells Dev.* 21:655–667. https://doi.org/10.1089/scd.2011.0517

Scotti, C., B. Tonnarelli, A. Papadimitropoulos, A. Scherberich, S. Schaeren, A. Schauerte, J. Lopez-Rios, R. Zeller, A. Barbero, and I. Martin. 2010. Recapitulation of endochondral bone formation using human adult mesenchymal stem cells as a paradigm for developmental engineering. *Proc. Natl. Acad. Sci. USA*. 107:7251–7256. https://doi.org/10.1073/pnas.1000302107

Scotti, C., E. Piccinini, H. Takizawa, A. Todorov, P. Bourgine, A. Papadimitropoulos, A. Barbero, M.G. Manz, and I. Martin. 2013. Engineering of a functional bone organ through endochondral ossification. *Proc. Natl. Acad. Sci. USA*. 110:3997–4002. https://doi.org/10.1073/pnas.1220108110

Seandel, M., J.M. Butler, H. Kobayashi, A.T. Hooper, I.A. White, F. Zhang, E.L. Vertes, M. Kobayashi, Y. Zhang, S.V. Shmelkov, et al. 2008. Generation of a functional and durable vascular niche by the adenoviral E4ORF1 gene. *Proc. Natl. Acad. Sci. USA*. 105:19288–19293. https://doi.org/10.1073/pnas.0805980105

Seke Etet, P.F., L. Vecchio, and A.H. Nwabo Kamdje. 2012. Interactions between bone marrow stromal microenvironment and B-chronic lymphocytic leukemia cells: any role for Notch, Wnt and Hh signaling pathways? *Cell. Signal.* 24:1433–1443. https://doi.org/10.1016/j.cellsig.2012.03.008

Sharma, M.B., L.S. Limaye, and V.P. Kale. 2012. Mimicking the functional hematopoietic stem cell niche in vitro: recapitulation of marrow physiology by hydrogel-based three-dimensional cultures of mesenchymal stromal cells. *Haematologica*. 97:651–660. https://doi.org/10.3324/haematol.2011.050500

Sheehy, E.J., T. Mesallati, T. Vinardell, and D.J. Kelly. 2015. Engineering cartilage or endochondral bone: a comparison of different naturally derived hydrogels. *Acta Biomater.* 13:245–253. https://doi.org/10.1016/j.actbio.2014.11.031

Shih, Y.-R., H. Kang, V. Rao, Y.-J. Chiu, S.K. Kwon, and S. Varghese. 2017. In vivo engineering of bone tissues with hematopoietic functions and mixed chimerism. *Proc. Natl. Acad. Sci. USA*. 114:5419–5424. https://doi.org/10.1073/pnas.1702576114

Shinkai, Y., G. Rathbun, K.P. Lam, E.M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A.M. Stall, et al. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell*. 68:855–867. https://doi.org/10.1016/0092-8674(92)90029-C

Shinkai, Y., S. Koyasu, K. Nakayama, K.M. Murphy, D.Y. Loh, E.L. Reinherz, and F.W. Alt. 1993. Restoration of T cell development in RAG-2-deficient mice by functional TCR transgenes. *Science*. 259:822–825. https://doi.org/10.1126/science.8430336

Shlush, L.I., A. Mitchell, L. Heisler, S. Abelson, S.W.K. Ng, A. Trotman-Grant, J.J.F. Medeiros, A. Rao-Bhatia, I. Jaciw-Zurakowsky, R. Marke, et al. 2017. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. *Nature*. 547:104–108. https://doi.org/10.1038/nature22993

Shultz, L.D., B.L. Lyons, L.M. Burzenski, B. Gott, X. Chen, S. Chaleff, M. Kotb, S.D. Gillies, M. King, J. Mangada, et al. 2005. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J. Immunol.* 174:6477–6489. https://doi.org/10.4049/jimmunol.174.10.6477

Siddappa, R., R. Licht, C. van Blitterswijk, and J. de Boer. 2007. Donor variation and loss of multipotency during in vitro expansion of human mesenchymal stem cells for bone tissue engineering. *J. Orthop. Res.* 25:1029–1041. https://doi.org/10.1002/jor.20402

Siddappa, R., A. Martens, J. Doorn, A. Leusink, C. Olivo, R. Licht, L. van Rijn, C. Gaspar, R. Fodde, F. Janssen, et al. 2008. cAMP/PKA pathway activation

in human mesenchymal stem cells in vitro results in robust bone formation in vivo. *Proc. Natl. Acad. Sci. USA.* 105:7281–7286. https://doi.org/10.1073/pnas.0711190105

Sieber, S., L. Wirth, N. Cavak, M. Koenigsmark, U. Marx, R. Lauster, and M. Rosowski. 2017. Bone marrow-on-a-chip: Long-term culture of human haematopoietic stem cells in a three-dimensional microfluidic environment. *J. Tissue Eng. Regen. Med.* https://doi.org/10.1002/term.2507

Skovseth, D.K., A.M. Küchler, and G. Haraldsen. 2007. The HUVEC/Matrigel assay: an in vivo assay of human angiogenesis suitable for drug validation. *Methods Mol. Biol.* 360:253–268. https://doi.org/10.1385/1-59745-165-7:253

Song, J., M.J. Kiel, Z. Wang, J. Wang, R.S. Taichman, S.J. Morrison, and P.H. Krebsbach. 2010. An in vivo model to study and manipulate the hematopoietic stem cell niche. *Blood.* 115:2592–2600. https://doi.org/10.1182/blood-2009-01-200071

Sontakke, P., M. Carretta, J. Jaques, A.Z. Brouwers-Vos, L. Lubbers-Aalders, H. Yuan, J.D. de Bruijn, A.C. Martens, E. Vellenga, R.W. Groen, and J.J. Schuringa. 2016. Modeling BCR-ABL and MLL-AF9 leukemia in a human bone marrow-like scaffold-based xenograft model. *Leukemia.* 30:2064–2073. https://doi.org/10.1038/leu.2016.108

Spiegel, A., S. Shivtiel, A. Kalinkovich, A. Ludin, N. Netzer, P. Goichberg, Y. Azaria, I. Resnick, I. Hardan, H. Ben-Hur, et al. 2007. Catecholaminergic neurotransmitters regulate migration and repopulation of immature human CD34+ cells through Wnt signaling. *Nat. Immunol.* 8:1123–1131. https://doi.org/10.1038/ni1509

Steele, J.P., R.D. Clutterbuck, R.L. Powles, P.L. Mitchell, C. Horton, R. Morilla, D. Catovsky, and J.L. Millar. 1997. Growth of human T-cell lineage acute leukemia in severe combined immunodeficiency (SCID) mice and nonobese diabetic SCID mice. *Blood.* 90:2015–2019.

Takagi, S., Y. Saito, A. Hijikata, S. Tanaka, T. Watanabe, T. Hasegawa, S. Mochizuki, J. Kunisawa, H. Kiyono, H. Koseki, et al. 2012. Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. *Blood.* 119:2768–2777. https://doi.org/10.1182/blood-2011-05-353201

Taussig, D.C., J. Vargaftig, F. Miraki-Moud, E. Griessinger, K. Sharrock, T. Luke, D. Lillington, H. Oakervee, J. Cavenagh, S.G. Agrawal, et al. 2010. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. *Blood.* 115:1976–1984. https://doi.org/10.1182/blood-2009-02-206565

Theocharides, A.P., A. Rongvaux, K. Fritsch, R.A. Flavell, and M.G. Manz. 2016. Humanized hemato-lymphoid system mice. *Haematologica.* 101:5–19. https://doi.org/10.3324/haematol.2014.115212

Thompson, E.M., A. Matsiko, E. Farrell, D.J. Kelly, and F.J. O'Brien. 2015. Recapitulating endochondral ossification: a promising route to in vivo bone regeneration. *J. Tissue Eng. Regen. Med.* 9:889–902. https://doi.org/10.1002/term.1918

Torisawa, Y.S., C.S. Spina, T. Mammoto, A. Mammoto, J.C. Weaver, T. Tat, J.J. Collins, and D.E. Ingber. 2014. Bone marrow-on-a-chip replicates hematopoietic niche physiology in vitro. *Nat. Methods.* 11:663–669. https://doi.org/10.1038/nmeth.2938

Traggiai, E., L. Chicha, L. Mazzucchelli, L. Bronz, J.C. Piffaretti, A. Lanzavecchia, and M.G. Manz. 2004. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science.* 304:104–107. https://doi.org/10.1126/science.1093933

Turgeman, G., D.D. Pittman, R. Müller, B.G. Kurkalli, S. Zhou, G. Pelled, A. Peyser, Y. Zilberman, I.K. Moutsatsos, and D. Gazit. 2001. Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy. *J. Gene Med.* 3:240–251.

Urist, M.R. 1965. Bone: formation by autoinduction. *Science.* 150:893–899. https://doi.org/10.1126/science.150.3698.893

Uusi-Rasi, K., L.M. Semanick, J.R. Zanchetta, C.E. Bogado, E.F. Eriksen, M. Sato, and T.J. Beck. 2005. Effects of teriparatide [rhPTH (1-34)] treatment on structural geometry of the proximal femur in elderly osteoporotic women. *Bone.* 36:948–958. https://doi.org/10.1016/j.bone.2005.03.003

Vaiselbuh, S.R., M. Edelman, J.M. Lipton, and J.M. Liu. 2010. Ectopic human mesenchymal stem cell-coated scaffolds in NOD/SCID mice: an in vivo model of the leukemia niche. *Tissue Eng. Part C Methods.* 16:1523–1531. https://doi.org/10.1089/ten.tec.2010.0179

Velten, L., S.F. Haas, S. Raffel, S. Blaszkiewicz, S. Islam, B.P. Hennig, C. Hirche, C. Lutz, E.C. Buss, D. Nowak, et al. 2017. Human haematopoietic stem cell lineage commitment is a continuous process. *Nat. Cell Biol.* 19:271–281. https://doi.org/10.1038/ncb3493

Visser, J., D. Gawlitta, K.E. Benders, S.M. Toma, B. Pouran, P.R. van Weeren, W.J. Dhert, and J. Malda. 2015. Endochondral bone formation in gelatin methacrylamide hydrogel with embedded cartilage-derived matrix particles. *Biomaterials.* 37:174–182. https://doi.org/10.1016/j.biomaterials.2014.10.020

Vormoor, J., T. Lapidot, F. Pflumio, G. Risdon, B. Patterson, H.E. Broxmeyer, and J.E. Dick. 1994. Immature human cord blood progenitors engraft and proliferate to high levels in severe combined immunodeficient mice. *Blood.* 83:2489–2497.

Wang, J.C., T. Lapidot, J.D. Cashman, M. Doedens, L. Addy, D.R. Sutherland, R. Nayar, P. Laraya, M. Minden, A. Keating, et al. 1998. High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. *Blood.* 91:2406–2414.

Wei, X., Y. Lai, B. Li, L. Qin, Y. Xu, S. Lin, S. Wang, Q. Wu, Q. Liang, G. Huang, et al. 2017. CRISPR/Cas9-Mediated Deletion of Foxn1 in NOD/SCID/IL2rg-/- Mice Results in Severe Immunodeficiency. *Sci. Rep.* 7:7720. https://doi.org/10.1038/s41598-017-08337-8

Woll, P.S., U. Kjällquist, O. Chowdhury, H. Doolittle, D.C. Wedge, S. Thongjuea, R. Erlandsson, M. Ngara, K. Anderson, Q. Deng, et al. 2014. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. *Cancer Cell.* 25:794–808. https://doi.org/10.1016/j.ccr.2014.03.036

Wunderlich, M., F.S. Chou, K.A. Link, B. Mizukawa, R.L. Perry, M. Carroll, and J.C. Mulloy. 2010. AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. *Leukemia.* 24:1785–1788. https://doi.org/10.1038/leu.2010.158

Yahata, T., K. Ando, T. Sato, H. Miyatake, Y. Nakamura, Y. Muguruma, S. Kato, and T. Hotta. 2003. A highly sensitive strategy for SCID-repopulating cell assay by direct injection of primitive human hematopoietic cells into NOD/SCID mice bone marrow. *Blood.* 101:2905–2913. https://doi.org/10.1182/blood-2002-07-1995

Yamazaki, S., H. Ema, G. Karlsson, T. Yamaguchi, H. Miyoshi, S. Shioda, M.M. Taketo, S. Karlsson, A. Iwama, and H. Nakauchi. 2011. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. *Cell.* 147:1146–1158. https://doi.org/10.1016/j.cell.2011.09.053

Yang, W., S.K. Both, G.J. van Osch, Y. Wang, J.A. Jansen, and F. Yang. 2015. Effects of in vitro chondrogenic priming time of bone-marrow-derived mesenchymal stromal cells on in vivo endochondral bone formation. *Acta Biomater.* 13:254–265. https://doi.org/10.1016/j.actbio.2014.11.029

Yoshimi, A., M.E. Balasis, A. Vedder, K. Feldman, Y. Ma, H. Zhang, S.C.-W. Lee, C. Letson, S. Niyongere, S.X. Lu, et al. 2017. Robust patient-derived xenografts of MDS/MPN overlap syndromes capture the unique characteristics of CMML and JMML. *Blood.* 130:397–407. https://doi.org/10.1182/blood-2017-01-763219

Yu, V.W., and D.T. Scadden. 2016. Heterogeneity of the bone marrow niche. *Curr. Opin. Hematol.* 23:331–338. https://doi.org/10.1097/MOH.0000000000000265

Yurino, A., K. Takenaka, T. Yamauchi, T. Nunomura, Y. Uehara, F. Jinnouchi, K. Miyawaki, Y. Kikushige, K. Kato, T. Miyamoto, et al. 2016. Enhanced Reconstitution of Human Erythropoiesis and Thrombopoiesis in an Immunodeficient Mouse Model with Kit(Wv) Mutations. *Stem Cell Reports.* 7:425–438. https://doi.org/10.1016/j.stemcr.2016.07.002

Zambetti, N.A., Z. Ping, S. Chen, K.J.G. Kenswil, M.A. Mylona, M.A. Sanders, R.M. Hoogenboezem, E.M.J. Bindels, M.N. Adisty, P.M.H. Van Strien, et al. 2016. Mesenchymal inflammation drives genotoxic stress in hematopoietic stem cells and predicts disease evolution in human pre-leukemia. *Cell Stem Cell.* 19:613–627. https://doi.org/10.1016/j.stem.2016.08.021

Zhao, M., J.M. Perry, H. Marshall, A. Venkatraman, P. Qian, X.C. He, J. Ahamed, and L. Li. 2014. Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. *Nat. Med.* 20:1321–1326. https://doi.org/10.1038/nm.3706

Zhou, B.O., R. Yue, M.M. Murphy, J.G. Peyer, and S.J. Morrison. 2014. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell.* 15:154–168. https://doi.org/10.1016/j.stem.2014.06.008