1 **Emerging trends in bone marrow organoid research: From hematopoietic**
2 **microenvironment reconstruction to translational and regenerative theranostic**
3 **applications**

4

5 Heebin Park[1], Jaemin Jeong[1,*]

6 [1]Department of Biohealth Convergence, College of Science and Convergence Technology, Seoul
7 Women's University, Seoul, 01797, Republic of Korea.

8

9

10 *Correspondence: Jaemin Jeong, Ph.D., Address: Science Building No. 1, Department of Biohealth
11 Convergence, College of Science and Convergence Technology, Seoul Women's University, Seoul,
12 01797, Republic of Korea, E-mail: jmj1103@swu.ac.kr

13

14 *Keywords:* Biomaterials, Bone marrow, Bone marrow environment, Disease model, Organoids

15

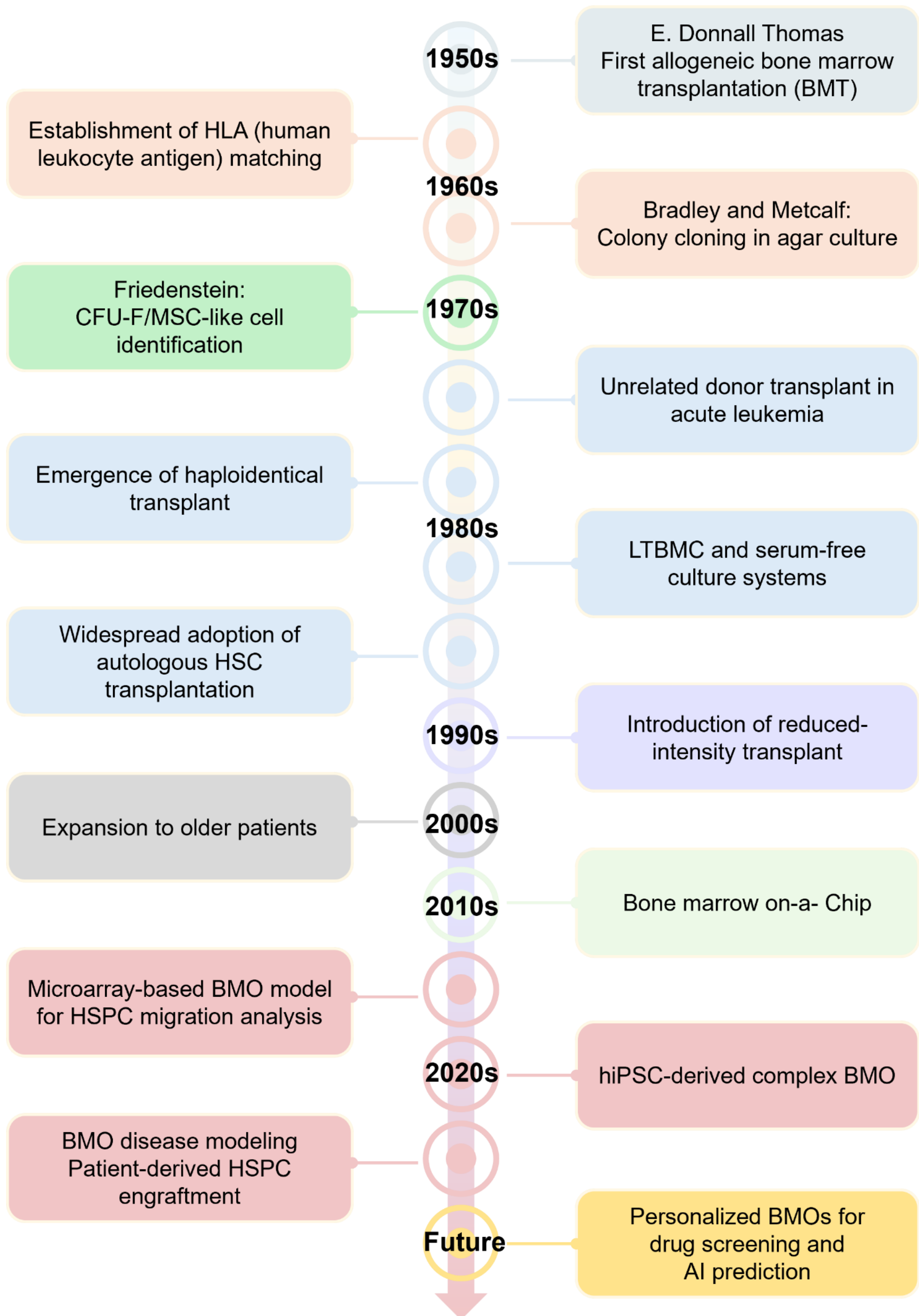
16 Highlights:

17 • Bone marrow organoids recapitulate the main anatomical and functional properties of the
18 native niche.
19 • The in vivo engraftment potential and therapeutic applicability of bone marrow organoids
20 have been discussed.
21 • Bone marrow organoids have become promising platforms for disease modeling and
22 translational research.

23

Abstract

Bone marrow organ is characterized as a dynamic tissue with a complex microenvironment wherein hematopoietic stem cell (HSC) homeostasis is maintained, and the generation of various hematopoietic cell subsets is regulated. Organoids technology has been applied as an alternative tool for modeling complex tissue microenvironments in a laboratory setting through the self-organization of cells. Recent studies have established a platform capable of studying complex cellular interactions by generating bone marrow organoids (BMOs) from iPSCs. Moreover, BMOs can emulate the architecture observed of the bone marrow, including the various cell types, containing vascular-like networks, HSCs, mesenchymal stromal cells (MSCs), and mature hematopoietic cells. This review aimed to summarize how BMOs can provide foundational data essential for understanding similarities in the microenvironment and the pathological mechanisms underlying bone marrow diseases, as well as for developing new treatments. Furthermore, BMO systems represent a cutting-edge platform for studying hematopoiesis, disease mechanisms, and therapeutic screening, highlighting the recent trend toward physiologically relevant organoid-based models in regenerative and hematopoietic research.



44

45 **Figure 1. History and major milestones in bone marrow research.** A timeline showing the
46 major milestones in bone marrow research, from early transplantation studies and in vitro bone
47 marrow culture systems to recent advances in BMO models. Early research has focused on
48 improving HSC memory and establishing experimental platforms for in vitro bone marrow
49 research, while recent research has expanded toward the microphysiological and organoid
50 based modeling.
51
52 **Introduction**

53 The bone marrow is a hematopoietic organ that contributes to blood cell production,
54 immune regulation, and niche-mediated support for HSCs [1-4] and supports continuous
55 hematopoiesis throughout life [1, 2]. An important concept in bone marrow physiology is the
56 HSC niche, a specialized microenvironment that regulates the self-renewal, quiescence, and
57 differentiation of HSCs [1, 5]. This niche comprises of diverse cellular and molecular
58 components that interact through complex signaling networks and regulate the fate and
59 function of hematopoietic stem and progenitor cells (HSPCs) [6, 7]. The interactions between
60 hematopoietic cells and multiple types of bone marrow niche cells, such as MSCs, endothelial
61 cells (ECs), and osteoblastic cells, ensure bone marrow homeostasis [5, 8]. Moreover, the
62 interactions between cells within the bone marrow microenvironment highlight that
63 hematopoiesis occurs within a complex, precisely regulated environment.

64 Bone marrow research has evolved as an important field for understanding HSCs,
65 hematologic physiology, and related diseases. Although various technologies have been
66 established to reduce immune rejection and improve transplantation outcomes, the
67 development of in vitro models that can reproduce the complex bone marrow

68 microenvironment in the laboratory is essential. Early colony-forming studies using mouse
69 bone marrow cells cultured in semi-solid agar, the identification of colony-forming unit-
70 fibroblasts (CFU-F), and the development of long-term bone marrow culture and serum-free
71 culture systems provided the conceptual and technical foundation for in vitro bone marrow
72 research (Figure 1) [9-11]. In parallel, advances in hematopoietic cell transplantation, including
73 allogeneic, unrelated donor, autologous, and haploidentical transplantation, emphasized the
74 importance of the bone marrow microenvironment in determining stem cell engraftment and
75 hematopoietic recovery (Figure 1) [12, 13]. Subsequently, microphysiological systems such as
76 bone marrow-on-a-chip partially reconstructed the vascular and stromal microenvironmental
77 functions ex vivo, further extending this line of research and ultimately laying the foundation
78 for future BMO studies (Figure 1) [14].

79 Since 2020, BMO research has evolved from proof-of-concept platforms to more
80 sophisticated human-derived models (Figure 1). Recent studies have shown that BMOs can
81 recapitulate the essential features of bone marrow biology, including vascular-like networks,
82 stromal organization, hematopoietic progenitor populations, and multilineage differentiation
83 (Figure 1) [4, 15-18]. In particular, the development of microarray-based BMOs for analyzing
84 HSPC migration represented an important milestone, followed by the establishment of human
85 BMO systems capable of disease modeling and therapeutic target validation [17]. Furthermore,
86 hiPSC-derived BMOs can be effectively used as a platform for reconstructing vascular and
87 stromal microenvironments, while patient-derived HSPC engraftment models demonstrate the
88 potential for the clinical and translational expansion of this field [4, 15, 16]. These advances
89 have positioned BMOs not only as helpful tools for studying microenvironment-dependent
90 hematopoiesis and bone marrow pathophysiology but also as promising platforms for patient-

91 specific disease modeling, therapeutic screening, and regenerative medicine applications.

92 The present study focused on the development of BMOs since 2020. We discussed major
93 advances in BMO generation technologies, their applications in hematopoietic function and
94 disease modeling, and the remaining challenges and future directions for establishing BMOs
95 systems with both physiological relevance and clinical usefulness. Building upon these
96 advancements, this review article aimed to summarize the current progress in BMOs research,
97 highlight its applications in modeling hematopoietic function and disease pathology, and
98 discuss new challenges and future directions toward translational and regenerative applications.

99

100 **Table 1. Bone marrow-derived hematopoietic cell lineages, markers and functional**
101 **characteristics.**

102 The representative bone marrow-derived hematopoietic cell populations, their commonly used
103 surface markers, and major functional characteristics.

Lineage	Cell	Markers	Features	References
Myeloid lineage	Neutrophils	$CD15^+$, $CD11b^+$, $CD66b^+$	Eliminate bacterial infection	[133, 134]
	Eosinophils	$CCR3^+$, $CD125^+$	Allergic reaction	[135, 136]
	Basophils	$CD125^+$, $CD193^+$	Allergic reaction	[137]
	Monocytes	$CD14^+$, $CD16^+$	Differentiation into macrophages	[138]
	Macrophages	$CD68^+$, $CD163^+$	Phagocytosis, antigen processing	[139]
	Megakaryocytes	$CD41^+$, $CD42b^+$	Platelet production	[140]
	Erythrocytes	$CD235a^+$	Red blood cell maturation	[141]
	Platelets	$CD41^+$, $CD61^+$, $CD42b^+$	Hemostasis	[142, 143]
	Dendritic cells	$CD11c^+$, MHC-II^+	Antigen presentation	[144]
Lymphoid lineage	B cells	$CD19^+$, $CD20^+$	Antibody production	[145]
	T cells	$CD3^+$	Cellular immunity	[146]
	NK cells	$CD56^+$, $CD16^+$	Nonspecific killing	[147]
	Plasma cells	$CD38^+$, $CD138^+$	Antibody production	[148]

104

105 **Cellular components of the bone marrow niche relevant to organoids design**

106 ***Hematopoietic stem cells (HSCs)***

107 The bone marrow stem cell niche provides a specialized microenvironment wherein the
108 HSCs reside. Within this niche, HSC fate decisions are regulated by both intrinsic and extrinsic
109 mechanisms [2, 19, 20]. HSCs are functionally defined as cells that can sustain over the long
110 term and reconstitute the hematopoietic system following an injury or ablation [21]. This
111 definition is based on the ability of HSCs to undergo self-renewal and facilitate the production
112 of all hematopoietic lineages, including both myeloid and lymphoid blood cells, indicating their
113 multipotent differentiation capacity [22, 23]. Within the bone marrow, HSCs tend to exhibit
114 long-term hematopoietic reconstitution, which refers to the stable maintenance of the
115 hematopoietic system throughout the lifespan rather than transient hematopoietic activities.
116 This long-term maintenance is enabled by specific anatomical regions within the bone marrow,
117 which are collectively referred to as the HSC niche. Recent studies have reported that many
118 HSCs are located near sinusoidal vessels, suggesting that the perivascular region, along with
119 the endosteal niche, can serve as an important microenvironment for the localization and
120 maintenance of HSCs [24, 25]. Within this niche, ECs and stromal cells cooperate to create a
121 microenvironment essential for hematopoietic homeostasis [2, 6, 25, 26]. Indeed, bone marrow
122 contains diverse microenvironments, and HSCs primarily reside in the perivascular region,
123 where ECs and stromal cells interact closely to create an environment that supports
124 hematopoietic regulation [27, 28]. Within this niche, the CXCL12–CXCR4 signaling axis plays
125 a central role in maintaining HSC quiescence and guiding their spatial localization [29, 30].
126 CXCL12-abundant reticular (CAR) cells are a subset of bone marrow stromal cells that secrete
127 high levels of CXCL12, are distributed in a reticular pattern around the HSCs niche and

128 regulate the establishment and maintenance of HSCs [15, 31-33]. Simultaneously, SCF
129 regulates HSCs adhesion and motility, acting in concert with CXCL12-mediated chemotactic
130 cues to maintain proper stem cell positioning and retention in the bone marrow [29, 34, 35].
131 Notably, membrane-bound SCF expressed by stromal and endothelial cells stabilizes the niche,
132 ensuring long-term maintenance of the quiescent HSCs pool [35-37]. Recent advances in three-
133 dimensional (3D) BMO models have allowed the faithful reconstruction of this regulatory
134 network [4, 15, 16, 18]. Importantly, organoid-derived HSPCs have been shown to differentiate
135 into myeloid and lymphoid progenitors, including granulocytes, monocytes, macrophages, and
136 megakaryocytes [4, 15, 16, 18]. In line with this, HSCs facilitate the production of myeloid
137 lineage cells in the bone marrow, including erythrocytes, neutrophils, eosinophils, basophils,
138 monocytes, and platelets, while differentiating into lymphoid lineage cells such as T cells, B
139 cells, NK cells, and plasma cells (Table 1). Within these systems, HSCs reside in the
140 perivascular regions and exhibit gene expression patterns and migratory behaviors that are
141 consistent with in vivo marrow physiology. Furthermore, the re-establishment of CXCL12–
142 CXCR4 and SCF-dependent interactions within the organoid validates the formation of a
143 functional hematopoietic niche capable of supporting physiological HSC maintenance and
144 multilineage differentiation [4].

145 ***Mesenchymal stromal cells (MSCs)***

146 MSCs are a central component of the bone marrow microenvironment and serve as
147 key regulators of hematopoietic homeostasis [5, 8]. These multipotent cells produce
148 extracellular matrix (ECM) proteins such as collagen, laminin, and fibronectin, which
149 constitute the structural framework necessary for the compartmentalization and organization
150 of hematopoietic cells and ECs in the bone marrow microenvironment [4, 38, 39]. Such ECM-

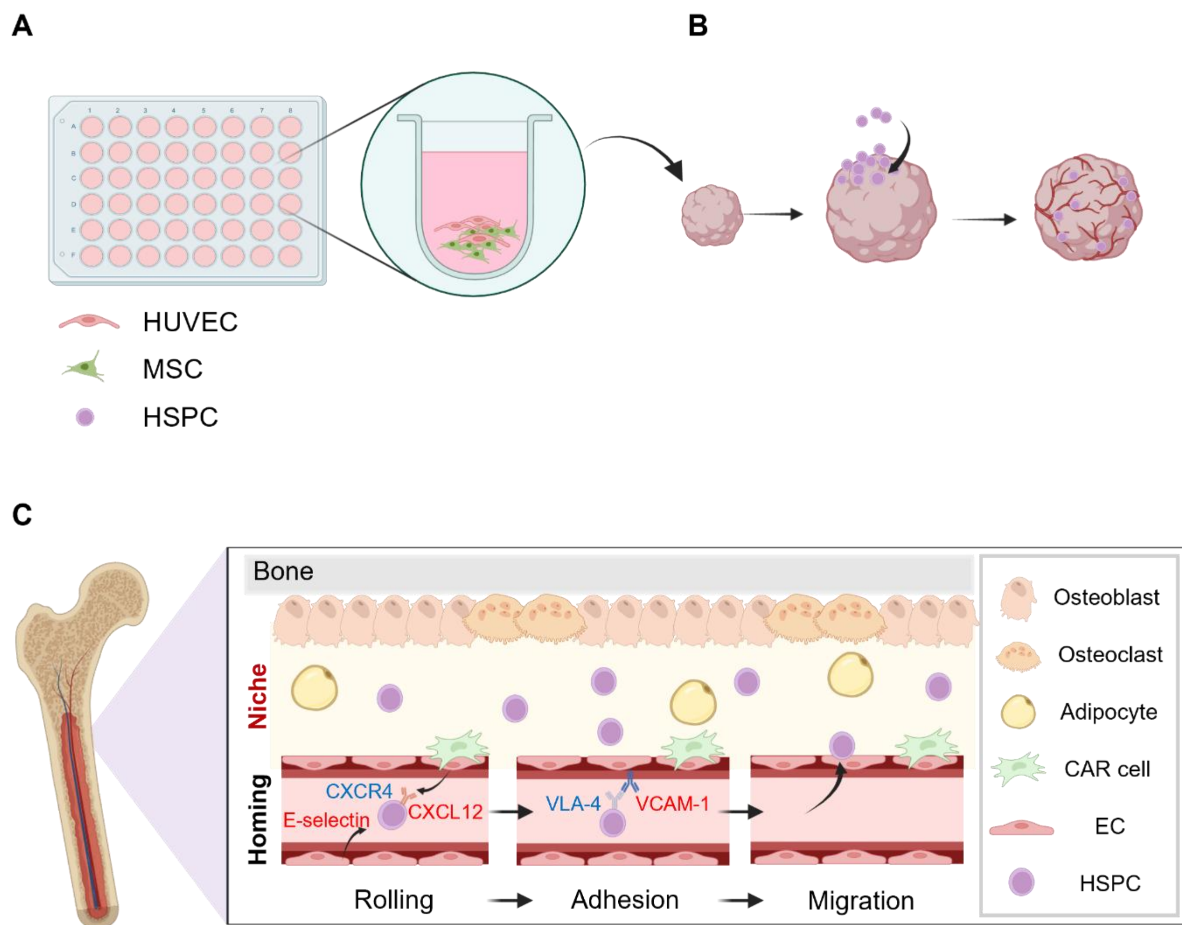
151 based architectures allow for the spatial organization of the bone marrow niche and provide the
152 foundation for sustaining stable hematopoietic function [40]. MSCs also regulate stem cell
153 function and the organization of the cells within the niche through direct interactions and
154 paracrine signaling with hematopoietic cells and ECs [41]. Moreover, MSCs that are localized
155 in the perivascular niche secrete CXCL12, which promotes HSC retention within the niche via
156 CXCR4 signaling and helps maintain their quiescence [42, 43]. They also produce ANG-1,
157 which is received through the Tie2 receptor on HSCs to reinforce quiescence and
158 simultaneously promote vascular stabilization within the niche [42, 44]. MSCs tends to
159 cooperate with ECs to promote angiogenesis, support HSC regeneration in a transplantation
160 setting, or regulate the stability of the vascular niche [45, 46]. In organoid models, they self-
161 organize into stromal and vascular compartments, recapitulating their in vivo role in regulating
162 hematopoietic activities [4, 15]. Within the bone marrow niche, MSCs integrate mechanical
163 and biochemical cues to maintain hematopoietic homeostasis and orchestrate the formation of
164 a physiologically relevant BMO microenvironment.

165 ***Endothelial cells (ECs)***

166 ECs constitute the vascular compartment of the bone marrow niche, which serves as a
167 structural scaffold and paracrine regulator that maintains hematopoietic homeostasis [19]. They
168 secrete angiocrine signals such as VEGF, and FLT4, which regulate HSC self-renewal,
169 differentiation, and mobilization [47, 48]. The vascular niche contains sinusoidal and arterial
170 endothelial compartments [48]. Sinusoidal ECs tend to secrete vasculogenic factors, such as
171 CXCL12, SCF, and VEGFA, and facilitate the maintenance and regeneration of HSCs through
172 interactions mediated by adhesion molecules, including E-selectin [20, 49, 50]. In the bone
173 marrow, sinusoidal ECs are characterized by high permeability and a relatively elevated ROS

174 environment, while they secrete factors such as CXCL12, VCAM1, and E-selectin to promote
175 the activation, regeneration, and differentiation of HSCs [51, 52]. In contrast, arteriolar ECs
176 are associated with a relatively low-ROS microenvironment and support the long-term
177 dormancy and maintenance of HSCs by providing antiproliferative and Notch signals,
178 including DLL4 and JAG-1 [53, 54]. These vascular EC subtypes establish distinct
179 microenvironmental niches that regulate the localization and functional status of HSCs.
180 Recently, 3D BMO systems have allowed for the partial in vitro reconstruction of these
181 vascular niches. In hiPSC-derived BMOs and MSC precursor-EC co-culture-based BMO
182 models, ECs self-organize into arterial-like networks and reproduce major vascular and stromal
183 interactions which constitute $CXCL12^{+}$ perivascular cells, $LepR^{+}$ and $Nestin^{+}$ stromal
184 populations, and megakaryocytes positioned near sinusoid-like vessels [4, 15, 18, 31, 32].
185 These niches recapitulate essential endothelial and stromal signaling, supporting the long-term
186 maintenance of HSPCs and multilineage hematopoiesis without exogenous cytokines [4]. The
187 ECs within 3D bone marrow niches serve as niche regulators, integrating structural, metabolic,
188 and biochemical signals that are crucial for hematopoietic regulation.

189



190

191 **Figure 2. A 3D stromal–vascular co-culture system for BMO generation and HSPC engraftment. (A)** The BMOs were generated by co-culturing MSCs and human umbilical vein endothelial cells (HUVECs). **(B)** Following transplantation, the HSPCs were delivered through the bloodstream and successfully engrafted into the organoids. **(C)** The homing process of HSPCs into the bone marrow niche. Created with BioRender.com.

196

197 **The evolution of bone marrow organoid systems**

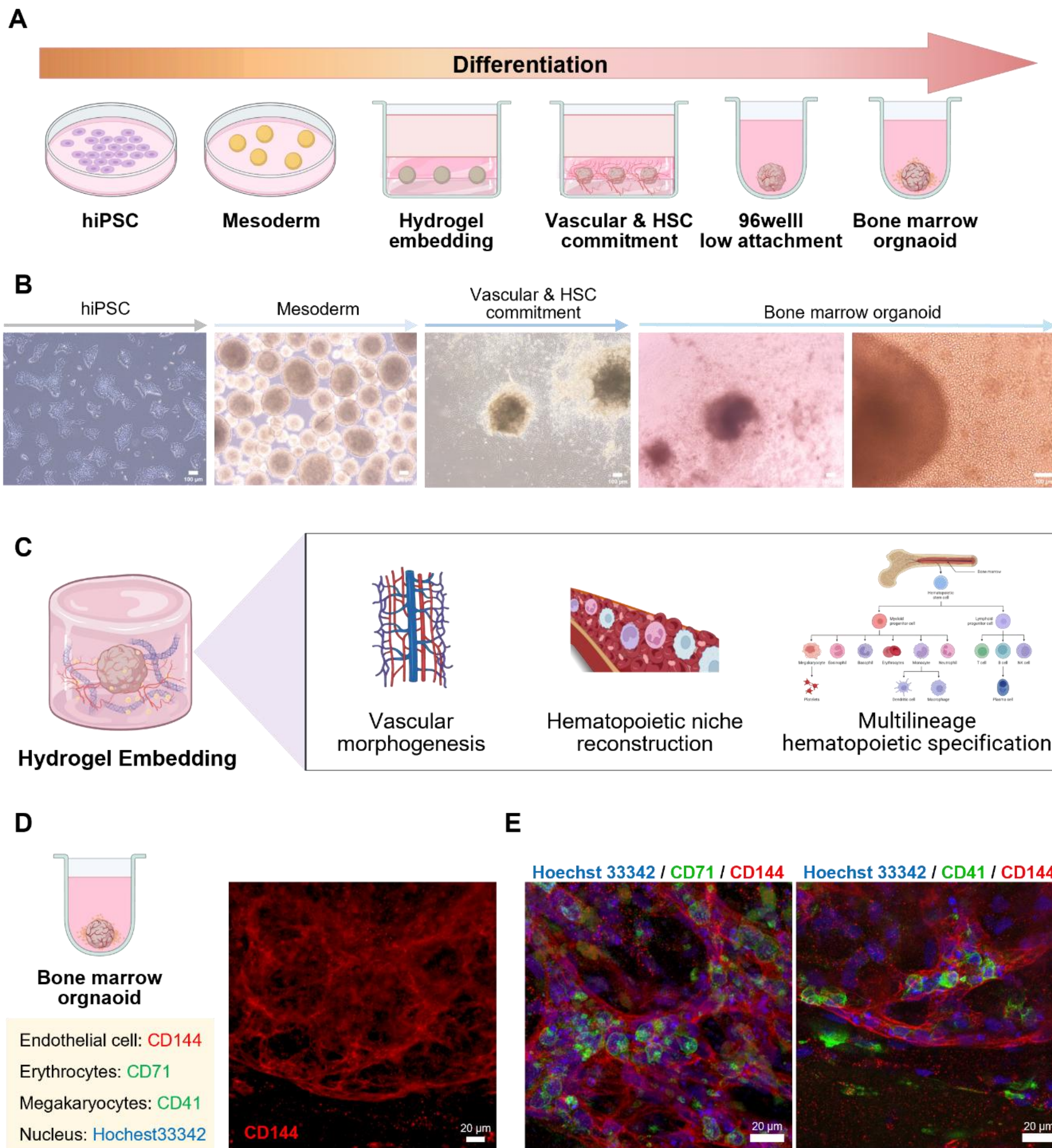
198 ***Early stromal–vascular co-culture models for HSPC homing***

199 A major advance in early BMOs research was the development of a 3D stromal-vascular co-culture system that allowed for an in vitro analysis of HSPC homing. Giger *et al.* presented a

201 3D matrix vascular co-culture system that allowed for an in vitro analysis of HSPC homing in
202 their initial BMO studies (Figure 2) [17]. HSPC homing is defined as the process by which
203 transplanted HSPCs migrate to specific sites in the bone marrow and initiate engraftment and
204 hematopoiesis [55]. This process involves the stage wherein HSPCs migrate through the
205 bloodstream; are implanted into the bone marrow microenvironmental; interact with various
206 components, including MSCs and ECs; and settle in the bone marrow microenvironment [34,
207 56]. ECs regulate the intravascular migration and initial adhesion of HSPCs on certain
208 molecules, such as E-selectin and VCAM1 [49, 57]. In particular, when CXCL12 binds to
209 CXCR4 on HSCs, integrin-mediated adhesion mechanisms, such as the activation of VLA-4-
210 VCAM1 allowed HSPCs to stably attach to the vascular endothelium and migrate to the
211 endothelium (Figure 2) [29, 56]. This process is supported by the chemotactic and adhesive
212 signals from MSCs and ECs [56, 58]. In this context, early studies on the recreation of the bone
213 marrow microenvironment have focused not only on interactions among MSCs, ECs, and
214 HSPCs but also on the reconstruction of the microenvironment that is conducive to these
215 interactions. The migration of HSPCs is dependent on the ratio of ECs and the degree of
216 vascular-like network formation, which implies that the endothelial structure is a functional
217 element regulating the migration of HSPCs beyond simple morphological features. A spatial
218 analysis revealed that HSPCs migrate into the organoid and localize adjacent to the $CD31^+$ and
219 $CD144^+$ endothelial networks. This is similar to the pattern of localization along in vivo
220 vascular niches [59]. Thus, the initial stromal-vascular co-culture model not only partially
221 reproduces the bone marrow-like tissue structure but also simultaneously recapitulates the core
222 functional features of HSPC homing.

223

224



225

226 **Figure 3. Extracellular matrix-supported 3D culture system for vascular morphogenesis**
227 **and multilineage hematopoietic specification**. **(A, B)** The differentiation of hiPSCs into
228 BMOs. Scale bar = 100 μm. **(C)** These ECM combinations provide a microenvironment
229 conducive to vascular morphogenesis and multilineage hematopoietic specification. **(D, E)** The
230 immunofluorescence analysis further confirmed the co-organization of $CD144^+$ ECs, $CD71^+$

erythrocytes, and $CD41^+$ megakaryocytes within the organoid. Scale bar = 20 μm. Created with BioRender.com.

Transition to hiPSC-derived organoids

Previous MSC-EC co-culture models marked an important step toward modeling the human hematopoietic microenvironment, but encountered several limitations in terms of long-term stability, scalability, and donor diversity [17]. To address the limitations of early co-culture systems, recent studies have established standardized protocols for generating hiPSC-derived BMOs, providing reproducible and developmentally coherent platforms for hematopoietic niche reconstruction. This approach involves embedding hiPSC-derived embryoids by binding ECM components, such as collagen type 1 or 4, to Matrigel or Geltrex-like substrates and culturing them under 3D conditions (Figure 3) [4, 15]. Consistent with this framework, our differentiation system also showed sequential morphological progression from early cellular aggregates to mature spheroid-like organoids during stepwise culture (Figure 3). Such composite matrix environments were determined as critical determinants for supporting vascular morphogenesis and multilineage hematopoietic specification (Figure 3). Moreover, the results of the immunofluorescence analysis confirmed that $CD71^+$ erythrocytes and $CD41^+$ megakaryocytes are organized around the organoid $CD144^+$ ECs (Figure 3), which supports the concept that hiPSC-derived BMOs can spatially reproduce the cellular components of the hematopoietic microenvironment.

Extracellular matrix guided self-organization and lineage balancing in bone marrow organoids

The ECM composition of the hiPSC-derived BMOs system plays a crucial role in

254 regulating angiogenesis and hematopoietic lineage differentiation. Collagen type I provides a
255 fibrous structural framework that supports tissue integrity and mechanical stability [60, 61].
256 Collagen type IV, which is a major component of basement membranes, has been shown to
257 create a microenvironment that is more permissive for bone marrow myeloid lineage
258 differentiation [62, 63]. Through comparative hydrogel studies, Khan *et al.* showed that a
259 composite matrix combining collagen types I and IV with Matrigel improves angiogenesis
260 compared with single-collagen conditions [4]. Importantly, this combined ECM environment
261 allows for the harmonious emergence of various myeloid–associated cell populations,
262 including HSPCs, ECs, MSCs, megakaryocytes, erythrocytes, and monocytes [64]. Similarly,
263 Frenz-Wiessner *et al.* applied a strategy in which vascular organoids were embedded within
264 hydrogels composed of collagen type I and Matrigel [15]. Under these conditions, the
265 persistence of ECs and the emergence of MSCs were confirmed. Single-cell analyses revealed
266 distinct clusters corresponding to populations of ECs, hematopoietic cells, and MSCs, and
267 confirmed that the hematopoietic population was further subdivided into myeloid populations
268 and HSPCs. Collectively, these studies demonstrate that multiple myeloid-related cell
269 populations can be harmoniously generated by incorporating mesenchymal-derived 3D cell
270 aggregates into a specific hydrogel environment. These results support the concept that
271 extracellular matrix composition plays not merely a structural role but also a directive function,
272 inducing multicellular self-organization and promoting the reorganization of a functional
273 perivascular hematopoietic microenvironment in vitro.

Bone marrow Organoid
Stem cell
Differentiation

A

Sinusoidal EC
Pericyte
HSC
CAR cell
Adipocyte
Weibel-Palade
Megakaryocyte
Platelet
Arteriolar EC
LepR⁺ stromal cell
NG2⁺ periarteriolar cell

B Vascular network

CD45⁺
PDGFRβ⁺
lumenized vessel

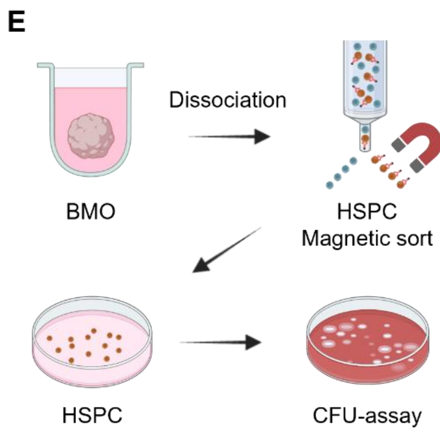
C Sinusoidal vessel

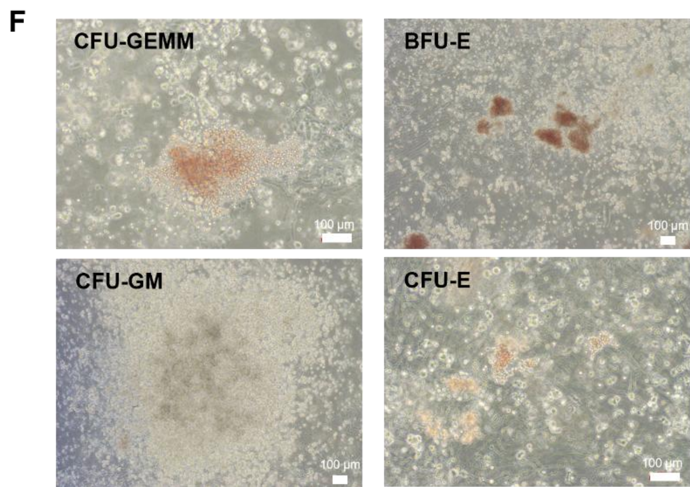
LepR⁺
SCF
CXCL12
CXCR4
CXCL12⁺
CXCL12
CXCR4
E-selectin
VCAM-1

D

Megakaryocyte-MSC
TGF-β
PDGF, FGF
VEGF family

Megakaryocyte-EC
NOTCH-centered
Vascular supportive
Adhesion and niche interface





274

275 **Figure 4. Structural and functional features of the bone marrow organoid vascular niche.**
276 **(A, B)** The bone marrow vascular microenvironment and its major cellular components,
277 including lumenized vessels, PDGFRβ^{+} perivascular cells, and surrounding CD45^{+} HSPCs. **(C)**
278 The sinusoidal vessels form a key hematopoietic niche in which LepR^{+} and CXCL12^{+} stromal
279 populations provide SCF and CXCL12, while E-selectin, VCAM-1, and CXCR4-mediated
280 signaling regulate the localization and retention of HSPCs. (**D**) Thrombopoiesis-related
281 signaling in the perisinusoidal niche. **(E, F)** A CFU assay was used to assess the hematopoietic
282 potential of HSPCs isolated from BMOS, along with the representative images of the CFU-
283 GEMM, BFU-E, CFU-GM, and CFU-E colonies. Scale bar = 100 μm. Created with
284 BioRender.com.

285

286 **Vascular architecture and niche specification in hiPSC-derived BMOs**

287 ***Structural reconstruction of the bone marrow vascular network***

288 Recent studies have established that HSPCs reside in the perivascular regions of the native
289 bone marrow, wherein endothelial and stromal components cooperate to regulate stem cell
290 maintenance and differentiation (Figure 4). In hiPSC-derived BMOs, a network of lumenized
291 vascular structures was established throughout the organoid, with hematopoietic cells
292 distributed both within and surrounding the endothelial-lined vessels [4, 15]. The perivascular
293 distribution of CD45^{+} cells in organoids suggests the in vitro functional reconstruction of a
294 hematopoietic microenvironment. Evidence of vascular maturation was further supported by
295 platelet-derived growth factor receptor-β (PDGFRβ) positive perivascular cells enveloping
296 endothelial structures, along with the formation of lumenized vessels. PDGFRβ expression is
297 characteristic of pericytes and stromal subsets that contribute to vascular stabilization and

298 endothelial survival (Figure 4) [65, 66]. The presence of Weibel–Palade bodies within ECs
299 further indicates acquisition of functional endothelial identity (Figure 4) [67]. Collectively,
300 these findings suggest that BMOs reconstruct a structurally organized vascular network
301 resembling the architecture of the native bone marrow.

302 ***Perivascular stromal organization and formation of CXCL12 abundant reticular cell like niches***

303 The perivascular niche represents a specialized microenvironment surrounding the bone
304 marrow vasculature that regulates the maintenance, quiescence, and trafficking of HSCs [68,
305 69]. This niche constitutes stromal populations, including LepR$^+$ MSCs, which support
306 hematopoiesis via the secretion of key factors such as CXCL12 and SCF (Figure 4) [69]. In
307 hiPSC-derived BMOs, Frenz-Wiessner *et al.* reported that LepR$^+$ cell populations were
308 localized adjacent to vascular structures, indicating the incorporation of a stromal compartment
309 within the reconstructed vascular niche [15]. In the native bone marrow, HSPCs primarily
310 reside in the perivascular region, where coordinated interactions between endothelial and
311 stromal cells regulate their maintenance and differentiation [25, 70, 71]. Thus, the perivascular
312 spatial organization of the MSC subsets in BMOs recapitulates the key structural features of
313 the physiological HSC niche. These stromal cells represent the key components of the HSC
314 niche and support hematopoietic maintenance through CXCL12-mediated signaling [29, 33].
315 Their spatial organization around vascular networks indicate the integration of an MSCs
316 compartment within the vascular niche [2, 25]. Moreover, CXCL12$^+$ cells tend to form a
317 reticular network structure along the vascular structures, supporting the formation of CAR cell-
318 like populations. In the naïve bone marrow, CAR cells are distributed around the HSCs and
319 regulate their establishment, quiescence, and spatial localization through the CXCL12-CXCR4
320 signaling axis [29, 31, 32]. Within the perivascular niche, CXCL12-CXCR4 signaling plays a

321 key role in maintaining HSC dormancy and inducing position maintenance [29]. Thus, the
322 presence of a similar $CXCL12^+$ reticular network in BMOs implies a functional reconfiguration
323 of the CAR-related perivascular microenvironment.

324 ***Endothelial patterning and sinusoidal features driven by vascular endothelial growth factor***
325 ***C (VEGFC)***

326 The sinusoidal ECs in the bone marrow are characterized by high permeability and a
327 relatively elevated ROS environment and secrete several factors such as CXCL12, VCAM1,
328 and E-selectin to promote the activation, regeneration, and differentiation of HSCs [51, 52].
329 These vascular endothelial subtypes establish distinct microenvironmental niches that
330 differentially regulate the localization and functional states of HSCs. Khan et al. suggested that
331 the addition of VEGFC during organoid differentiation reconstituted a sinusoidal-like bone
332 marrow microenvironment in vitro, recapitulating key molecular and functional features of
333 sinusoidal ECs [4]. The transcriptional profiling of these conditions revealed an overall
334 upregulation of VEGF receptors, including FLT4, which encodes VEGFR3, a marker that is
335 abundant in sinusoidal ECs [72, 73]. Simultaneously, the expression of HSC adhesion
336 molecules, including VCAM1 and integrin subunit alpha 4 (ITGA4), was increased, as was the
337 expression of HSC support growth factors and chemotactic cytokines such as CXCR4 and
338 FGF4 [4]. Collectively, the sinusoidal-like vascular niche is enhanced in BMOs, and the
339 structural and functional characteristics that support the adhesion, retention, migration, and
340 survival of HSPCs are evident [29, 74]. These organoids form lumenized vascular networks
341 containing hematopoietic cells, and HSPCs are mainly localized in the perivascular regions.
342 Megakaryocytes were observed adhering to endothelial barriers and extending proplatelets into
343 the vascular lumen, which accurately reflects an important aspect of platelet production in the

344 bone marrow [75]. Taken together, these findings suggest that BMOs support vascular
345 morphogenesis and reconstruct a structurally organized and functionally competent sinusoidal-
346 like microenvironment capable of sustaining hematopoietic regulation.

347 ***Crosstalk among megakaryocytes endothelial cells and stromal cells within the perivascular niche***

348 Khan *et al.* reported that hiPSC-derived BMOs recapitulate the major structural and
349 molecular interactions among megakaryocytes, ECs, and MSCs within the bone marrow
350 microenvironment [4]. In particular, megakaryocytes were observed in proximity to the
351 endothelial barrier, suggesting that the organoid system recapitulates the spatial architecture of
352 the perivascular microenvironment. This spatial arrangement is also consistent with the
353 physiological context in the bone marrow, where megakaryocytes closely associate with
354 arterial endothelium and extend proplatelets toward the vascular lumen during platelet
355 production (Figure 4) [76, 77]. Consistent with these structural features, extensive receptor-
356 ligand interactions were identified among megakaryocytes, ECs, and MSCs, indicating that the
357 BMOs model not only maintains hematopoietic cell maintenance but also dynamic intercellular
358 communication within the stromal microenvironment. The bidirectional signaling axes
359 detected between megakaryocytes and ECs could be functionally categorized into Notch-
360 centered cell state regulatory signaling, vascular supportive signaling involving the VEGF/
361 ANGPT/ PDGF/ KITLG pathways, and signaling associated with adhesion and niche interface
362 regulation, including SELP-CD34 and FGF2-CD44 [4, 54, 78-80]. These signals suggest that
363 direct contact with ECs regulates megakaryocyte positioning, maintenance, survival, and
364 functional maturation within the vascular niche [81, 82]. In addition, the increased expression
365 of FLT4, ANGPT2, and DLL4 demonstrate that the arterial endothelial program is enhanced in
366 organoid ECs. These vascular characteristics serve as an important basis for the localization of

367 perivascular megakaryocytes and the formation of a microenvironment suitable for platelet
368 production [4, 83]. Collectively, these interactions suggest that ECs may provide instructive
369 cues that regulate the positioning, maintenance, survival, and functional maturation of
370 megakaryocytes within the vascular microenvironment. Meanwhile, the interactions between
371 MSCs and megakaryocytes were centered on TGF-β, PDGF, FGF, and VEGF family members
372 and their corresponding receptors [4]. The growth factors such as PDGF, TGF-β, and FGF
373 stored in the α-granules of megakaryocytes can regulate the state and differentiation potential
374 of MSCs, while MSCs can respond to these signals to provide a substrate environment
375 conducive to the maintenance and maturation of megakaryocytes [76, 84]. Collectively, these
376 findings suggest that BMOs recapitulate essential features of the megakaryocyte-associated
377 vascular microenvironment and suggest that this platform may also support
378 microenvironmental programs relevant to thrombopoiesis.

379 ***Myeloid lineage differentiation***

380 The human bone marrow contains HSPCs that can differentiate into multiple blood cell
381 lineages [64]. Intramedullary HSPCs can potentially mature along the myeloid differentiation
382 pathway via common myeloid progenitor cells [23, 64]. This is precisely tuned by interactions
383 in the bone marrow niche, and for BMOs to reflect the physiological functions of the human
384 bone marrow, it must be possible to reproduce not only the presence of HSPCs but also the
385 ability to differentiate into various myeloid lineage progenitors. Khan *et al.* suggested that aside
386 from merely containing HSPCs, BMOs can also structurally and functionally recapitulate the
387 key aspects of myeloid differentiation observed in the human bone marrow [4]. Specifically,
388 bone marrow myelomonocytic, megakaryocytic, and erythroid cells were identified within the
389 organoids, and the differentiation trajectory analysis showed that major differentiation axes,

390 including the megakaryocyte–erythroid and monocyte–neutrophil lineages, were reproduced
391 [4, 23, 85]. This suggests that BMOs can serve as a significant model reflecting the myeloid
392 differentiation program of the human bone marrow. Moreover, Park *et al.* isolated BMO-
393 derived HSPCs and assessed their functional potential using a colony-forming unit (CFU) assay
394 [18]. The results demonstrated the differentiation into erythroid, granulocytic, and
395 monocyte/macrophage lineages, validating the usefulness of BMOs as a platform for assessing
396 both myeloid differentiation capacity and HSPC function [86]. In concordance with these
397 reports, the CFU assays performed on the cells dissociated from our BMOs yielded multiple
398 colony subtypes, including colony-forming unit-granulocyte, erythrocyte, monocyte, and
399 megakaryocyte (CFU-GEMM), burst-forming unit-erythroid (BFU-E), colony-forming unit-
400 granulocyte/macrophage (CFU-GM), and colony-forming unit-erythroid (CFU-E) (Figure 4),
401 further confirming the multilineage progenitor potential of organoid-derived hematopoietic
402 cells.

403 ***Lymphoid lineage differentiation***

404 Stem cells derived from the bone marrow are important immune organs that produce and
405 mature white blood cells and various immune cells that are at the core of the immune response
406 [87]. Within the bone marrow, immune cells, including T cells, B cells, neutrophils, dendritic
407 cells, and NK cells, closely interact with HSCs to maintain HSC function and regulate
408 inflammatory responses and tissue repair [4, 15, 18, 87]. A recently developed BMOs system
409 has successfully reconstructed these complex immune cell lineages and niche interactions in a
410 physiologically relevant architecture [15, 18]. In the BMOs, both myeloid and lymphoid
411 progenitor cells differentiated, and major immune cells such as monocytes, macrophages,
412 neutrophils, basophils, eosinophils, and mast cells, were observed in a compartmentalized form

413 [15, 18]. This suggests that BMO is a model that reflects the cellular composition of
414 multilineage hematopoiesis. Frenz-Wiessner *et al.* reported that BMOs faithfully recapitulate
415 granulopoiesis, indicating a sequential maturation of neutrophil precursors into immature and
416 mature neutrophils closely resembling those in the human bone marrow [15]. Notably, this
417 process occurred without the exogenous G-CSF, suggesting that intrinsic niche cues regulate
418 myeloid maturation. Upon LPS stimulation, BMOs exhibited increased secretion of
419 inflammatory cytokines IL-6, IL-8, and G-CSF, accompanied by expansion of the preNeu
420 population, effectively modeling human-like emergency granulopoiesis [15]. Furthermore, the
421 potential for T-cell lineage differentiation was confirmed in an artificial thymic organoid (ATO)
422 environment constructed using BMO-derived HSCs and MS5-DLL4, demonstrating that the
423 organoid-based microenvironment enables lymphoid and myeloid differentiation. These
424 research results suggest that BMOs can be a potential research platform for diseases induced
425 by immune cells. This functional responsiveness is useful for studying the pathophysiology of
426 diseases caused by unregulated immune-stromal interactions. Furthermore, in bone marrow
427 diseases characterized by the abnormal signaling between immune and stromal cells, organoids
428 can incorporate patient-derived cells to recapitulate inflammatory cytokine production, stromal
429 reorganization, and immune-stromal dysfunction [88].

430 **In vivo evaluation of bone marrow organoids**

431 ***Structural engraftment of intact bone marrow organoids***

432 Ren *et al.* transplanted intact BMOs beneath the kidney capsule of immunodeficient NSG
433 mice to determine whether these organoids can sustain long-term structural and functional
434 stability within a physiological environment [16]. The subcapsular kidney space has a rich
435 vascular distribution and excellent graft survival [89]. A histological examination conducted 1

month after transplantation confirmed that the organoids had successfully integrated into the host tissue, with clear evidence of engraftment beneath the renal capsule and the presence of vascular lumens surrounding the erythrocytes. The formation of vascular lumens in the mouse model suggests the capacity for physiological hematopoietic maintenance and confirms the potential for intercellular niche communication. Flow cytometric monitoring further revealed the persistent presence of human CD45^{+} leukocytes and CD235a^{+} erythroid cells for up to 11 months, indicating the long-term survival and hematopoietic activity of the transplanted BMOs in vivo. These findings underscore BMOs. These findings underscore the significance of BMOs as a platform capable of achieving long-term vascular integration and sustaining multilineage hematopoiesis following transplantation.

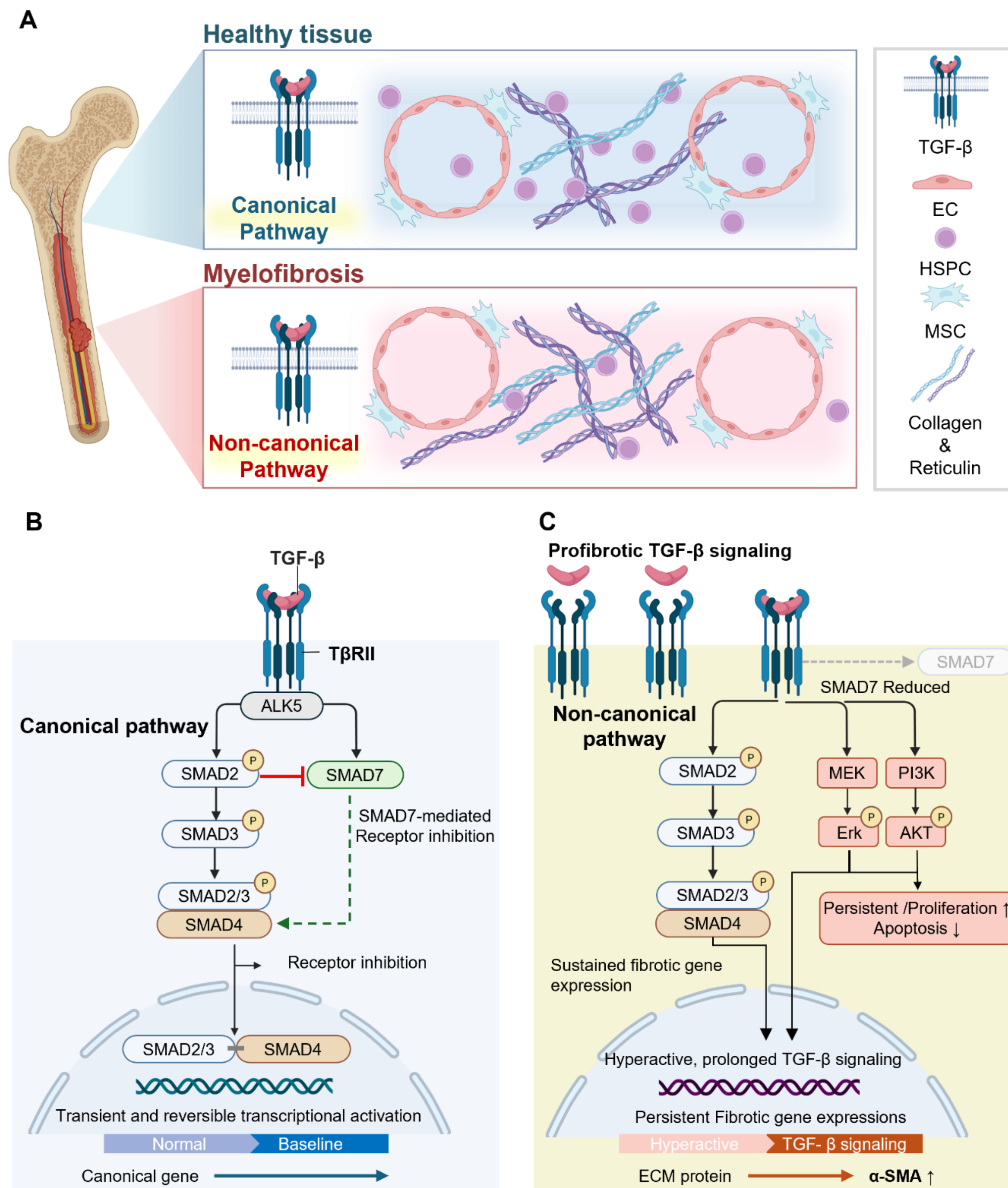
Hematopoietic reconstitution by bone marrow organoids-derived HSPCs

Frenz-Wiessner *et al.* isolated CD34^{+} cells from BMOs by FACS and intravenously injected them into NSG-HLA-DQ8 mice. Serial peripheral blood analyses detected human CD45^{+} cells at weeks 5–10 post-transplantation, indicating partial engraftment [15]. Analysis of the bone marrow niche demonstrated that the transplanted cells survived for 10–12 weeks, with some animals exhibiting increased proportions of human CD45^{+} cells over time. Furthermore, the engrafted human cells differentiated into multiple hematopoietic lineages, including CD20^{+} B cells and CD33^{+} myeloid cells. These findings suggest that BMO-derived HSPCs possess functional hematopoietic reconstitution capacity *in vivo*.

Therapeutic potential of bone marrow organoid based transplantation

Park et al. generated BMOs from hiPSCs and reported that this model can replicate the key elements of the human bone marrow microenvironment [18]. The generated BMOs formed an organized stromal-vascular structure and proved the maintenance and differentiation of

459 hematopoietic progenitor cell populations. These suggest that the BMOs successfully
460 replicated the key elements of the functional human bone marrow microenvironment. In
461 subsequent studies, the research team assessed the potential of BMOs for regenerative therapy
462 through in vivo transplantation experiments using immunodeficient mice. After establishing a
463 model of severe bone marrow injury by irradiating NSG mice with gamma rays, BMOs were
464 transplanted into the irradiated recipients, and the recovery of the hematopoietic system was
465 analyzed. As a result, the survival rates improved in the transplant group, and the partial
466 engraftment of human hematopoietic cells was confirmed. Collectively, this study confirmed
467 the potential of BMO-based bone marrow structures as a therapeutic platform that not only
468 reproduces the pathological characteristics of the human hematopoietic microenvironment but
469 also supports the regeneration of the damaged hematopoietic system.



470

471 **Figure 5. Canonical and non-canonical TGF-β signaling in healthy bone marrow and**

472 **myelofibrosis. (A)** The differential regulation of TGF-β signaling in a normal bone marrow

473 and myelofibrosis. Myelofibrosis is characterized by the accumulation of fibrotic fibers,

474 including collagen and reticulin, together with a reduction in HSPCs within the bone marrow

475 niche. **(B)** In the normal bone marrow, TGF-β signaling is tightly regulated, leading to
476 transient and reversible transcriptional activation that maintains tissue homeostasis. **(C)** In
477 myelofibrosis, non-canonical TGF-β signaling becomes dominant, accompanied by reduced
478 SMAD7-mediated inhibition, prolonged pathway activation, and persistent fibrotic gene
479 expression. Created with BioRender.com.
480

481 **Disease modeling of bone marrow organoids**

482 ***Myelofibrosis: pathological basis***

483 Myelofibrosis, a hematological malignancy, is characterized by an excessive
484 accumulation of reticulin and collagen in the bone marrow microenvironment (Figure 5) [90,
485 91]. A central driver of marrow fibrosis is the sustained activation of TGF-β signaling [91].
486 Activated TβRII subsequently recruits the type I receptor ALK5 to form a functional receptor
487 complex [91, 92]. Upon complex formation, ALK5 phosphorylates and activates SMAD2/3,
488 allowing for these regulatory SMADs to associate with SMAD4 and translocate into the
489 nucleus, where they modulate the transcription of fibrosis-related target genes (Figure 5) [92,
490 93]. The activation of this canonical, SMAD-dependent TGF-β signaling pathway drives the
491 differentiation of fibroblasts into α-SMA–expressing myofibroblasts and markedly increases
492 the production of ECM components, including collagen type I/III and fibronectin [91, 94]. The
493 inhibitory SMAD7 attenuates receptor activity and limits SMAD2/3 nuclear signaling,
494 maintaining ECM homeostasis (Figure 5) [92, 95]. In myelofibrosis, sustained TGF-β signaling
495 and impaired negative feedback result in prolonged SMAD activation, persistent fibrotic
496 transcriptional programs, and excessive ECM deposition [92, 96]. The resulting excessive
497 matrix deposition and increased microenvironmental stiffness amplify the inflammatory

498 signaling and progressively disrupt the hematopoietic niche, ultimately impairing normal
499 hematopoiesis and contributing to the development and progression of bone marrow fibrosis
500 [97].

501 ***Modeling acquired myelofibrosis using bone marrow organoids***

502 Khan *et al.* treated BMOs with TGF-β to model the pathological process of myelofibrosis
503 in vitro [4]. This stimulation significantly increased the fibrosis-related markers such as α-SMA,
504 IL-11, and COL1A1, and induced the major molecular changes of myelofibrotic progression
505 within BMOs [4, 98, 99]. Importantly, fibrosis marker expression was significantly reduced
506 when TGF-β signaling was inhibited with SB431542 or when treated with the bromodomain
507 inhibitor JQ1 [100, 101]. This suggests that the model is not merely a simulation of pathology
508 but can be used as an experimental platform to assess responses to antifibrotic therapy. Beyond
509 induced fibrosis, the researchers further assessed the engraftment capacity of patient-derived
510 HSPCs within BMOs [4]. Both healthy donor and myelofibrosis patient-derived HSPCs
511 successfully engrafted and interacted with the stromal matrix. The organoids seeded with
512 patient-derived cells showed an increased deposition of fibrotic factors, confirming disease-
513 relevant remodeling at the tissue level. Furthermore, they reported that mutant hematopoietic
514 clones can preserve clonal heterogeneity within organoids and mimic malignant stem cell
515 niches in vitro, highlighting the potential of BMOs for disease-specific microenvironmental
516 interactions and therapeutic studies in hematological malignancies.

517 ***Modeling VPS45 associated inherited myelofibrosis using bone marrow organoids***

518 BMOs were used to model hereditary myelofibrosis associated with *VPS45* deficiency
519 [15]. *VPS45* mutations are clinically associated with severe congenital neutropenia, progressive
520 bone marrow failure, and myelofibrosis [102, 103]. Frenz-Wiessner *et al.* established BMOs

521 from iPSCs carrying a homozygous *VPS45 Thr224Asn* mutation and observed stromal
522 abnormalities resembling myelofibrosis, including proliferation of α-SMA–positive
523 myofibroblast-like cells and increased reticulin deposition confirmed via Gomori staining [15].
524 Moreover, altered granulopoiesis, with increased mature neutrophil populations, was detected
525 in mutant organoids. *VPS45* mutant BMOs are characterized by reticular fibrillar deposition
526 and an expanded population of α-SMA-expressing stromal cells, consistent with the patient
527 biopsy findings [15, 99, 102]. Collectively, these studies suggest that hiPSC-derived BMOs
528 can model both acquired and inherited myelofibrosis, can be used in mechanistic studies of
529 fibrotic transformation, and can provide a platform for therapeutic evaluation.

530 ***Modeling radiation induced hematopoietic syndrome using bone marrow organoids***

531 Hematopoietic syndrome is a radiation-induced disorder resulting from the depletion of
532 HSPCs within the bone marrow following exposure to high-dose ionizing radiation [104, 105].
533 Ionizing radiation not only directly induces DNA double-strand breaks but also generates
534 excessive ROS, thereby resulting in additional base lesions, single-strand breaks, and
535 chromosomal instability (Figure 6) [104, 106]. The accumulation of DNA damage induces
536 replication stress in proliferating HSPCs, promoting replication fork stalling and collapse,
537 thereby resulting in the excessive activation of the ATM/ATR– p53–mediated DNA damage
538 response [107, 108]. This damage results in the severe impairment of marrow function, which
539 manifests as rapid declines in leukocytes, lymphocytes, and other blood cell populations,
540 increasing susceptibility to infection, hemorrhage, and immune dysfunction [108, 109]. Park
541 et al. exposed BMOs to ionizing radiation at doses of 3, 6, and 9 Gy to model this condition ex
542 vivo [18]. The irradiated organoids showed structural disintegration, closely resembling the
543 architectural collapse observed in irradiated bone marrow in vivo. Correspondingly,

544 accompanied by the increased expression of apoptotic and DNA damage markers, including

545 phosphorylated *γH2AX*, *BAD*, and *AIF*, as well as elevated numbers of *TUNEL*-positive cells

546 [18]. Notably, the expression of AIF and BAD, which promotes mitochondrial membrane

547 permeabilization was significantly upregulated, confirming radiation-induced cytotoxic and

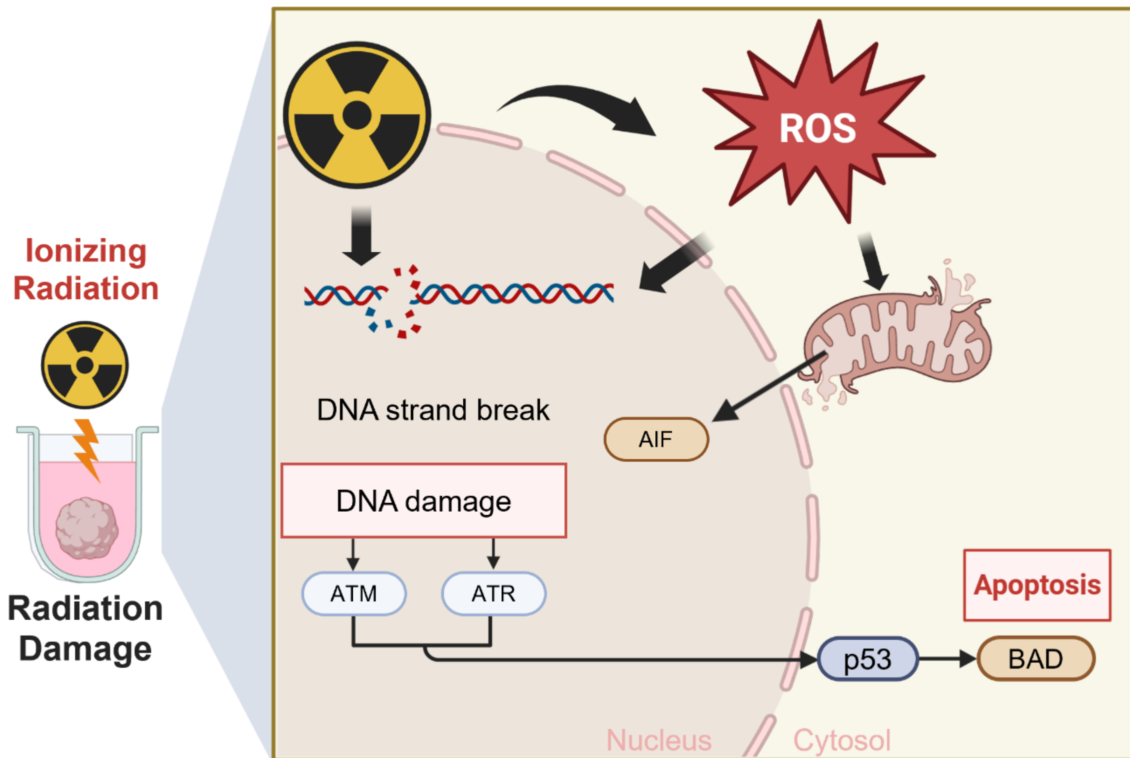
548 genotoxic [110, 111]. These findings establish the BMO as a physiologically relevant platform

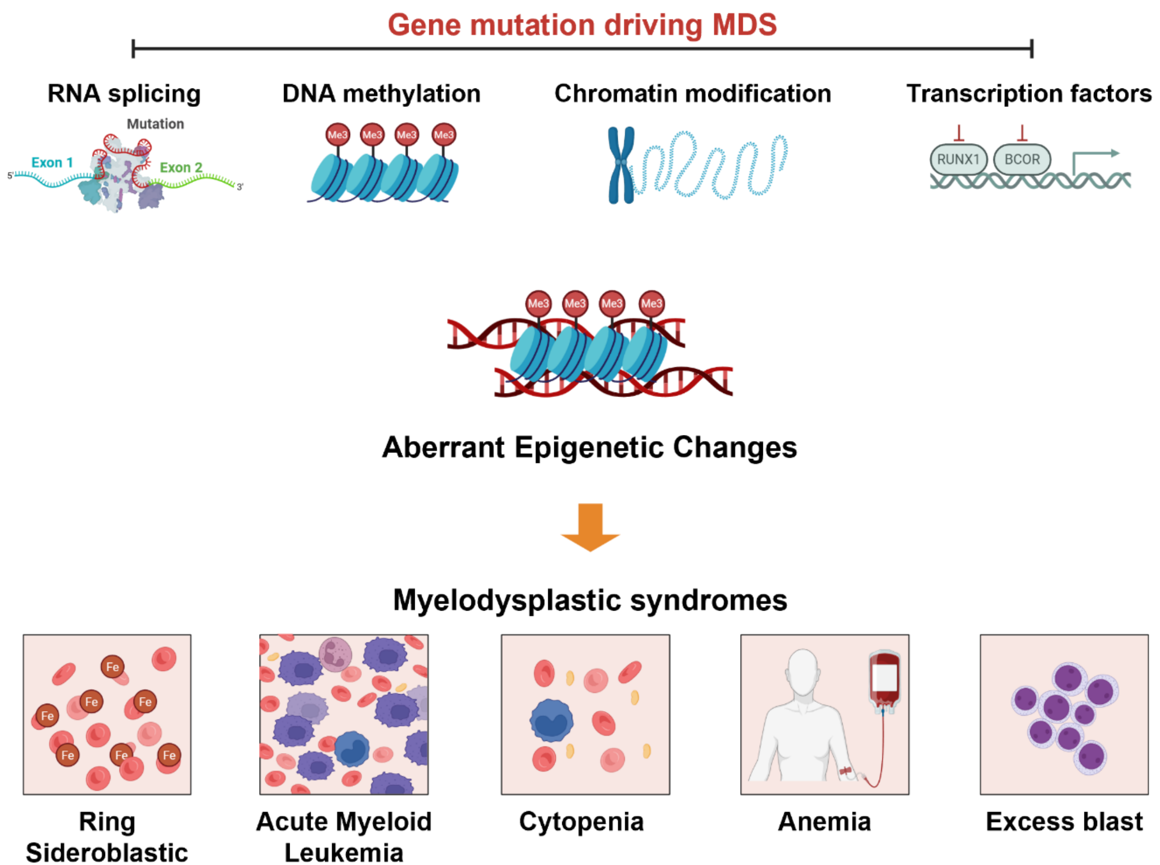
549 for modeling radiation-induced hematopoietic failure, offering potential applications in

550 studying marrow regeneration and radioprotective therapeutic strategies.

551

552





553

554 **Figure 6. Mechanisms underlying radiation-induced hematopoietic syndrome and gene**
555 **mutation-driven myelodysplastic syndrome (MDS). (A)** ROS generation and DNA damage
556 induced by ionizing radiation activate the ATM, ATR, and p53 pathways, leading to
557 mitochondrial dysfunction and apoptosis, thereby mediating radiation-induced hematopoietic
558 injury. **(B)** Gene mutations involving aberrant splicing, DNA methylation, chromatin
559 modification, and transcription factor dysregulation disrupt hematopoiesis and ultimately give
560 rise to the major clinical features of MDS. Created with BioRender.com.
561

562 **Table 2. Gene mutations in myelodysplastic syndromes.**

563 The table summarizes recurrent mutations in MDS, categorized by their principal biological
564 functions, including RNA splicing, DNA methylation, chromatin modification, and
565 transcriptional regulation. These mutations contribute to ineffective hematopoiesis, cytopenia,
566 multilineage dysplasia, ring sideroblast formation, and progression to AML.

Functional category	Gene	Primary function	Clinical significance in MDS	Reference
RNA splicing	*SF3B1*	Core spliceosome component required for branch point recognition and accurate pre-mRNA splicing.	Abnormal mitochondrial iron accumulation, ring sideroblasts, and defective erythropoiesis	[116, 149-153]
	SRSF2	Regulates sequence-specific exon recognition via binding to exonic splicing enhancer motifs.	High AML progression	
	U2AF1	Essential splicing factor that recognizes the 3′ splice site during pre-mRNA splicing to ensure functional mRNA generation.	Anemia, multilineage dysplasia, and leukocytosis	
DNA methylation	*TET2*	Regulates DNA demethylation to maintain proper HSC self-renewal and lineage differentiation.	Mild anemia without marked leukopenia or thrombocytopenia	[116, 154, 155]

	DNMT3A	Functions as a DNA methyltransferase responsible for de novo DNA methylation.	Higher risk of leukemia transformation	
	IDH2	Inhibits TET2-dependent demethylation by generating 2-hydroxyglutarate.	Mild anemia without marked leukopenia	
Chromatin modification	*ASXL1*	Controls chromatin remodeling via histone modification regulation.	Clonal hematopoiesis, myeloid malignancies	[116, 156, 157]
	EZH2	Encodes a core component of the PRC2 complex.	Anemia, thrombocytopenia, and neutropenia	
Transcription factors	*RUNX1*	Hematopoietic transcription factor required for normal blood cell development and differentiation.	Multilineage dysplasia, cytopenia with excess blasts and thrombocytopenia	[116, 158-160]
	BCOR	Epigenetic corepressors required for transcriptional repression and regulation of myeloid cell proliferation and differentiation	multilineage dysplasia and cytopenia with excess blasts	

567

568 ***Myelodysplastic syndrome***

569 Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic disorders driven
570 by somatic mutations in HSCs, resulting in ineffective hematopoiesis, increased apoptosis, and
571 peripheral cytopenias [112]. These defects cause cytopenias, including anemia, neutropenia,
572 and thrombocytopenia [113, 114]. In MDS, recurrent mutations commonly involve genes that
573 can be broadly classified into several functional categories, including RNA splicing, DNA
574 methylation, chromatin modification, and transcription factors, all of which contribute to
575 disordered hematopoietic differentiation and clonal expansion (Figure 6 and Table 2) [115, 116].
576 The gene mutations commonly associated with MDS include *TET2*, *DNMT3A*, *TP53*, *SF3B1*,
577 *ASXL1*, *U2AF1*, and several additional genes, among others [117]. Ren et al. modeled MDS
578 using BMOs seeded with $CD34^+$ HSCs obtained from three patients harboring distinct genetic
579 mutations and clinical phenotypes [16]. *TET2* mutations, clinically present with mild anemia

580 without marked leukopenia or thrombocytopenia, a pattern that is characteristic of some *TET2*-
581 mutated cases. *TET2* mutations result in hematopoietic dysfunction by decreasing DNA
582 demethylation activity and resulting in the persistence of a stem cell-like state with impaired
583 differentiation [112, 115, 118]. *SF3B1* mutation induces spliceosome dysfunction resulting in
584 abnormal mitochondrial iron accumulation, ring sideroblast formation, and defective
585 erythropoiesis—a hallmark phenotype associated with this mutation [118-120]. These defects
586 interfere with normal red blood cell maturation and act as a key cause of persistent anemia in
587 *SF3B1*-mutant MDS. *U2AF1* mutations also cause extensive abnormalities throughout RNA
588 splicing, which consequently disrupts inflammatory pathways, membrane protein expression,
589 and hematopoiesis-related signaling [118, 121]. As a result, lineage differentiation is impaired,
590 resulting in the accumulation of immature progenitors and clinical manifestations such as
591 anemia, multilineage dysplasia, and leukocytosis [122]. The patient-derived $CD34^+$ cells were
592 successfully engrafted within BMOs and maintained over time. These organoids reproduced
593 cytopenia and hematopoietic dysfunction as observed in the MDS patients, suggesting that
594 BMOs can reflect patient-specific hematopoietic defects. The exome DNA sequencing results
595 showed that the patient-specific mutant clones within the organoids maintained similar allele
596 frequencies in vivo. This implies that BMOs can also be used to study somatic mutation
597 maintenance and clonal evolution. Consequently, these MDS organoid models can potentially
598 serve as precision disease modeling platforms for patient-specific genetic and functional
599 pathophysiological studies, the analysis of hematopoietic microenvironmental changes, and
600 validation of novel therapeutic targets.

601 ***Conclusions and future perspectives***

602 hiPSC-derived BMO models can be used as physiologically relevant in vitro models that

603 recapitulate the human bone marrow microenvironment to study hematopoietic development
604 and hematopoietic diseases. This approach reduces the reliance on immunodeficient mouse
605 models and provides a platform for the efficient transplantation and maintenance of HSCs
606 derived from patients with diverse disease backgrounds, allowing for the investigation of
607 patient-specific disease characteristics [123, 124]. By generating and isolating HSPCs within
608 BMOs and validating their multilineage differentiation potential, functional studies can be
609 conducted using HSPCs generated within the organoid system itself [18]. This capability
610 represents a significant advance in the study of the mechanisms of human hematopoiesis and
611 the pathophysiology of blood diseases. Furthermore, by faithfully recapitulating the bone
612 marrow microenvironment, the use of BMOs allows for the in-depth analysis of stem cell niche
613 regulation and associated molecular pathways. Specifically, these systems facilitate the study
614 of cell-to-cell and cell-to-extracellular matrix interactions that regulate HSC behaviors within
615 the bone marrow microenvironment.

616 One future application of BMO is the development of personalized BMOs. Since BMOs
617 can be generated from patient-derived stem or progenitor cells, they provide the advantage of
618 reproducing individual disease phenotypes within physiologically relevant microenvironments
619 [125]. When patient-derived HSPCs with different genetic mutations are transplanted into
620 BMOs, it is possible that disease-specific characteristics associated with each mutation will be
621 reproduced within the organoid [16]. Furthermore, it has been reported that when HSPCs
622 derived from myelofibrosis patients are transplanted into BMOs, disease characteristics related
623 to fibrosis are reproduced within the organoid microenvironment [4]. Thus, such patient-
624 specific platforms can provide significant advantages to precision medicine by allowing for the
625 prediction of individual drug responses, the identification of disease-causing mechanisms, and

626 the identification of new therapeutic targets.

627 Another major application of BMOs is drug screening. Because these systems better 628 mimic the human bone marrow microenvironment than conventional two-dimensional culture 629 models, they provide a more predictable platform for evaluating therapeutic efficacy and 630 toxicity [126]. Furthermore, BMOs can reduce the time and cost of preclinical evaluation by 631 enabling rapid screening of large drug libraries in a patient-specific manner. Indeed, fibrosis 632 BMOs models have already demonstrated responsiveness to therapeutic interventions, with 633 TGF-β inhibitor treatment reducing fibrosis-related phenotypes [4]. These results demonstrate 634 the potential of BMOs as a translational platform for testing therapeutic agents in hematological 635 and bone marrow diseases.

636 Artificial intelligence (AI)-assisted prediction is also likely to become an important future 637 direction in BMOs research. By integrating imaging, transcriptomic, and functional readouts 638 from BMOs, AI-based approaches may improve the prediction of drug responses and enhance 639 precision medicine strategies [127-129]. Moreover, these models may help identify early 640 events involved in the transition from normal hematopoiesis to pathological hematopoiesis and 641 predict whether specific mutational combinations are likely to drive disease progression within 642 the bone marrow microenvironment. AI could also be applied to organoid quality control, 643 including the prediction of organoid maturation, HSPCs maintenance capacity, and 644 experimental success rates based on early morphological or functional features, ultimately 645 helping to reduce batch-to-batch variability [129].

646 Finally, future studies may extend beyond BMOs alone toward integrated BMO systems. 647 One possible direction is the development of models incorporating an osteogenic outer scaffold 648 or shell that mimics the structural properties of the bone, with an inner marrow-like

649 compartment containing hematopoietic and stromal elements [130]. Another approach would
650 be to co-culture BMOs with osteogenic cell populations to better reproduce endosteal and
651 marrow niche interactions. Such integrated bone–marrow models may better recapitulate the
652 in vivo relationship between bone and marrow and further improve the physiological relevance
653 of organoid-based systems. Currently, BMO-based disease modeling studies are limited to a
654 few disease groups, such as myelofibrosis, myelodysplastic syndrome, and radiation-induced
655 hematopoietic syndrome. BMO systems may also be expanded as platforms for studying the
656 pathophysiology of a broader range of bone marrow diseases. For example, bone marrow
657 failure syndromes, in which the bone marrow fails to produce sufficient blood cells, and
658 infectious and inflammatory bone marrow diseases could become targets for future BMO-
659 based modeling and analysis [131]. These diseases involve the complex dysregulation of the
660 bone marrow environment, including altered hematopoietic signaling, immune responses, and
661 interactions between hematopoietic and stromal cells [132]. Thus, BMO platforms could be
662 further developed to model a broader range of bone marrow disorders beyond the disease
663 settings currently studied. In conclusion, BMOs have rapidly evolved into versatile platforms
664 for modeling hematopoiesis, bone marrow pathology, and patient-specific disease features.
665 Furthermore, continued advances in personalized organoid design, drug screening, AI-assisted
666 prediction, and integrated bone–marrow modeling are expected to further expand their value
667 in basic research, translational studies, and regenerative medicine.

668

669 **Abbreviations**

670 CFU-F: colony-forming unit-fibroblast; LTBMC: long-term bone marrow culture; LTHR:
671 long-term hematopoietic reconstitution; CAR: CXCL12-abundant reticular; SCF: stem cell

672 factor; 3D: three-dimensional; NK: natural killer; ECM: extracellular matrix; ANG-1:
673 angiopoietin-1; Tie2: TEK receptor tyrosine kinase; VCAM1: vascular cell adhesion molecule
674 1; LepR: leptin receptor; Nestin: neuroepithelial stem cell protein; VLA-4: very late antigen-4;
675 PDGFRβ: platelet-derived growth factor receptor β; VEGFR3: vascular endothelial growth
676 factor receptor 3; ITGA4: integrin subunit alpha 4; FGF4: fibroblast growth factor 4; ANGPT:
677 angiopoietin; PDGF: platelet-derived growth factor; KITLG: KIT ligand; FGF2: fibroblast
678 growth factor 2; ANGPT2: angiopoietin 2; TGF-β: transforming growth factor-β; CFU: colony-
679 forming unit; ATO: artificial thymic organoid; TβRII: transforming growth factor-β type II
680 receptor; ALK5: activin receptor-like kinase 5; α-SMA: alpha-smooth muscle actin; IL-11:
681 interleukin-11; VPS45: vacuolar protein sorting 45 homolog; p53: protein 53; γH2AX:
682 phosphorylated gamma H2AX; BAD: BCL2-associated agonist of cell death; AIF: apoptosis-
683 inducing factor; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; MDS:
684 myelodysplastic syndromes; DNMT3A: DNA methyltransferase 3 alpha; TP53: tumor protein
685 p53.

686 **Acknowledgements**

687 This work was supported by Genome editing research program funded from the Korea
688 government (MSIT) (RS-2023-00260529), the Basic Science Research Program through the
689 National Research Foundation of Korea (NRF), funded by the Ministry of Education (RS-2025-
690 25434724), the Institute of Information & Communications Technology Planning & Evaluation
691 (IITP) grant funded by the Korea government (MSIT) (RS-2025-02304986), and a research
692 grant from Seoul Women's University (2026-0031). The authors acknowledge the use of
693 BioRender.com for the preparation of figures. ChatGPT (OpenAI) was used solely as an AI-
694 assisted language editing tool to improve English grammar and sentence clarity during

695 manuscript preparation. ChatGPT was not used to generate scientific content, figures, images,
696 data, analyses, interpretations, or conclusions.

697 **Author contributions**

698 H.P., and J.J. wrote the manuscript and generated figures and table. J.J. designed study and
699 supervised the manuscript. All the authors have read and approved the review article.

700 **Competing Interests**

701 The authors declare no competing interests.

702

703 **References**

704 1. Khattab S, El Sorady M, El-Ghandour A, Visani G, Piccaluga PP. Hematopoietic and
705 leukemic stem cells homeostasis: the role of bone marrow niche. Explor Target Antitumor Ther.
706 2024; 5: 1027.
707 2. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells.
708 Nature. 2014; 505: 327-34.
709 3. Shafat MS, Gnaneswaran B, Bowles KM, Rushworth SA. The bone marrow
710 microenvironment–home of the leukemic blasts. Blood Rev. 2017; 31: 277-86.
711 4. Khan AO, Rodriguez-Romera A, Reyat JS, Olijnik A-A, Colombo M, Wang G, et al.
712 Human bone marrow organoids for disease modeling, discovery, and validation of therapeutic
713 targets in hematologic malignancies. Cancer Discov. 2023; 13: 364-85.
714 5. Huang Z, Iqbal Z, Zhao Z, Liu J, Alabsi A, Shabbir M, et al. Cellular crosstalk in the
715 bone marrow niche. J Transl Med. 2024; 22: 1096.
716 6. Sánchez-Lanzas R, Kalampalika F, Ganuza M. Diversity in the bone marrow niche:

717 classic and novel strategies to uncover niche composition. Br J Haematol. 2022; 199: 647-64.
718 7. Pinho S, Frenette PS. Haematopoietic stem cell activity and interactions with the niche.
719 Nat Rev Mol Cell Biol. 2019; 20: 303-20.
720 8. Pimenta DB, Varela VA, Datoguia TS, Caraciolo VB, Lopes GH, Pereira WO. The
721 bone marrow microenvironment mechanisms in acute myeloid leukemia. Front Cell Dev Biol.
722 2021; 9: 764698.
723 9. Bradley T, Metcalf D, Robinson W. Stimulation by leukaemic sera of colony formation
724 in solid agar cultures by proliferation of mouse bone marrow cells. Nature. 1967; 213: 926-7.
725 10. Phinney DG. Alexander Friedenstein, mesenchymal stem cells, shifting paradigms and
726 euphemisms. Bioengineering. 2024; 11: 534.
727 11. Hocking WG, Golde DW. Long-term human bone marrow cultures. Blood. 1980; 56:
728 118-24.
729 12. Nikolousis E, Sakia T, Horgan C, Ahmed M. History of bone marrow transplantation.
730 In: Chandy M, Radhakrishnan VS, Sukumaran RK, Eds. Contemporary Bone Marrow
731 Transplantation, 1st ed. Cham: Springer; 2021: 3-26.
732 13. Pedrazzoli P, Ledermann J, Lotz J-P, Leyvraz S, Aglietta M, Rosti G, et al. High dose
733 chemotherapy with autologous hematopoietic stem cell support for solid tumors other than
734 breast cancer in adults. Ann Oncol. 2006; 17: 1479-88.
735 14. Torisawa Y-S, Spina CS, Mammoto T, Mammoto A, Weaver JC, Tat T, et al. Bone
736 marrow-on-a-chip replicates hematopoietic niche physiology in vitro. Nat Methods. 2014; 11:
737 663-9.
738 15. Frenz-Wiessner S, Fairley SD, Buser M, Goek I, Salewskij K, Jonsson G, et al.
739 Generation of complex bone marrow organoids from human induced pluripotent stem cells.
740 Nat Methods. 2024; 21: 1-14.

741 16. Ren K, Li E, Aydemir I, Liu Y, Han X, Bi H, et al. Development of iPSC-derived
742 human bone marrow organoid for autonomous hematopoiesis and patient-derived HSPC
743 engraftment. Blood Adv. 2025; 9: 54-65.
744 17. Giger S, Hofer M, Miljkovic-Licina M, Hoehnel S, Brandenberg N, Guiet R, et al.
745 Microarrayed human bone marrow organoids for modeling blood stem cell dynamics. APL
746 Bioeng. 2022; 6; 036101.
747 18. Park H, Yu I, Kim T-J, Jang H, Kim HB, Lee SB, et al. Human bone marrow niche
748 organoids for disease modeling and therapeutic application in hematopoietic syndrome.
749 Biomaterials. 2026; 327: 123744.
750 19. Asada N. Regulation of malignant hematopoiesis by bone marrow microenvironment.
751 Front Oncol. 2018; 8: 119.
752 20. He N, Zhang L, Cui J, Li Z. Bone marrow vascular niche: home for hematopoietic
753 stem cells. Bone Marrow Res. 2014; 2014: 128436.
754 21. Seita J, Weissman IL. Hematopoietic stem cell: self-renewal versus differentiation.
755 Wiley Interdiscip Rev Syst Biol Med. 2010; 2: 640-53.
756 22. Huang X, Cho S, Spangrude GJ. Hematopoietic stem cells: generation and self-
757 renewal. Cell Death Differ. 2007; 14: 1851-9.
758 23. Iwasaki H, Akashi K. Myeloid lineage commitment from the hematopoietic stem cell.
759 Immunity. 2007; 26: 726-40.
760 24. Donnelly H, Ross E, Xiao Y, Hermantara R, Taqi AF, Doherty-Boyd WS, et al.
761 Bioengineered niches that recreate physiological extracellular matrix organisation to support
762 long-term haematopoietic stem cells. Nat Commun. 2024; 15: 5791.
763 25. Comazzetto S, Shen B, Morrison SJ. Niches that regulate stem cells and hematopoiesis
764 in adult bone marrow. Dev Cell. 2021; 56: 1848-60.

765 26. Anthony BA, Link DC. Regulation of hematopoietic stem cells by bone marrow
766 stromal cells. Trends Immunol. 2014; 35: 32-7.
767 27. Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during
768 homeostasis and regeneration. Nat Med. 2014; 20: 833-46.
769 28. Mitroulis I, Kalafati L, Bornhäuser M, Hajishengallis G, Chavakis T. Regulation of the
770 bone marrow niche by inflammation. Front Immunol. 2020; 11: 1540.
771 29. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem
772 cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches.
773 Immunity. 2006; 25: 977-88.
774 30. Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, et al. Rapid
775 mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a
776 CXCR4 antagonist. J Exp Med. 2005; 201: 1307-18.
777 31. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and
778 pathological perspectives, problems, and promises. Dev Cell. 2011; 21: 193-215.
779 32. Xie L, Zeng X, Hu J, Chen Q. Characterization of nestin, a selective marker for bone
780 marrow-derived mesenchymal stem cells. Stem Cells Int. 2015; 2015: 762098.
781 33. Omatsu Y. Cellular niches for hematopoietic stem cells in bone marrow under normal
782 and malignant conditions. Inflamm Regen. 2023; 43: 15.
783 34. Whetton AD, Graham GJ. Homing and mobilization in the stem cell niche. Trends Cell
784 Biol. 1999; 9: 233-8.
785 35. Driessen RL, Johnston HM, Nilsson SK. Membrane-bound stem cell factor is a key
786 regulator in the initial lodgment of stem cells within the endosteal marrow region. Exp Hematol.
787 2003; 31: 1284-91.
788 36. Hao J, Zhou H, Nemes K, Yen D, Zhao W, Bramlett C, et al. Membrane-bound SCF

789 and VCAM-1 synergistically regulate the morphology of hematopoietic stem cells. J Cell Biol.
790 2021; 220: e202010118.
791 37. Li J. Quiescence regulators for hematopoietic stem cell. Exp Hematol. 2011; 39: 511-
792 20.
793 38. Heo JH, Kang D, Seo SJ, Jin Y. Engineering the extracellular matrix for organoid
794 culture. Int J Stem Cells. 2022; 15: 60-9.
795 39. Zhang P, Zhang C, Li J, Han J, Liu X, Yang H. The physical microenvironment of
796 hematopoietic stem cells and its emerging roles in engineering applications. Stem Cell Res
797 Ther. 2019; 10: 327.
798 40. Crippa S, Bernardo ME. Mesenchymal stromal cells: role in the BM niche and in the
799 support of hematopoietic stem cell transplantation. Hemasphere. 2018; 2: e151.
800 41. Shiozawa Y, Havens A, Pienta K, Taichman R. The bone marrow niche: habitat to
801 hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites.
802 Leukemia. 2008; 22: 941-50.
803 42. Woods K, Guezguez B. Dynamic changes of the bone marrow niche: mesenchymal
804 stromal cells and their progeny during aging and leukemia. Front Cell Dev Biol. 2021; 9:
805 714716.
806 43. Wei Q, Frenette PS. Niches for hematopoietic stem cells and their progeny. Immunity.
807 2018; 48: 632-48.
808 44. Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. Tie2/angiopoietin-
809 1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 2004;
810 118: 149-61.
811 45. Mangialardi G, Cordaro A, Madeddu P. The bone marrow pericyte: an orchestrator of
812 vascular niche. Regen Med. 2016; 11: 883-95.

813 46. Kiel MJ, Yilmaz ÖH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family
814 receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for
815 stem cells. Cell. 2005; 121: 1109-21.
816 47. Apte RS, Chen DS, Ferrara N. VEGF in signaling and disease: beyond discovery and
817 development. Cell. 2019; 176: 1248-64.
818 48. Shahrabi S, Rezaeeyan H, Ahmadzadeh A, Shahjahani M, Saki N. Bone marrow blood
819 vessels: normal and neoplastic niche. Oncol Rev. 2016; 10: 306.
820 49. Winkler IG, Barbier V, Nowlan B, Jacobsen RN, Forristal CE, Patton JT, et al. Vascular
821 niche E-selectin regulates hematopoietic stem cell dormancy, self-renewal and
822 chemoresistance. Nat Med. 2012; 18: 1651-7.
823 50. Lee C, Kim M-J, Kumar A, Lee H-W, Yang Y, Kim Y. Vascular endothelial growth
824 factor signaling in health and disease: from molecular mechanisms to therapeutic perspectives.
825 Signal Transduct Target Ther. 2025; 10: 170.
826 51. Hassanshahi M, Hassanshahi A, Khabbazi S, Su Y-W, Xian CJ. Bone marrow
827 sinusoidal endothelium as a facilitator/regulator of cell egress from the bone marrow. Crit Rev
828 Oncol Hematol. 2019; 137: 43-56.
829 52. Prieto-Bermejo R, Romo-González M, Pérez-Fernández A, Ijurko C, Hernández-
830 Hernández Á. Reactive oxygen species in haematopoiesis: leukaemic cells take a walk on the
831 wild side. J Exp Clin Cancer Res. 2018; 37: 125.
832 53. Kunisaki Y, Bruns I, Scheiermann C, Ahmed J, Pinho S, Zhang D, et al. Arteriolar
833 niches maintain haematopoietic stem cell quiescence. Nature. 2013; 502: 637-43.
834 54. Mercher T, Cornejo MG, Sears C, Kindler T, Moore SA, Maillard I, et al. Notch
835 signaling specifies megakaryocyte development from hematopoietic stem cells. Cell Stem Cell.
836 2008; 3: 314-26.

837 55. Frimberger AE, Stering AI, Quesenberry PJ. An in vitro model of hematopoietic stem
838 cell homing demonstrates rapid homing and maintenance of engraftable stem cells. Blood.
839 2001; 98: 1012-8.
840 56. Hasan T, Pasala AR, Hassan D, Hanotaux J, Allan DS, Maganti HB. Homing and
841 engraftment of hematopoietic stem cells following transplantation: a pre-clinical perspective.
842 Curr Oncol. 2024; 31: 603-16.
843 57. Ehninger A, Trumpp A. The bone marrow stem cell niche grows up: mesenchymal
844 stem cells and macrophages move in. J Exp Med. 2011; 208: 421-8.
845 58. Suresh S, Venkatesan V, Thangavel S, Marepally S. Exploring MSC and HSPC
846 interactions: new frontiers in hematopoiesis and transplant medicine. Stem Cell Res Ther. 2025;
847 16: 640.
848 59. Braham MVJ, Li Yim ASP, Garcia Mateos J, Minnema MC, Dhert WJA, Öner FC, et
849 al. A human hematopoietic niche model supporting hematopoietic stem and progenitor cells in
850 vitro. Adv Healthc Mater. 2019; 8: 1801444.
851 60. Chen Z, Du C, Liu S, Liu J, Yang Y, Dong L, et al. Progress in biomaterials inspired
852 by the extracellular matrix. Giant. 2024; 19: 100323.
853 61. Sutherland TE, Dyer DP, Allen JE. The extracellular matrix and the immune system:
854 A mutually dependent relationship. Science. 2023; 379: eabp8964.
855 62. Feng Q, Shabrani N, Thon JN, Huo H, Thiel A, Machlus KR, et al. Scalable generation
856 of universal platelets from human induced pluripotent stem cells. Stem Cell Reports. 2014; 3:
857 817-31.
858 63. Lee-Thedieck C, Schertl P, Klein G. The extracellular matrix of hematopoietic stem
859 cell niches. Adv Drug Deliv Rev. 2022; 181: 114069.
860 64. Lucas D. Structural organization of the bone marrow and its role in hematopoiesis.

861 Curr Opin Hematol. 2021; 28: 36-42.

862 65. Smyth LC, Highet B, Jansson D, Wu J, Rustenhoven J, Aalderink M, et al.
863 Characterisation of PDGF-BB: PDGFRβ signalling pathways in human brain pericytes:
864 evidence of disruption in Alzheimer's disease. Commun Biol. 2022; 5: 235.

865 66. Jiang Z, Feng T, Lu Z, Wei Y, Meng J, Lin C-P, et al. PDGFRb+ mesenchymal cells,
866 but not NG2+ mural cells, contribute to cardiac fat. Cell Rep. 2021; 34: 108697.

867 67. Kaufman DP, Sanvictores T, Costanza M. Weibel Palade Bodies. In: StatPearls
868 [Internet]. Treasure Island, FL: StatPearls Publishing; 2022.

869 68. Yushkov B, Chereshnev V, Korneva E, Yushkova V, Sarapultsev A. Stem-cell niches
870 in health and disease: microenvironmental determinants of regeneration and pathology. Cells.
871 2025; 14: 981.

872 69. Xiao Y, McGuinness CS, Doherty-Boyd WS, Salmeron-Sanchez M, Donnelly H,
873 Dalby MJ. Current insights into the bone marrow niche: from biology in vivo to bioengineering
874 ex vivo. Biomaterials. 2022; 286: 121568.

875 70. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells
876 maintain haematopoietic stem cells. Nature. 2012; 481: 457-62.

877 71. Greenbaum A, Hsu Y-MS, Day RB, Schuettpelz LG, Christopher MJ, Borgerding JN,
878 et al. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell
879 maintenance. Nature. 2013; 495: 227-30.

880 72. Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M, et al. Engraftment
881 and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of
882 sinusoidal endothelial cells. Cell Stem Cell. 2009; 4: 263-74.

883 73. Sung DC, Chen M, Dominguez MH, Mahadevan A, Chen X, Yang J, et al. Sinusoidal
884 and lymphatic vessel growth is controlled by reciprocal VEGF-C-CDH5 inhibition. Nat

885 Cardiovasc Res. 2022; 1: 1006-21.

886 74. Singh P, Mohammad KS, Pelus LM. CXCR4 expression in the bone marrow
887 microenvironment is required for hematopoietic stem and progenitor cell maintenance and
888 early hematopoietic regeneration after myeloablation. Stem Cells. 2020; 38: 849-59.

889 75. Junt T, Schulze H, Chen Z, Massberg S, Goerge T, Krueger A, et al. Dynamic
890 visualization of thrombopoiesis within bone marrow. Science. 2007; 317: 1767-70.

891 76. Goette NP, Borzone FR, Lupi ADD, Chasseing NA, Rubio MF, Costas MA, et al.
892 Megakaryocyte-stromal cell interactions: effect on megakaryocyte proliferation, proplatelet
893 production, and survival. Exp Hematol. 2022; 107: 24-37.

894 77. Fu W, Ishikawa-Ankerhold H, Gaertner F. Homeostasis of megakaryocytes: balancing
895 tissue residency and consumptive platelet production. Trends Cell Biol, in press. doi:
896 10.1016/j.tcb.2025.11.002. 78. Zhou B, Lin W, Long Y, Yang Y, Zhang H, Wu K, et al. Notch
897 signaling pathway: architecture, disease, and therapeutics. Signal Transduct Target Ther. 2022;
898 7: 95.

899 79. Tamma R, Ribatti D. Bone niches, hematopoietic stem cells, and vessel formation. Int
900 J Mol Sci. 2017; 18: 151.

901 80. Georgescu A, Oved JH, Galarraga JH, Cantrell T, Mehta S, Dulmovits BM, et al. Self-
902 organization of the hematopoietic vascular niche and emergent innate immunity on a chip. Cell
903 Stem Cell. 2024; 31: 1847-64.e6.

904 81. Zhan H, Kaushansky K. Megakaryocytes as the regulator of the hematopoietic vascular
905 niche. Front Oncol. 2022; 12: 912060.

906 82. Zheng K, Wei Z, Li W. Ecological insights into hematopoiesis regulation: unraveling
907 the influence of gut microbiota. Gut Microbes. 2024; 16: 2350784.

908 83. Pereira AL, Galli S, Nombela-Arrieta C. Bone marrow niches for hematopoietic stem

909 cells. Hemasphere. 2024; 8: e133.

910 84. Ng F, Boucher S, Koh S, Sastry KS, Chase L, Lakshmipathy U, et al. PDGF, TGF-β,
911 and FGF signaling is important for differentiation and growth of mesenchymal stem cells:
912 transcriptional profiling can identify markers and signaling pathways important in
913 differentiation of MSCs into adipogenic, chondrogenic, and osteogenic lineages. Blood. 2008;
914 112: 295-307.

915 85. Di Buduo CA, Aguilar A, Soprano PM, Bocconi A, Miguel CP, Mantica G, et al. Latest
916 culture techniques: cracking the secrets of bone marrow to mass-produce erythrocytes and
917 platelets ex vivo. Haematologica. 2021; 106: 947-57. 86. Revenfeld ALS, Møller AM, Tylvad
918 M, Bill M, Rosenberg CA, Møller BK. Exploring a flow cytometry-based CFU assay for
919 functional assessment of human HSPCs: a robust alternative to morphological colony
920 evaluation. Stem Cell Rev Rep. 2026; 22: 1007-22.

921 87. Schirrmacher V. Bone marrow: the central immune system. Immuno. 2023; 3: 289-329.

922 88. Curto-Garcia N, Harrison C, McLornan DP. Bone marrow niche dysregulation in
923 myeloproliferative neoplasms. Haematologica. 2020; 105: 1189-1200.

924 89. Shultz LD, Goodwin N, Ishikawa F, Hosur V, Lyons BL, Greiner DL. Subcapsular
925 transplantation of tissue in the kidney. Cold Spring Harb Protoc. 2014; 2014: pdb.prot078089.

926 90. Kuter DJ, Bain B, Mufti G, Bagg A, Hasserjian RP. Bone marrow fibrosis:
927 pathophysiology and clinical significance of increased bone marrow stromal fibres. Br J
928 Haematol. 2007; 139: 351-62.

929 91. Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. Growth
930 Factors. 2011; 29: 196-202.

931 92. Shi Y, Massagué J. Mechanisms of TGF-β signaling from cell membrane to the nucleus.
932 Cell. 2003; 113: 685-700.

933 93. Zhang K, Zhang Q, Deng J, Li J, Li J, Wen L, et al. ALK5 signaling pathway mediates
934 neurogenesis and functional recovery after cerebral ischemia/reperfusion in rats via Gadd45b.
935 Cell Death Dis. 2019; 10: 360.
936 94. Ignotz RA, Massague J. Transforming growth factor-beta stimulates the expression of
937 fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem.
938 1986; 261: 4337-45.
939 95. Yan X, Liu Z, Chen Y. Regulation of TGF-β signaling by Smad7. Acta Biochim
940 Biophys Sin. 2009; 41: 263-72.
941 96. Chen F, Lyu L, Xing C, Chen Y, Hu S, Wang M, et al. The pivotal role of TGF-β/Smad
942 pathway in fibrosis pathogenesis and treatment. Front Oncol. 2025; 15: 1649179.
943 97. Kramann R, Schneider RK. The identification of fibrosis-driving myofibroblast
944 precursors reveals new therapeutic avenues in myelofibrosis. Blood. 2018; 131: 2111-9.
945 98. O'Reilly S. Interleukin-11 and its eminent role in tissue fibrosis: a possible therapeutic
946 target. Clin Exp Immunol. 2023; 214: 154-61.
947 99. Rao B, Malathi N, Narashiman S, Rajan ST. Evaluation of myofibroblasts by
948 expression of alpha smooth muscle actin: a marker in fibrosis, dysplasia and carcinoma. J Clin
949 Diagn Res. 2014; 8: ZC14-7.
950 100. Yamamoto K, Tateishi K, Kudo Y, Hoshikawa M, Tanaka M, Nakatsuka T, et al.
951 Stromal remodeling by the BET bromodomain inhibitor JQ1 suppresses the progression of
952 human pancreatic cancer. Oncotarget. 2016; 7: 61469-84.
953 101. Inman GJ, Nicolás FJ, Callahan JF, Harling JD, Gaster LM, Reith AD, et al. SB-
954 431542 is a potent and specific inhibitor of transforming growth factor-β superfamily type I
955 activin receptor-like kinase receptors ALK4, ALK5, and ALK7. Mol Pharmacol. 2002; 62: 65-
956 74.

957 102. Stepensky P, Saada A, Cowan M, Tabib A, Fischer U, Berkun Y, et al. The Thr224Asn
958 mutation in the VPS45 gene is associated with congenital neutropenia and primary
959 myelofibrosis of infancy. Blood. 2013; 121: 5078-87.
960 103. Vilboux T, Lev A, Malicdan MCV, Simon AJ, Järvinen P, Racek T, et al. A congenital
961 neutrophil defect syndrome associated with mutations in VPS45. N Engl J Med. 2013; 369: 54-
962 65.
963 104. Srivastava NN, Shukla SK, Yashavarddhan M, Devi M, Tripathi RP, Gupta ML.
964 Modification of radiation-induced DNA double-strand break repair pathways by chemicals
965 extracted from Podophyllum hexandrum: an in vitro study in human blood leukocytes. Environ
966 Mol Mutagen. 2014; 55: 436-48.
967 105. Yashavarddhan M, Sharma AK, Chaudhary P, Bajaj S, Singh S, Shukla SK.
968 Development of hematopoietic syndrome mice model for localized radiation exposure. Sci Rep.
969 2021; 11: 89.
970 106. Vignard J, Mirey G, Salles B. Ionizing-radiation-induced DNA double-strand breaks:
971 a direct and indirect lighting up. Radiother Oncol. 2013; 108: 362-9.
972 107. Zeman MK, Cimprich KA. Causes and consequences of replication stress. Nat Cell
973 Biol. 2014; 16: 2-9.
974 108. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation.
975 Antioxid Redox Signal. 2014; 20: 1447-62.
976 109. Kiang JG, Smith JT, Anderson MN, Swift JM, Christensen CL, Gupta P, et al.
977 Hemorrhage exacerbates radiation effects on survival, leukocytopenia, thrombopenia,
978 erythropenia, bone marrow cell depletion and hematopoiesis, and inflammation-associated
979 microRNAs expression in kidney. PLoS One. 2015; 10: e0139271.
980 110. Candé C, Cohen I, Daugas E, Ravagnan L, Larochette N, Zamzami N, et al. Apoptosis-

981 inducing factor: a novel caspase-independent death effector released from mitochondria.
982 Biochimie. 2002; 84: 215-22.
983 111. Danial NN, Gramm CF, Scorrano L, Zhang C-Y, Krauss S, Ranger AM, et al. BAD
984 and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis.
985 Nature. 2003; 424: 952-6.
986 112. Nimer SD. Myelodysplastic syndromes. Blood. 2008; 111: 4841-51.
987 113. Samiev D, Bhatt VR, Armitage JD, Maness LJ, Akhtari M. A primary care approach
988 to myelodysplastic syndromes. Korean J Fam Med. 2014; 35: 111-8.
989 114. Hasserjian RP, Germing U, Malcovati L. Diagnosis and classification of
990 myelodysplastic syndromes. Blood. 2023; 142: 2247-57.
991 115. Villaume MT, Savona MR. Pathogenesis and inflammaging in myelodysplastic
992 syndromes. Haematologica. 2025; 110: 283-99.
993 116. Saygin C, Godley LA. Genetics of myelodysplastic syndromes. Cancers. 2021; 13:
994 3380.
995 117. Cook MR, Karp JE, Lai C. The spectrum of genetic mutations in myelodysplastic
996 syndrome: should we update prognostication? EJHaem. 2022; 3: 301-13.
997 118. Nagata Y, Maciejewski JP. The functional mechanisms of mutations in
998 myelodysplastic syndrome. Leukemia. 2019; 33: 2779-94.
999 119. Jiang M, Chen M, Liu Q, Jin Z, Yang X, Zhang W. SF3B1 mutations in
1000 myelodysplastic syndromes: a potential therapeutic target for modulating the entire disease
1001 process. Front Oncol. 2023; 13: 1116438.
1002 120. Dolatshad H, Pellagatti A, Liberante FG, Llorian M, Repapi E, Steeples V, et al.
1003 Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-
1004 mutant myelodysplastic syndromes. Leukemia. 2016; 30: 2322-31.

1005 121. Shirai CL, Ley JN, White BS, Kim S, Tibbitts J, Shao J, et al. Mutant U2AF1
1006 expression alters hematopoiesis and pre-mRNA splicing in vivo. Cancer Cell. 2015; 27: 631-
1007 43.
1008 122. Markiewicz M, Kopacz A, Blajer-Olszewska B, Mazur M, Warzybok K, Szarawarska
1009 M, et al. Rapid development of unclassified myeloid lineage acute leukaemia with trisomy 6
1010 and U2AF1 mutation. J Cell Mol Med. 2025; 29: e70461.
1011 123. Song Y, Rongvaux A, Taylor A, Jiang T, Tebaldi T, Balasubramanian K, et al. A highly
1012 efficient and faithful MDS patient-derived xenotransplantation model for pre-clinical studies.
1013 Nat Commun. 2019; 10: 366.
1014 124. Lang Y, Lyu Y, Tan Y, Hu Z. Progress in construction of mouse models to investigate
1015 the pathogenesis and immune therapy of human hematological malignancy. Front Immunol.
1016 2023; 14: 1195194.
1017 125. Goetz LH, Schork NJ. Personalized medicine: motivation, challenges, and progress.
1018 Fertil Steril. 2018; 109: 952-63.
1019 126. Zhao Y, Li S, Zhu L, Huang M, Xie Y, Song X, et al. Personalized drug screening using
1020 patient-derived organoid and its clinical relevance in gastric cancer. Cell Rep Med. 2024; 5:
1021 101627.
1022 127. Wu Y, Ma L, Li X, Yang J, Rao X, Hu Y, et al. The role of artificial intelligence in drug
1023 screening, drug design, and clinical trials. Front Pharmacol. 2024; 15: 1459954.
1024 128. Lee B, Yoon S, Lee JW, Kim Y, Chang J, Yun J, et al. Statistical characterization of the
1025 morphologies of nanoparticles through machine learning-based electron microscopy image
1026 analysis. ACS Nano. 2020; 14: 17125-33.
1027 129. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: construction,
1028 analysis, and application. Bioact Mater. 2024; 31: 525-48.

1029 130. Hsiung N, Ju Y, Yang K, Yang P, Zeng W, Zhao H, et al. Organoid-based tissue
1030 engineering for advanced tissue repair and reconstruction. Mater Today Bio. 2025; 33: 102093.
1031 131. Leguit RJ, van den Tweel JG. The pathology of bone marrow failure. Histopathology.
1032 2010; 57: 655-670.
1033 132. Kastrinaki MC, Pavlaki K, Batsali AK, Kouvidi E, Mavroudi I, Pontikoglou C,
1034 Papadaki HA. Mesenchymal stem cells in immune-mediated bone marrow failure syndromes.
1035 Clin Dev Immunol. 2013; 2013: 265608.
1036 133. Gysemans C, Beya M, Pedace E, Mathieu C. Exploring neutrophil heterogeneity and
1037 plasticity in health and disease. Biomedicines. 2025; 13: 597.
1038 134. McKenna E, Mhaonaigh AU, Wubben R, Dwivedi A, Hurley T, Kelly LA, et al.
1039 Neutrophils: need for standardized nomenclature. Front Immunol. 2021; 12: 602963.135.
1040 Sanchez Santos A, Socorro Avila I, Galvan Fernandez H, Cazorla Rivero S, Lemes
1041 Castellano A, Cabrera Lopez C. Eosinophils: old cells, new directions. Front Med. 2025; 11:
1042 1470381.
1043 136. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: characteristics and
1044 functions. Front Med. 2017; 4: 101.
1045 137. Sonder SU, Plassmeyer M, Loizou D, Alpan O. Towards standardizing basophil
1046 identification by flow cytometry. Front Allergy. 2023; 4: 1133378.
1047 138. Dash SP, Gupta S, Sarangi PP. Monocytes and macrophages: origin, homing,
1048 differentiation, and functionality during inflammation. Heliyon. 2024; 10: e29686.
1049 139. Wei Q, Deng Y, Yang Q, Zhan A, Wang L. The markers to delineate different
1050 phenotypes of macrophages related to metabolic disorders. Front Immunol. 2023; 14: 1084636.
1051 140. Wang H, He J, Xu C, Chen X, Yang H, Shi S, et al. Decoding human megakaryocyte
1052 development. Cell Stem Cell. 2021; 28: 535-49.e8.

1053 141. Karsten U, Butschak G, Stahn R, Goletz S. A novel series of anti-human glycophorin
1054 A antibodies defining five extra- and intracellular epitopes. Int Immunopharmacol. 2010; 10:
1055 1354-60.
1056 142. Bodrova VV, Shustova ON, Khaspekova SG, Mazurov AV. Laboratory markers of
1057 platelet production and turnover. Biochemistry (Mosc). 2023; 88: S39-51.
1058 143. Cracolici V, Parilla M, Henriksen KJ, Cipriani NA. An evaluation of CD61
1059 immunohistochemistry in identification of vascular invasion in follicular thyroid neoplasms.
1060 Head Neck Pathol. 2020; 14: 399-405.
1061 144. Patente TA, Pinho MP, Oliveira AA, Evangelista GC, Bergami-Santos PC, Barbuto JA.
1062 Human dendritic cells: their heterogeneity and clinical application potential in cancer
1063 immunotherapy. Front Immunol. 2019; 9: 3176.
1064 145. Forsthuber TG, Cimbora DM, Ratchford JN, Katz E, Stüve O. B cell-based therapies
1065 in CNS autoimmunity: differentiating CD19 and CD20 as therapeutic targets. Ther Adv Neurol
1066 Disord. 2018; 11: 1756286418761697.
1067 146. Call ME, Wucherpfennig KW. Molecular mechanisms for the assembly of the T cell
1068 receptor-CD3 complex. Mol Immunol. 2004; 40: 1295-305.
1069 147. Amand M, Iserentant G, Poli A, Sleiman M, Fievez V, Sanchez IP, et al. Human
1070 CD56dimCD16dim cells as an individualized natural killer cell subset. Front Immunol. 2017;
1071 8: 699.
1072 148. Robillard N, Wuillème S, Moreau P, Béné MC. Immunophenotype of normal and
1073 myelomatous plasma-cell subsets. Front Immunol. 2014; 5: 137.
1074 149. Tseng C-C, Obeng EA. RNA splicing as a therapeutic target in myelodysplastic
1075 syndromes. Semin Hematol. 2024; 61: 431-41.
1076 150. Pellagatti A, Armstrong RN, Steeples V, Sharma E, Repapi E, Singh S, et al. Impact of

1077 spliceosome mutations on RNA splicing in myelodysplasia: dysregulated genes/pathways and
1078 clinical associations. Blood. 2018; 132: 1225-40.
1079 151. Kim E, Ilagan JO, Liang Y, Daubner GM, Lee SC-W, Ramakrishnan A, et al. SRSF2
1080 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. Cancer
1081 Cell. 2015; 27: 617-30.
1082 152. Cilloni D, Itri F, Bonuomo V, Petiti J. SF3B1 mutations in hematological malignancies.
1083 Cancers. 2022; 14: 4927.
1084 153. Zhao Y, Cai W, Hua Y, Yang X, Zhou J. The biological and clinical consequences of
1085 RNA splicing factor U2AF1 mutation in myeloid malignancies. Cancers. 2022; 14: 4406.
1086 154. Ferrone CK, Blydt-Hansen M, Rauh MJ. Age-associated TET2 mutations: common
1087 drivers of myeloid dysfunction, cancer and cardiovascular disease. Int J Mol Sci. 2020; 21: 626.
1088 155. Lin M-E, Hou H-A, Tsai C-H, Wu S-J, Kuo Y-Y, Tseng M-H, et al. Dynamics of
1089 DNMT3A mutation and prognostic relevance in patients with primary myelodysplastic
1090 syndrome. Clin Epigenetics. 2018; 10: 42.
1091 156. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape
1092 of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014; 28: 241-
1093 7.
1094 157. Hamed NAM, El Ghandour A, Elwafa RA, Rezk MR, Ghallab O. Expression of
1095 enhancer of zeste homolog 2 gene in acute myeloid leukemia. Asian Pac J Cancer Prev. 2023;
1096 24: 81.
1097 158. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its
1098 clinical relevance. Blood. 2013; 122: 4021-34.
1099 159. de Bruijn M, Dzierzak E. Runx transcription factors in the development and function
1100 of the definitive hematopoietic system. Blood. 2017; 129: 2061-9.

1101 160. Cao Q, Gearhart MD, Gery S, Shojaee S, Yang H, Sun H, et al. BCOR regulates
1102 myeloid cell proliferation and differentiation. Leukemia. 2016; 30: 1155-65.
1103
1104
1105
1106
1107
1108