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# Myelodysplastic syndrome patient-derived xenografts: from no options to many

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## Introduction

According to the recently updated tumor classification by the World Health Organization, myelodysplastic syndrome (MDS) constitutes a heterogeneous group of blood disorders characterized by cytopenia and dysplasia in at least one of the myeloid lineages.<sup>1</sup> MDS is most common in the elderly and is caused by inefficient hematopoiesis and increased apoptosis within the bone marrow (BM). It is a genetically heterogeneous disorder and individual cases generally harbor two to three mutations in one of approximately 30 driver genes which are recurrently mutated in MDS.<sup>2,3</sup> Of importance, many of these genes have also been found to be mutated in acute myeloid leukemia (AML), with frequencies of mutations differing between the two diseases.<sup>2,4</sup> The spectrum of survival of patients with MDS is broad and high-risk MDS is associated with an increased propensity to progression to AML.<sup>5</sup>

There has been considerable emphasis on the development of genetically engineered mouse models in attempts to study MDS. These include strains harboring lesions in the most commonly mutated genes in MDS, such as *SF3B1*,<sup>6</sup> *TET2*,<sup>7,8</sup> *ASXL1*<sup>9</sup> and *SRSF2*.<sup>10</sup> The phenotypic properties of these models have been reviewed in detail previously<sup>11-13</sup> and although they all present with several phenotypic features of MDS, they clearly have some limitations with respect to their abilities to recapitulate human MDS biology. As an example, *Sf3b1*<sup>K700E</sup> mutant mice develop anemia and display expansion of the long-term hematopoietic stem cell compartment, consistent with an MDS phenotype. However, the *Sf3b1*<sup>K700E</sup> mutant line fails to present with ring sideroblasts which are normally found in patients with *SF3B1* mutations.<sup>14</sup> Another likely contributor to the inability of current genetically engineered mouse model lines to fully recapitulate the phenotypic spectrum of MDS is the fact that most models typically harbor one genetic lesion and, therefore, not the full mutational complement observed in MDS patients. Thus, there is a clear need for better models of MDS biology, including patient-derived xenografts (PDX), in order to recapitulate the disease's biology and complexity better.

## The history of myelodysplastic syndrome patient-derived xenografts

The first PDX models of AML were established more than 40 years ago by subcutaneously engrafting patient material into immune-deprived mice.<sup>15</sup> More physiologically relevant models were developed over the next decade via the use of tail vein injection and improved immune-deficient strains.<sup>16,17</sup> In contrast, it was not until the beginning of this millennium that cells from MDS patients were demonstrated to engraft functionally in immune-compromised mice.<sup>18-20</sup> However, only cells from a limited number of patients could be engrafted and a study with a large number of patients demonstrated that engraftment was sustained by residual normal cells and not by the MDS clone(s).<sup>21</sup> During the last decade, several laboratories have published a number of complementary approaches for the generation of MDS PDX.<sup>22-34</sup> Importantly, these combined efforts have demonstrated the engraftment capacity of most MDS subtypes,<sup>25-28,34</sup> that the expanded cells retain the genetic and phenotypic features of the primary tumor,<sup>24-27,29,30,32,34</sup> that these PDX models also sustain engraftment in secondary recipients<sup>24,27,29,34</sup> and that they allow evaluation of new therapies.<sup>32,33</sup> Nevertheless, as summarized in Table 1 and Figure 1, these models are quite heterogeneous. Specifically, several immune-compromised murine strains have been used (NOG, NSG, NSG-S or MISTRG) and injected at different ages (from newborn pups to adult animals). Moreover, a number of different cell

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Table 1. Summary of published patient-derived xenograft models from myelodysplastic syndrome patients.

Year	Reference	Mice				MDS patient cells			MSC co-injected?	Patients tested	Expansion time (weeks)
		Strain	Age	Irradiation?	Injection route	MDS subtype / features	Injected cells	Cell numbers			
2002	Nilsson <i>et al.</i> <sup>18</sup>	NOD/LtSz-SCID or NOD/LtSz-SCID $\beta 2m^{-/-}$	8-12 weeks	yes, 350-375 cGy	tail vein	trisomy 8+	BM CD34 <sup>+</sup> BM CD34 <sup>+</sup> CD38	1.68-5 x10 <sup>6</sup> 1-1.4x10 <sup>4</sup>	No No	3	6-8 weeks
2003	Benito <i>et al.</i> <sup>21</sup>	NOD/SCID	6-8 weeks	300-350-375 cGy	tail i.v or i.p	15 RA, 11 RAEB, 6 RAEBt, 5 RARS	BM cells	0.4-3.5x10 <sup>7</sup>	No	37	n.s
2004	Thanopoulou <i>et al.</i> <sup>20</sup>	NOD/SCID $\beta 2m^{-/-}$ or NOD/SCID $\beta 2m^{-/-}$ /3/GM/SF	8-10 weeks	yes, 350 cGy	tail vein or i.p	1 RA, 1 RARS, 2 RAEB, 3 RAEBT, 4 CMML	BM cells	4-17x10 <sup>6</sup>	No	11	5-23 weeks
2004	Kerbaux <i>et al.</i> <sup>19</sup>	NOD/SCID $\beta 2m^{-/-}$	n.s	n.s	intrafemoral	n.s	BM MNC + MSC cell lines HS5 & HS27a	10 <sup>7</sup> MNC + 10 <sup>5</sup> HS5 & HS27a	yes, HS5 & HS27a	6	4-17 weeks
2010	Martin <i>et al.</i> <sup>27</sup>	NSG	n.s	yes, 250 cGy	i.v. (retro-orbital) or intra-tibial	low risk	BM cells BM cells T cells depleted	5x10 <sup>5</sup> -5x10 <sup>6</sup> 1.8-5x10 <sup>6</sup>	No	5 5	7-12 weeks
					i.v. (retro-orbital)		BM cells CD34 <sup>+</sup>	5x10 <sup>6</sup> /2x10 <sup>6</sup>		3	
2011	Muguruma <i>et al.</i> <sup>22</sup>	NOG	n.s	yes, 250 cGy	intrafemoral	3 RA, 3 RAEB, 5 RAEB-T	BM CD34 <sup>+</sup>	1.4-5x10 <sup>5</sup>	Yes	6	n.s
2013	Pang <i>et al.</i> <sup>23</sup>	NSG	P0-P3 newborn pups	sublethal (100 rads)	anterior facial vein	low risk (monosomy 7)	HSC-like (Lin CD34 <sup>+</sup> CD38 CD90 <sup>+</sup> CD45RA)	1.5-3x10 <sup>3</sup>	No	4	16
2014	Medyouf <i>et al.</i> <sup>24</sup>	NSG or NSG-S	6-8 weeks	yes, 200 cGy	intrabone	low or intermediate risk	BM CD34 <sup>+</sup> + BM MSC	10 <sup>6</sup>	Yes	20	16-28 weeks
2015	Mian <i>et al.</i> <sup>25</sup>	NSG (females)	8-12 weeks	yes, 375 cGy	intra-BM (tibia)	RARS	BM CD34 <sup>+</sup>	0.65-2 10 <sup>5</sup>	No	4	18-20 weeks
2017	Rouault-Pierre <i>et al.</i> <sup>26</sup>	NSG or NSG-S	n.s	yes, 330-375 cGy	intra-BM (tibia)	8 RCMD, 3 RCMD-RS, 7 RAEB, 6 RARS, 1 MDS/MPN, 3 CMML	CD34 <sup>+</sup> BM cells +/- BM MSC	1-2x10 <sup>6</sup> (1:1 for MSC)	Yes		12-18 weeks
							CD3 BM cells +/- BM MSC (2:1 for CD3)	10 <sup>6</sup> CD3- (2:1 for MSC)	Yes	28	
2017	Yoshimi <i>et al.</i> <sup>27</sup>	NSG-S	6-10 weeks	yes, 200-250 cGy	intrafemoral tail i.v	CMML JMML	BM CD34 <sup>+</sup> BM or PB MNC	0.2-1.18x10 <sup>6</sup> 2.2-4x10 <sup>6</sup> (BM) or 1.3-2x10 <sup>6</sup> (PB)	No No	8 4	3-11 weeks 2-7 weeks
2017	Zhang <i>et al.</i> <sup>28</sup>	NSG or NSG-S	6-8 weeks	yes, 250 cGy	i.v. (retro-orbital)	CMML	BM or PB CD34 <sup>+</sup>	3x10 <sup>4</sup> -1.2x10 <sup>6</sup>	No	16	10-16 weeks
2018	Krewata <i>et al.</i> <sup>29</sup>	NSG or NSG-S	6-8 weeks	yes, 250cGy	intrafemoral	3 low-risk, 4 high-risk	BM MNC +/- BM MSC	10 <sup>6</sup> BM MNC +/- 10 <sup>5</sup> BM MSC	Yes	7	8-32 weeks
2018	Meunier <i>et al.</i> <sup>30</sup>	NSG	8-12 weeks	no, 25mg/kg busulfan d-1 i.p	intra-BM (tibia)	1 RAEB, 2 RAEB1, 1 RARS	CD34 <sup>+</sup> BM cells + BM MSC	5x10 <sup>5</sup> CD34 <sup>+</sup> & 1.5x10 <sup>6</sup> MSC (1:3)	Yes	4	6 months
2018	Shastri <i>et al.</i> <sup>31</sup>	NSG	n.s	yes, 200cGy	tail i.v	2 int-2 risk, 1 high-risk, 1 MPN	BM/PB MNC	2-5x10 <sup>6</sup>	No	4	3 weeks
2018	Stevens <i>et al.</i> <sup>32</sup>	NSG-S	n.s	no, 25mg/kg busulfan d-1	tail i.v	8 high-risk	BM MNC	0.8-1x10 <sup>6</sup>	No	4	6-10 weeks
2019	Smith <i>et al.</i> <sup>33</sup>	NSG	n.s	yes, 200cGy	tail i.v	U2AF1 mutants	BM MNC	2-5x10 <sup>6</sup>	No	2	3 weeks

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2019	Song et al. <sup>34</sup>	MISTRG or NSG	1-3 days	yes, 2x150cGy intrahepatically (MISTRG) or 1x100cGy (NSG)	1 del(5q), 3 MLD, 2 RS-SLD, 1 RS-MLD, 1 MPN-RS-T, 3 EB-1, 3 EB-2	BM CD34 <sup>+</sup>	0.2-2.75x10 <sup>6</sup>	No	22	12-31 weeks
					1 MLD, 2 EB-1, 1 EB-2	BM CD3 <sup>-</sup>	1.15-6x10 <sup>6</sup>	No	4	13-15 weeks

The main characteristics of the protocols published to generate patient-derived xenograft (PDX) models from patients with myelodysplastic syndromes (MDS) are presented. NOD/SCID: non-obese diabetic mice with *Prkdc*<sup>scid</sup> mutation;  $\beta 2m^{-/-}$ : beta2-microglobulin null; 3/GM/SF: constitutive expression of human interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF) and stem cell factor (SF); NOG: NOD/Shi-scid *Il2rg*<sup>tm1.1</sup>; NSG: NOD.Cg-*Prkdc*<sup>scid</sup> *Il2rg*<sup>tm1.1</sup>; NSG-S: NSG with human IL-3, GM-CSF and SF constitutive expression; MISTRG: humanized macrophage colony-stimulating factor, IL-3/GM-CSF/SIRP alpha and thrombopoietin combined with Rag2<sup>+</sup>, IL2R $\gamma$ <sup>+</sup>; i.v.: intravenous injection; i.p.: intraperitoneal injection; cGy: centigray; BM: bone marrow; PB: peripheral blood; MNC: mononuclear cells; MSC: mesenchymal stromal cells; HSC: hematopoietic stem cells; Lin-: lineage negative; RA: refractory anemia; RAEB: refractory anemia with excess blasts; RAEB-I: RAEB type 1; RAEB-II: RAEB type 2; RAEB-T: RAEB in transformation; RARS: refractory anemia with ringed sideroblasts; RARS-T: RARS with thrombocytosis; RS: ringed sideroblasts; RCMD: refractory cytopenia with multilineage dysplasia; RCMD-RS: RCMD and ringed sideroblasts; del(5q): MDS associated with isolated deletion of chromosome 5q; MDS/MPN: myelodysplastic/myeloproliferative neoplasms; CMML: chronic myelomonocytic leukemia; JMML: juvenile myelomonocytic leukemia; MLD: multilineage dysplasia; SLD: single lineage dysplasia; EB: excess blasts; MDS/MPN-RS-T: MDS/MPN with ring sideroblasts and thrombocytosis; n.s.: not specified.

sources have been employed (BM or peripheral blood mononuclear cells, CD3-depleted BM cells, CD34<sup>+</sup> BM cells) which were injected in different quantities, in the presence or absence of BM-derived mesenchymal stromal cells (MSC) and in different anatomical locations (intravenous, intrafemoral, intrahepatic). Not surprisingly, this resulted in very different disease latencies (from 3 to 32 weeks post-injection). A number of conclusions can be drawn from this extensive work:

(i) With respect to selection of the recipient strain, an immunodeficient background is necessary. The most commonly used recipient for the generation of PDX is the NSG strain which harbors mutations in *Prkdc* and *Il2g* leading to the absence of B, T and NK cells.<sup>35</sup> The constitutive expression of the human cytokines interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF) and stem cell factor (SCF) on this background (NSG-S, also designated NSG-SGM3) does not lead to enhanced engraftment of most MDS subtypes, except for chronic myelomonocytic leukemia,<sup>28</sup> in contrast to the situation in AML.<sup>24,26,29,36</sup> On the other hand, the recently developed MISTRG strain, expressing human macrophage colony-stimulating factor (M-CSF), IL-3, GM-CSF, signal regulatory protein alpha (SIRP $\alpha$ ) and thrombopoietin at physiological levels on a different immunodeficient background (Rag2<sup>+</sup>, IL2R $\gamma$ <sup>+</sup>), was recently demonstrated to be a promising host for engraftment of MDS patients' material.<sup>34</sup> Not only could cells from patients with various subtypes of MDS be expanded in this line, but the levels of engraftment were increased, with a higher percentage of CD33<sup>+</sup> myeloid cells than in NSG mice. Moreover, long-term engraftment of these myeloid cells was also improved in this strain as CD33<sup>+</sup> cells constituted more than 80% of the hCD45<sup>+</sup> compartment in secondary recipients, compared to 30% in NSG mice. Additionally, MDS cells engrafted in MISTRG mice generated erythroid and megakaryocytic lineages at a higher frequency than in the NSG counterpart.<sup>34</sup>

(ii) T-cell depletion of the primary MDS tumor, either by treatment with a human CD3 antibody or by physical separation, is a prerequisite to limit graft-versus-host disease.<sup>26,34,36-38</sup> Indeed, one of the first attempts to generate MDS PDX failed mainly because of the predominant growth of human CD3<sup>+</sup> T cells, leading to graft-versus-host disease in most of the recipient animals.<sup>37</sup>

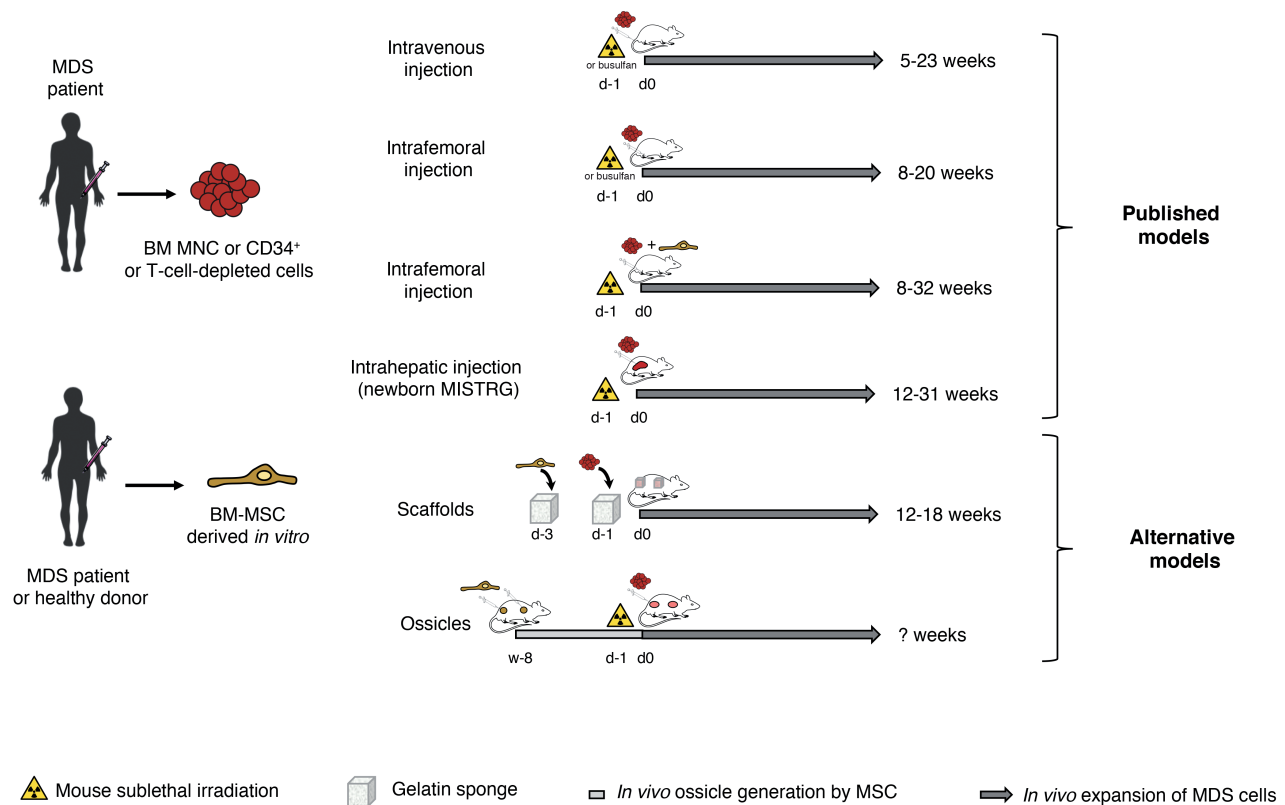
(iii) Intrafemoral injections result in better engraftment in NSG mice compared to an intravenous route of injection.

(iv) Co-injection of MSC leads to variable results in terms of promoting the engraftment of MDS samples, with some laboratories reporting some enhancement,<sup>22-24</sup> whereas others have not found this effect.<sup>26,29</sup> The underlying reasons for this variation are not clear. However, as human MSC only survive for 2-4 weeks in the murine BM,<sup>24,26</sup> this variation could potentially reflect patient-specific differences in the ability of MSC to promote the initial seeding and engraftment of MDS cells in the murine BM.

(v) Engraftment capacity does not seem to be related to MDS subtypes, but rather appears to be specific to the individual samples, as indicated in studies with large numbers of patients.<sup>24,26,34</sup>

## Alternative strategies

Despite extensive efforts in several laboratories, this cumulative work has only produced a total of approximately 100 MDS PDX so far. There is, therefore, a strong need for alternative systems that could enhance the generation of MDS PDX. Interestingly, descriptions of a number of humanized bone marrow-like structure (hBMLS) models have been published recently. These models enable the expansion of AML patients' cells that failed to engraft with conventional methods.<sup>39-41</sup> They are all based on the use of BM MSC and can be separated into two categories. In the first category, which we will define as "scaffold" models, *in vitro*-expanded MSC are seeded in a gelatin sponge and cultured for a couple of days. Next, human leukemic cells are injected into the sponge which is subsequently introduced subcutaneously into non-irradiated immunocompromised mice<sup>41</sup> (Figure 1). In the second approach, BM MSC are first mixed with Matrigel and introduced subcutaneously into immune-deficient mice in which they develop a so-called "ossicle" after 2-3 months, which constitutes an exterior bone structure surrounding a hematopoietic core. Following sublethal irradiation, human leukemic cells are injected into the ossicle where they expand<sup>39</sup> (Figure 1). Another ossicle-like approach combines osteogenic priming of MSC with a physical support consisting of two or three biphasic calcium phosphate particles, prior to subcutaneous insertion into mice and subsequent ossicle development.<sup>40</sup> Importantly, up to four hBMLS per animal can be introduced,<sup>39-41</sup> and Reinisch *et al.* have elegantly demonstrated that tumor cells can cir-



**Figure 1. Key features of published and alternative patient-derived xenograft models of myelodysplastic syndrome.** The left panel depicts the sources of cells from patients with myelodysplastic syndrome (MDS) which are injected to generate MDS patient-derived xenografts. Tumor cells (red circles) are constituted of bone marrow (BM) cells, mononuclear cells (MNC), or CD34<sup>+</sup> purified or T-cell depleted BM cells. Supporting cells (yellow) are BM-derived mesenchymal stromal cells (MSC) derived from patients or healthy donors. The time periods for ossicle development and engraftment of MDS cells are illustrated by light and dark gray bars, respectively. The time of conditioning of the animal, by either irradiation or busulfan treatment is indicated, and the injection route is illustrated by a syringe.

culate between ossicles leading to engraftment of leukemic cells in non-injected hBMLS, thereby allowing for increased expansion of the original material from patients.<sup>39</sup>

In the previously described MDS PDX models, engraftment and expansion of the MDS material occur mainly in the recipient BM. In contrast, the hBMLS approaches exploit a humanized version of the BM niche, since at least bone, cartilage and MSC present in the niche are of human origin.<sup>42</sup> Of note, these hBMLS constitute a preferential homing niche for leukemic cells when compared to murine BM because leukemic cells injected intravenously expand earlier and at higher frequency in hBMLS than in the BM of mice.<sup>39,40</sup> Moreover, as the BM microenvironment has been reported to play an important role in the onset and development of MDS as well as the response to therapy, these hBMLS models are likely to be superior in mimicking key disease parameters.<sup>43,44</sup>

### Is a standardized approach possible?

As discussed above, a plethora of approaches has been or could be used to generate PDX from MDS patients (Table 1 and Figure 1). However, these approaches are quite heterogeneous, and use different murine strains, injection sites, types and numbers of cells injected. In order to facilitate a comparison between different studies, it would be

helpful if the field could agree on a more limited set of robust experimental protocols. In our opinion, two options are quite attractive. Our first candidate is the MISTRG model which has been demonstrated to mediate the engraftment of material from patients with different subtypes of MDS and appears relatively simple to implement. Moreover, in the published research, in which patients' cells have been injected intrahepatically into irradiated pups, this line appears to be superior to NSG in terms of engraftment frequency and myeloid percentages.<sup>34</sup> One note of caution is the reported development of anemia in this strain, which is also a characteristic of human MDS.<sup>45,46</sup> This may potentially make it complicated to determine whether the anemia observed in MDS PDX is caused by defects in MDS hematopoietic stem cells or by the intrinsic phenotype of the MISTRG strain. Moreover, the intrahepatic route of injection in newborn pups may not only raise some logistic challenges, but could also potentially influence tumor behavior, because this system constitutes a "young" niche, in contrast to the BM niche of elderly MDS patients. It is to be hoped that further generation of AML/MDS PDX with this mouse model by additional laboratories will strengthen the relevance of this model.

Even though the ossicle strategy is extremely seducing as it allows engraftment of patients' cells into a mature humanized BM-like environment, our own experience indicates that a very high proportion of MSC batches fail to sustain ossicle development (11/12, *unpublished observa-*



tions). Moreover, to our knowledge, AML PDX models based on this approach have only been described by one laboratory so far.<sup>39,42</sup> Therefore, our second proposed model is hBMLS based on gelatin scaffolds. This technique is quite simple and, as for ossicles, up to four scaffolds can be inserted per animal. Moreover, this strategy does not involve a long period of *in vivo* incubation in order to generate ossicles and, importantly, does not require pre-conditioning with irradiation.<sup>41</sup> Using this technique, we have succeeded in generating MDS PDX models covering several MDS subtypes in both our laboratories. A limitation of this approach, as for other hBMLS models, is the use of BM-derived MSC because these MSC have various alterations compared to those derived from healthy donors, such as DNA methylation status<sup>47,48</sup> and *in vitro* proliferation/differentiation capacity.<sup>47</sup> There is therefore a risk that the use of healthy allogeneic MSC may affect the behavior of the MDS clone(s) *in vivo*. Encouragingly, the few studies that have compared the use of healthy and patient-specific MSC have not suggested a major impact of the MSC origin on the engraftment levels of MDS in immunocompromised mice receiving intra-femoral injections.<sup>26,29</sup> Nevertheless, MDS-derived BM MSC do have an impact on the survival and differentiation capacities of CD34<sup>+</sup> hematopoietic stem and progenitor cells *in vitro* and *in vivo*,<sup>47,49</sup> and they can also respond favorably to the hypomethylating agent azacytidine, the current treatment regimen for high-risk MDS.<sup>49</sup> Consequently, investigation are needed to determine whether autologous MDS-BM MSC would be better at recapitulating the complexity of the disease in this model rather than BM MSC from healthy donors.

A major unresolved issue for the hBMLS approaches is that MSC display significant donor-to-donor variations and it would therefore be extremely useful to have a standardized source of MSC, i.e. in the form of BM MSC lines. Importantly, such cell lines have been generated recently and it would be of paramount importance to determine whether they retain their capacity to generate hBMLS *in vivo*<sup>50</sup> and whether MDS material could engraft and expand in these structures. As MDS MSC have been shown to have a strong impact on the *in vivo* potential of CD34<sup>+</sup> hematopoietic stem and progenitor cells, notably by showing altered extracellular signaling such as reduced CXCL12 expression,<sup>48,49</sup> such a cell line should either retain the features of MDS MSC or be receptive to “education” by MDS cells. However, if a MSC cell line that robustly retains these features could be obtained, this would provide an experimental platform for genetic manipulation of niche-derived cells, thereby facilitating studies into niche-MDS cell interactions.

## Conclusions and perspectives

MDS is a very heterogeneous group of blood disorders, associated with lesions in dozens of driver genes.<sup>2,3</sup> Genetically engineered mouse models harboring mutations in the most common MDS driver genes display several characteristics of MDS<sup>11-13</sup> but remain imperfect as an experimental tool since they generally only recapitulate a subset of the phenotypes associated with human MDS. During the past few decades, in particular during the past 5 years, we have seen several improvements in the toolbox available for the generation of MDS PDX.<sup>18-20,22-27,29,31,34</sup> Moreover, various alternative methods, especially hBMLS models, appear to be extremely promising in terms of facilitating a more robust generation of MDS PDX.<sup>39-41</sup> This is important since an increase in the number of MDS PDX models will allow us to cover the broad genetic and phenotypic spectra of human MDS more comprehensively and provide tools to address key aspects of MDS biology.

Despite the recent developments in MDS PDX, these models may be further improved by incorporating additional human niche cells, such as endothelial cells. Indeed, these cells are functional in hBMLS settings<sup>51,52</sup> and endothelial cells from low-risk MDS patients influence hematopoietic stem cell behavior *in vitro*.<sup>53</sup> However, the recent developments of hBMLS models already provide an excellent opportunity to characterize the interaction between MDS tumor cells and their microenvironment better. As indicated above, the tumor microenvironment plays a key role in the pathogenesis of MDS and if we could manipulate MSC in the hBMLS models, we would have a precise tool to discern the biological importance of the niche. Finally, the increasing armory of MDS PDX also holds great promise as preclinical translational models for the development and validation of novel therapies as well as for personalized medicine along the lines already occurring in solid cancers.

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