Myelodysplastic syndrome patient-derived xenografts
from no options to many
Côme, Christophe; Balhuizen, Alexander; Bonnet, Dominique; Porse, Bo T.

Published in:
Haematologica

DOI:
10.3324/haematol.2019.233320

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC

Citation for published version (APA):
Côme, C., Balhuizen, A., Bonnet, D., & Porse, B. T. (2020). Myelodysplastic syndrome patient-derived xenografts: from no options to many. Haematologica, 105(4), 864-869. https://doi.org/10.3324/haematol.2019.233320
Myelodysplastic syndrome patient-derived xenografts: from no options to many

Christophe Côme1,2,3 Alexander Balhuizen,1,2,3 Dominique Bonnet4 and Bo T. Porse1,2,3

1The Finsen Laboratory, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Denmark; 2Biotech Research and Innovation Center (BRIC), University of Copenhagen, Copenhagen, Denmark; 3Danish Stem Cell Center (DanStem), Faculty of Health Sciences, University of Copenhagen, Denmark and 4Haematopoietic Stem Cell Laboratory, The Francis Crick Institute, London, UK

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.1 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.2,3 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.4 The spectrum of survival of patients with MDS is broad and high-risk MDS is associated with an increased propensity to progression to AML.5

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 SF3B1,6 TET2,7 ASXL1,8 and SRSF2.9 The phenotypic properties of these models have been reviewed in detail previously10-12 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, SF3B1mutant mice develop anemia and display expansion of the long-term hematopoietic stem cell compartment, consistent with an MDS phenotype. However, the SF3B1mutant line fails to present with ring sideroblasts which are normally found in patients with SF3B1 mutations.13 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.14 More physiologically relevant models were developed over the next decade via the use of tail vein injection and improved immune-deficient strains.15,16 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.17-20 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).21 During the last decade, several laboratories have published a number of complementary approaches for the generation of MDS PDx.22-24 Importantly, these combined efforts have demonstrated the engraftment capacity of most MDS subtypes,23,25 that the expanded cells retain the genetic and phenotypic features of the primary tumor,26-29 and that these PDx models also sustain engraftment in secondary recipients24,27,30,31 and that they allow evaluation of new therapies.32,33 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
Table 1. Summary of published patient-derived xenograft models from myelodysplastic syndrome patients.

| Year | Reference | Strain | Age | Irradiation? | Injection route | MDS subtype / features | MDS patient cells | Cell numbers | MSC co-injected? | Patients | Expansion |
|------|-----------|--------|-----|--------------|-----------------|------------------------|------------------|--------------|------------------|----------|-----------|
| 2002 | Nilsson et al. | NOD/LtSz-SCID or NOD/LtSz-SCID β2m−/− | 8-12 weeks | yes, 350-375 cGy | tail vein | trisomy 8+ | BM CD34+ CD38 | 1.85-5x10⁶ | No | No | 3-6 - 8 weeks |
| 2003 | Benito et al. | NOD/SCID | 6-8 weeks | yes, 300-350-375 cGy | tail iv or i.p | 15 RA, 11 RAEB, 6 RAEBt, 5 RAS | BM cells | 0.4-3.5x10⁶ | No | 37 | n.s |
| 2004 | Thanopoulou et al. | NOD/SCID β2m−/− or NOD/SCID β2m−/−/SCIDβ2m−/− | 8-10 weeks | yes, 350 cGy | tail vein or i.p | 1 RA, 1 RARS, 2 RAEB, 3 RAEBt, 4 CMML | BM cells | 4-17x10⁶ | No | 11 | 5-23 weeks |
| 2004 | Kerbauy et al. | NOD/SCID β2m−/− | n.s | n.s | intrafemoral | n.s | BM MNC + MSC cell lines HSS & HSS27a | 10⁵ MNC + 10⁴ HSS & HSS27a | yes, HSS & HSS27a | 6 | 4-17 weeks |
| 2010 | Martin et al. | NSG | n.s | yes, 250 cGy | i.v. (retro-orbital) or intra-tibial | low risk | BM cells | 5x10⁵-5x10³ | No | 5 | 7-12 weeks |
| 2011 | Muguruma et al. | NOG | n.s | yes, 250 cGy | i.v. (retro-orbital) | HSC-like (Lin-CD34+ CD38+ CD45RRA-) | BM CD34⁺ | 1.4-5x10⁵ | Yes | 6 | n.s |
| 2013 | Pang et al. | NSG | NSG or NSG-S | P0-P3 newborn pups | sublethal (100 rads) | anterior facial vein | low risk (monosomy 7) | BM CD34⁺ | 5x10⁵/2x10⁵ | No | 3 | n.s |
| 2014 | Medyouf et al. | NSG or NSG-S | 6-8 weeks | yes, 200 cGy | intrabone | low or intermediate risk | BM CD34⁺ + BM MSC | 10⁵ | Yes | 20 | 16-28 weeks |
| 2015 | Mian et al. | NSG (females) | 8-12 weeks | yes, 375 cGy | intra-BM (tibia) | RARS | BM CD34⁺ | 0.65-2 10⁷ | No | 4 | 18-20 weeks |
| 2017 | Rouault-Pierre et al. | 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⁺ BM cells / BM MSC | BM CD34⁺ | 1.2-10⁷ (1:1 for MSC) | Yes | 28 | |
| 2017 | Yoshimi et al. | NSG-S | 6-10 weeks | yes, 200-250 cGy | intrafemoral tail iv | CMLM JMML | BM CD34⁺ BM or PB MNC | 0.2-1.18x10⁷ 2.2-4x10⁷ (BM) or 1.3-2x10⁷ (PB) | No | 8 | 3-11 weeks |
| 2017 | Zhang et al. | NSG or NSG-S | 6-8 weeks | yes, 250 cGy | i.v. (retro-orbital) | CMML | BM or PB CD34⁺ | 3x10⁵ / 1.2x10⁷ | No | 16 | 10-16 weeks |
| 2018 | Krevata et al. | NSG or NSG-S | 6-8 weeks | yes, 250cGy | intrafemoral | 3 low-risk, 4 high-risk | BM MNC / BM MSC | 10⁶ BM MNC / 10⁵ BM MSC | Yes | 7 | 8-32 weeks |
| 2018 | Meinier et al. | NSG | 8-12 weeks | no, 25mg/kg busulfan d-1 i.p | intra-BM (tibia) | 1 RAEB, 2 RAEBt, 2 RARS | CD34⁺ BM cells + BM MSC | 5x10³ CD34⁻ & 1.5x10⁵ MSC (1:3) | Yes | 4 | 6 months |
| 2018 | Shastri et al. | NSG | n.s | yes, 200cGy | tail iv | 2 int-2 risk, 1 high-risk, 1 MPN | BM/PB MNC | 2-5x10⁴ | No | 4 | 3 weeks |
| 2018 | Stevens et al. | NSG | n.s | no, 25mg/kg busulfan d-1 | tail iv | 8 high-risk | BM MNC | 0.8-1x10⁷ | No | 4 | 6-10 weeks |
| 2019 | Smith et al. | NSG | n.s | yes, 200cGy | tail iv | U2AF1 mutants | BM MNC | 2-5x10⁴ | No | 2 | 3 weeks |

continued on the next page
sources have been employed (BM or peripheral blood mononuclear cells, CD3-depleted BM cells, CD34+ 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 Prkdcscid and Il2rg leading to the absence of B, T and NK cells. 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, in contrast to the situation in AML. 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 (SIRPα) and thrombopoietin at physiological levels on a different immunodeficient background (Rag2−/−, Il2rγc−/−), was recently demonstrated to be a promising host for engraftment of MDS patients’ material. 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 CD34+ myeloid cells than in NSG mice. Moreover, long-term engraftment of these myeloid cells was also improved in this strain as CD34+ cells constituted more than 80% of the hCD45+ 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.

(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. Indeed, one of the first attempts to generate MDS PDX failed mainly because of the predominant growth of human CD3− T cells, leading to graft-versus-host disease in most of the recipient animals.

(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, whereas others have not found this effect. The underlying reasons for this variation are not clear. However, as human MSC only survive for 2-4 weeks in the murine BM, 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.

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. 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 (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 (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. Importantly, up to four hBMLS per animal can be introduced, and Reisch et al. have elegantly demonstrated that tumor cells can cir-
calculate between ossicles leading to engraftment of leukemic cells in non-injected hBMLs, thereby allowing for increased expansion of the original material from patients.39

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.42 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.39,40 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.41,44

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.34 One note of caution is the reported development of anemia in this strain, which is also a characteristic of human MDS.45,46 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-
Moreover, to our knowledge, AML PDX models based on this approach have only been described by one laboratory so far. Therefore, our second proposed model is hBM LS 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. 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 hBM LS 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 and in vitro proliferation/differentiation capacity. 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. Nevertheless, MDS-derived BM MSC do have an impact on the survival and differentiation capacities of CD34+ hematopoietic stem and progenitor cells in vitro and in vivo, and they can also respond favorably to the hypomethylating agent azacytidine, the current treatment regimen for high-risk MDS. 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 hBM LS 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 hBM LS in vivo 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+ hematopoietic stem and progenitor cells, notably by showing altered extracellular signaling such as reduced CXCL12 expression, 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. Genetically engineered mouse models harboring mutations in the most common MDS driver genes display several characteristics of MDS 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. Moreover, various alternative methods, especially hBM LS models, appear to be extremely promising in terms of facilitating a more robust generation of MDS PDX. 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 hBM LS settings and endothelial cells from low-risk MDS patients influence hematopoietic stem cell behavior. However, the recent developments of hBM LS 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 hBM LS 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.

Acknowledgement

Work in the Pose laboratory was supported through a center grant from the Novo Nordisk Foundation (Novo Nordisk Foundation Center for Stem Cell Biology, DanStem; Grant Number NNF17CC0027852). The present work is also part of the Danish Research Center for Precision Medicine in Blood Cancers funded by the Danish Cancer Society (grant n. R223-A130741) and Greater Copenhagen Health Science Partners. Work in the Bonnet laboratory was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001045), The UK Medical Research Council (FC001045) and the Wellcome Trust (FC001045).

References

1. Arber DA, Oztun A, Hassennaj R. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. 2016;127(20):2391-2405.
2. Papamannuel E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2015;125(22):3616-3627.
3. Hatzlach T, Nogata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241-247.
4. Papamannuel E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209-2221.
5. Strupp C, Nachtikamp K, Hildebrandt B, et al. New proposals of the WHO working group (2016) for the diagnosis of myelodysplastic syndromes (MDS): Characteristics of refined MDS types. Leuk Res. 2017;57:77-84.
6. Obeng EA, Chappell RJ, Seiler M, et al. Physiologic expression of SF3B1K700E causes impaired erythropoiesis, aberrant splicing, and sensitivity to therapeutic spliceosome modulation. Cancer Cell. 2016;30(5):404-417.
7. Moran-Crusio K, Reavie L, Shih A, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell. 2011;20(1):11-24.
8. Hasegawa N, Oshima M, Sashida G, et al. Impact of combinatorial dysfunctions of Tet2 and EzH2 on the epigenome in the pathogenesis of myelodysplastic syndrome. Leukemia. 2017;31(4):861-871.
9. Wang J, Li Z, He Y, et al. Loss of Axl1 leads to myelodysplastic syndrome-like disease in
Establishing of a xenograft model of NOD/SCID mice transplanted with marrow from patients with myelodysplastic syndrome human candidate genes, how relevant are they? Haematologica. 2012;98(1):10-22.

Zhou T, Kinney MC, Scott LM, et al. Revisiting the case for genetically engineered mouse models in human myelodysplastic syndrome research. Blood. 2015;126(9):1057-1068.

Malcovati L, Papaemmanuil E, Bouwen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118(24):6239-6246.

Franky CE, Rohrbach D, Bollard C, et al. Growth of acute myeloid leukaemia as discrete subcutaneous tumours in immunodeficient mice. Br J Cancer. 1977;35(5):697-701.

Lapidot T, Sirad C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645-648.

Bonnet D, Dick JE. Human acute myeloid leukaemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730-737.

Nilsson L, Astrand-Grundstrom I, Anderson K, et al. Involvement and functional impairment of the CD34(+)/CD38(-)/Thy-1(+) hematopoietic stem cell pool in myelodysplastic syndromes with trisomy 8. Blood. 2002;100(1):259-267.

Kerbauy DM, Lesnikov V, Torok-Storb B, et al. Engraftment of distinct clonal MDS-derived hematopoietic precursors in NOD/SCID-beta2-microglobulin-deficient mice after intramedullary transplantation of hematopoietic and stromal cells. Blood. 2004;104(7):2202-2208.

Thanopoulos E, Cadman J, Kakagianne T, et al. Engraftment of NOD/SCID-2 microglobulin null mice with multilineage neoplastic cells from patients with myelodysplastic syndrome. Blood. 2004;103(11):4285-4295.

Benito AI, Bryant E, Loken MR, et al. NOD/SCID mice transplanted with marrow from patients with myelodysplastic syndrome (MDS) show long-term propagation of normal but not clonal human precursors. Leuk Res. 2003;27(5):425-436.

Muguruma Y, Matsushita H, Yahata T, et al. Establishment of a xenograft model of human myelodysplastic syndromes. Haematologica. 2011;96(4):543-551.

Fang WW, Pavlinicz JV, Price EA, et al. Hematopoietic stem cell and progenitor cell mechanisms in myelodysplastic syndromes. Proc Natl Acad Sci U S A. 2013;110(8):3011-3016.

Medyof H, Mossner M, Jann JC, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell. 2014;14(6):624-637.

Mian SA, Rouault-Ferre K, Smith AE, et al. SF3B1 mutant MDS-initiating cells may arise from the haematopoietic stem cell compartment. Nature Commun. 2015;6:10004.

Rouault-Ferre K, Mian SA, Goudard M, et al. Freclinal modeling of myelodysplastic syndromes. Leukemia. 2017;31(12):2702-2708.

Yoshimi A, Balasie ME, Vedder A, et al. Robust patient-derived xenografts of MDS/MPN overlap syndromes capture the unique characteristic of CAML and JMLM. Blood. 2017;130(4):387-407.

Zhang Y, He L, Selimoglu-Buet D, et al. Engraftment of chronic myelomonocytic leukemia cells in immunocompromised mice supports disease dependency on cytokines. Blood Adv. 2017;1(11):972-979.

Krevvata M, Shan X, Zhou C, et al. Cytokines increase engraftment of human acute myeloid leukemia cells in immunocompromised mice but not engraftment of human myelodysplastic syndrome cells. Haematologica. 2018;103(6):959-971.

Meunier MA, Del S, S, Cazzola M, et al. Molecular dissociation of engraftment in a xenograft model of myelodysplastic syndromes. Oncotarget. 2018;9(19):14993-15000.

Shastri A, Choudhary G, Teixeira M, et al. Antisense STAT5 inhibitor decreases viability of myelodysplastic and leukemia stem cells. J Clin Invest. 2018;128(12):5479-5488.

Stevens BM, Khan N, D’Alessandro A, et al. Characterization and targeting of malignant stem cells in patients with advanced myelodysplastic syndromes. Nature Commun. 2018;9(1):360.

Smith MA, Choudhary GS, Pellagatti A, et al. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol. 2019;21(5):640-650.

Song Y, Rongvaux A, Taylor A, et al. A highly efficient and faithful MDS patient-derived xenotransplantation model for pre-clinical studies. Nature Commun. 2019;10(1):366.

Shulze LD, Lyons BU, Burzenski LM, et al. Human lymphoid and myeloid cell development in NOD/1.Tsz-scid IL2R null mice engrafted with mobilized human hematopoietic stem cells. J Immunol. 2005;174(4):4677-4689.

Wunderlich M, Chou FS, Link KA, et al. AML xenotransplantation model for preclinical studies. Exp Hematol. 2017;55:3-18.

Zhang Y, Brooks RA, Panchal R, et al. Limited engraftment of low-risk myelodysplastic syndrome cells in NOD/SCID gamma-C chain knockout mice. Leukemia. 2010;24(9):1662-1666.

Wunderlich M, Brooks RA, Fanchal P, et al. OKT3 prevents xenogeneic GVHD and allows reliable xenograft initiation from unfractionated human hematopoietic tissues. Blood. 2014;123(24):e134-e144.

Reinsch A, Thomas D, Corces MR, et al. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. Nat Med. 2016;22(7):812-821.

Antonelli A, Noott WA, Jaques J, et al. Establishing human leukemia xenograft mouse models by implanting human bone marrow–like scaffold-based niches. Blood. 2016;128(25):2949-2959.

Abarrategi A, Foster K, Hamilton A, et al. Versatile humanized niche model enables study of normal and malignant human hematopoiesis. J Clin Invest. 2017;127(2):543-546.

Reinsch A, Hernandez DC, Schallmoser K, et al. Generation and use of a humanized bone-marrow-ossicle niche for hematopoietic xenotransplantation into mice. Nat Protoc. 2017;12(10):2169-2188.

Li AJ, Calvi LM. The microenvironment in myelodysplastic syndromes: niche-mediated disease initiation and progression. Exp Hematol. 2017;55:3-18.

Pronk E, Raaikamers MHG. The mesenchymal niche in MDS. Blood. 2019;133 (10):1031-1038.

Rongvaux A, Willinger T, Martinek J, et al. Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 2014;32(4):564-572.

Hemrdl-Brandstetter D, Shan L, Yao Y, et al. Humanized mouse model supports development, function, and tissue residency of human natural killer cells. Proc Natl Acad Sci U S A. 2017;114(45):E9626-E9634.

Geyh S, Orz S, Caldeudo RF, et al. Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia. 2015;27(9):1841-1851.

Schoeder T, Geyh S, Gering U, et al. Mesenchymal stromal cells in myeloid malignancies. Blood Res. 2016;51(4):225-232.

Poon Z, Dighe N, Venkatesan SS, et al. Bone marrow MSCs in MDS: contribution towards dysfunctional hematopoiesis and potential targets for disease response to hypomethylating therapy. Leukemia. 2019;33(6):1487-1500.

James S, Fox J, Asfar E, et al. Multiparameter Analysis of human bone marrow stromal cells identifies distinct immunomodulatory and differentiation-competent subtypes. Stem Cell Reports. 2015;4(6):1004-1015.

Chen Y, Jacamo R, Shi YX, et al. Human extramedullary bone marrow in mice: a novel in vivo model of genetically controlled hematopoietic microenvironment. Blood. 2012;119(21):4971-4980.

Fassaro D, Abarrategi A, Foster K, et al. Bioengineering of human bone marrow microenvironments in mouse and their visualization by live imaging. J Vis Exp. 2017;126(25):e55914.

Teofili L, Martini M, Nuzzolo ER, et al. Endothelial progenitor cell dysfunction in myelodysplastic syndromes: possible contribution of a defective vascular niche to myelodysplasia. Neoplasia. 2015;17(5):401-409.