Murine models based on acute myeloid leukemia-initiating stem cells xenografting

Cristina Mambet, Mihaela Chivu-Economescu, Lilia Matei, Laura Georgiana Necula, Denisa Laura Dragu, Coralia Bleotu, Carmen Cristina Diaconu

Abstract
Acute myeloid leukemia (AML) is an aggressive malignant disease defined by abnormal expansion of myeloid blasts. Despite recent advances in understanding AML pathogenesis and identifying their molecular subtypes based on somatic mutations, AML is still characterized by poor outcomes, with a 5-year survival rate of only 30%-40%, the majority of the patients dying due to AML relapse. Leukemia stem cells (LSC) are considered to be at the root of chemotherapeutic resistance and AML relapse. Although numerous studies have tried to better characterize LSCs in terms of surface and molecular markers, a specific marker of LSC has not been found, and still the most universally accepted phenotypic signature remains the surface antigens CD34+CD38- that is shared with normal hematopoietic stem cells. Animal models provides the means to investigate the factors responsible for leukemic transformation, the intrinsic differences between secondary post-myeloproliferative neoplasm AML and de novo AML, especially the signaling pathways involved in inflammation and hematopoiesis. However, AML proved to be one of the hematological malignancies that is difficult to engraft even in the most immunodeficient mice strains, and numerous ongoing attempts are focused to develop “humanized mice” that can support the engraftment of LSC. This present review is aiming to in-
introduce the field of AML pathogenesis and the concept of LSC, to present the current knowledge on leukemic blasts surface markers and recent attempts to develop best AML animal models.

Key words: Acute myeloid leukemia; Leukemia-initiating stem cells; Antigen markers; Murine models; Xenografts

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The review is aiming to introduce the field of acute myeloid leukemia (AML) pathogenesis, the concept of leukemic stem cells, and also to present the current attempts to develop best AML animal models as means to investigate the factors responsible for leukemic transformation. Due to difficulties in engraftment of less aggressive AML samples, it is currently being attempted to develop humanized mice by introducing supporting human stromal cells as a source of proper cytokines, in a challenge to mimic an appropriate bone marrow niche able to support leukemic stem cells engraftment.

Mambet C, Chivu-Economescu M, Matei L, Necula LG, Dragu DL, Bleotu C, Diaconu CC. Murine models based on acute myeloid leukemia-initiating stem cells xenografting. World J Stem Cells 2018; 10(6): 57-65. Available from: URL: http://www.wjgnet.com/1948-0210/full/v10/i6/57.htm DOI: http://dx.doi.org/10.4252/wjsc.v10.i6.57

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive cancer characterized by unrestricted proliferation of functionally immature myeloid cells. High heterogeneity and variable expansion capacity of multiple clones within each patient[1,2], clinical and molecular differences between de novo and secondary AML, complicate even more treatment choices and make targeted therapy a goal yet to far to reach without using models that are able to simplify the multitude of mechanisms that might be involved in leukemogenesis.

In the last decades, murine models become very important tools in the field of preclinical research in oncology, hematology, and immunology, providing a platform for study of tumor biology and for in vivo evaluation of drugs in patient-derived xenograft tumors (PDX). Nowadays, a large variety of immunodeficient mice strains have emerged, able to support the xenografting and development of a complex human hematolymphoid system. The most difficult to reproduce is the immune system and the bone marrow (BM) microenvironment, mostly because of the differences between the signaling molecules responsible for the maturation of different hematopoietic cell populations[3]. Although the field of animal models has experienced a recent exponential growth through the development of IL2rgnull immunodeficiency mice, AML remains one of the hematologic malignancies difficult to engraft into the existing strains of mice due to the lack of a proper BM niche and absence of specific human growth factors and supporting stromal cells[4]. As a result several attempts were made to develop “humanized mice” that can better support myeloid leukemia-initiating stem cells xenografting.

This review is aiming to introduce AML pathogenesis and the concept of leukemic stem cells and the current most advanced strategies to overcome challenges in obtaining AML murine models.

AML PATHOGENESIS AND THE CONCEPT OF LEUKEMIC STEM CELLS

AML is a heterogeneous hematopoietic malignancy defined by clonal expansion of abnormally differentiated or undifferentiated myeloid progenitors (blasts) that accumulate in the BM and impair hematopoiesis, leading to multi-lineage cytopenias[5,6]. Blasts can also migrate from BM into peripheral blood and infiltrate other tissues[5].

AML can be divided in 3 categories taking into account their clinical ontology: Secondary AML (s-AML) occurred after leukemic transformation of a pre-existing myelodysplastic syndrome or myeloproliferative neoplasm, therapy-related AML (t-AML) developed in patients that received leukemogenic chemotherapy for antecedent non-myeloid malignancies and de novo AML generated in the absence of a previous stem cell disorder or a therapeutic exposure to cytotoxic drugs[7].

Despite recent progress in understanding AML pathogenesis and recognizing molecular subtypes of AML that have prognostic impact, AML is still characterized by poor outcomes, with a 5-year survival rate of only 30%-40%. The dismal prognosis is mainly related to high rate of relapse and refractory disease[2,8]. Patients with s-AML and t-AML display even a much worse prognosis, the median overall survival rate being 7 months. Notably, somatic mutations in SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCO, or STAG2 proved to be highly specific for s-AML. They are acquired early in leukemogenesis and tend to persist during clonal remissions[7].

Increasing evidence support the concept that a minor population of stem cells, named leukemia stem cells (LSCs), is responsible for leukemia initiation, disease progression and relapse, as well as drug resistance[9]. AML was among the first diseases in which the existence of cancer stem cells was documented using xenograft animal models[10].

LSCs are derived from transformed hematopoietic stem cells (HSCs) or downstream committed progenitors[11]. They are able to initiate the disease after transplantation into immunodeficient mice and are characterized by both unlimited self-renewal potential inducing disease in serial transplantation and capacity to
Although LSCs and non-LSC blasts harbor a common CXCL12-CXCR4 axis promotes retaining of LSCs within LSCs might escape apoptosis through upregulation of NF-κB or downregulation of Fas/CD95. Additionally, CXCL12-CXCR4 axis promotes retaining of LSCs within the protective BM microenvironment.

It is thought that, similarly to normal hematopoietic system, AML displays a hierarchical organization with LSCs on the top, being able to generate the whole population of AML blast cells. Signaling pathways that control self-renewal of HSCs, such as Wnt/β-catenin, PI3K/Akt/mTOR, or Hedgehog, are also involved in LSC survival and expansion and can serve as therapeutic targets to facilitate eradication of LSCs. Moreover, LSCs might escape apoptosis through up-regulation of NF-κB or down-regulation of Fas/CD95. Additionally, CXCL12-CXCR4 axis promotes retaining of LSCs within the protective BM microenvironment.

The existence of a preleukemic stage in AML was proven by isolating from leukemia patients a population of HSCs that was found to bear some, but not all, of the mutations identified in the downstream leukemia. These preleukemic HSCs, that can be distinguished from LSCs by the surface antigen markers, TIM3 and CD99, are capable to generate bi-lineage engraftment in NOD.Cg-PrkdcsidIl2rgtm1Wjl (NSG) mice and the derived lymphoid and myeloid progeny display the same preleukemia mutations. According to the currently proposed model of preleukemic clonal evolution, the first leukemia-related mutation has to occur in a cell that possesses self-renewal capacity or, alternatively, must confer self-renewal properties to a more differentiated progenitor. By single-cell analysis it was shown that during the process of leukemogenesis, the preleukemic HSCs gradually acquire somatic mutations in a nonrandom pattern. Thus, in the early phases of AML evolution there is enrichment for mutations in epigenetic modifiers such as TET2, DNMT3A, IDH1/2, and ASXL1. On the other hand, mutations in genes involved in signaling pathways and proliferation, such as FLT3 and KRAS occur in later stages. Other leukemogenic mutations in genes like NPM1, CEBPA, and WT1 can be found in preleukemic phase as well as in later stages.

The preleukemic HSCs that eventually give rise to AML persist in patient samples at diagnosis and are resistant to current chemotherapy, thus representing a source of disease recurrence.

LEUKEMIA-INITIATING STEM CELLS AND BLASTS

The identification of LSCs in AML plays an important role in disease diagnosis, prognosis and AML therapy monitoring, and also represents an important step in development of targeted therapy and drug discovery. Although initial studies suggested that LSCs were partially differentiated into non-LSC blasts that lack self-renewal properties and possibility of engraftment. However, later studies proved that LSCs are characterized by increased or decreased expression of surface markers of normal myeloid precursors (CD34, CD38, CD33, CD13, CD117, and CD123), asynchronous expression of antigens determined by AML morphological subtype and by the LSC stage of differentiation (CD4, CD11b, CD14, CD15, CD36, CD61, CD64, CD71, etc.), as well as by aberrant expression of lymphoid antigens (cross-lineage expression) (CD2, CD5, CD7, CD19, CD22, CD56, Tim3, etc.). LSCs reside mainly in CD34+CD38- population, but may be present also in other cellular fractions, usually CD34+CD38+, and in some cases, in CD34- population. Additional markers, more specific for the advanced characterization of cellular subpopulations in AML, include: CD90, CD96, CD123, CD125, CD47, C-type lectin-like molecule-1 (CLL1), aldehyde dehydrogenase etc. Currently, standard diagnosis and sub-classification of AML integrate the study of cell morphology, genetics/cytogenetics and multi-parametric immuno-phenotyping. The antibody panels for surface markers used for sub-classification of each AML group are showed in Figure 1.

Methods commonly used to assess HSC properties are colony-forming cell (CFC) assay, long term culture (LTC), flow cytometry and competitive repopulation. The CFC assay is an important tool used to evaluate the ability of the progenitor cells to proliferate and differentiate into multiple lineages. In order to produce colonies, cells are cultured in a semisolid medium, in the presence of appropriate cytokines for 7-14 d. Colonies are counted and characterized according to morphologic and phenotypic criteria. Although this short-term colony assay can determine the frequencies of hematopoietic progenitor cells in analyzed populations, still, it is not able to detect more immature progenitors or HSCs/LSCs. To overcome this limitation, the cells can be cultured for 5-8 wk on a stromal feeder layer that can provide a substrate and a source of cytokines and growth factors, in effort to mimic the in vivo niche conditions. The long-term culture-initiating cells (LTC-ICs) can be evaluated by their capacity to generate CFCs in culture supernatant after 5 wk. This period allows CFCs present in the inoculum to terminally differentiate and the remaining CFCs may represent the progeny of LTC-ICs. Subsequent limiting dilution tests can be performed to determine the LTC-IC frequency. Although this method facilitates the detection of more immature progenitor cells, it is time consuming and the presence of stromal cells can induce procedure variations and different outcomes. Competitive repopulation represents the best method to assess the functional abilities of immature progenitor/stem cells by serial transplants in immunocompromised mice. This method is based on the ability...
of cells that are investigated to compete with non-manipulated standard cells to repopulate the BM of an irradiated recipient\[43\].

MURINE MODELS - WHICH ARE THE BEST CHOICE?

Animal models are used as replacement for human biological niches due to ethical restrictions in the use of human tissue samples from donors. Moreover, animal models accurately recapitulate human disease and have been an important tool in advancing the understanding of human pathology, and development of pre-clinical therapy. Small animals, such as mice and rats, are often used as a model for various diseases because of their ease in breeding, maintenance, and manipulation. In spite of these many advantages, there are limitations due to the disparities between the murine and human biological systems. Human immune system and the BM microenvironment are the most difficult to be reproduced in mouse models because of the differences in the signaling molecules responsible for the maturation of various hematopoietic cell populations\[3\]. As a result, many malignant hematopoietic and other hematologic disorders do not successfully engraft in conventional mouse models.

AML is one of these hematologic malignancies that fail to properly graft into the existing strains of mice due to the lack of a proper BM niche, homing elements, absence of specific human growth factors and supporting stromal cells\[41\]. As a result, several attempts have been made to develop murine models that reproduce with fidelity human hematopoiesis, particularly the development of the myeloid line.

Early attempts to increase the support for myelopoiesis involved the use of mice injected with IL-3, GM-CSF, SCF\[44\], mice producing human TPO\[45\] or MISTRG mice strain which produces human tumor necrosis factor and IL-6\[46\]. These confirmed that the introduction of human genes into mice led to the production of functional proteins capable of supporting engraftment and proliferation of human grafts.

The following attempts were aimed to develop next-generation mouse models genetically engineered to support myeloid differentiation from human HSC. Thus, it was necessary to act at three major levels in order to induce tolerance in the murine host, provide a supportive niche, and support hematopoiesis/proliferation with appropriate growth factors and cytokines.

The first request was fulfilled by the development of mice that lacked the adaptive and innate immune compartment like NSG and NODShi.Cg-Prkdcsod Il2rg<sup>ms1Sug</sup> mice strains. These strains were developed on NOD scid immunodeficient mice by modifying them to bear mutations in the IL-2 receptor gamma chain gene th-
The median human CD45+ count in non-irradiated NBSGW mice was similar to the count in irradiated NSG. In BM, a significant increase in CD45+ was observed in non-irradiated NBSGW mice compared to non-irradiated NSG (97%) compared to non-irradiated NSG (30%).

The third constraint regarding the need for supportive myeloid cytokines was overcome using animal models with transgenic expression of hSCF, hGM-CSF and hIL-3 on the NOD SCID background resulting the NSG-SGM3 mouse strain [also known as NOD.Cg-Prkrdc1il2rg-1<sup>tm1Wj1</sup>Tg(CMV-IL3,CSF2,KITLG)/1Eav]]. Wunderlich et al. reported the development and use of NSG-SGM3 mouse for the engraftment of normal CD34+ and AML xenografts. The results showed an improvement in the expansion of normal human myeloid cells, as well as an enhanced engraftment of primary human AML samples. They injected five samples of primary AML in sub-lethally irradiated NGS and NSG-SGM3 mice. Only three samples from five engrafted in NSG mice, compared to five samples in NSG-SGM3 mice, showing variability in the engraftment potential across the AML samples, and that NSG-SGM3 strain was a better host for some subsets of AML relative to NSG mice. Moreover, three of five samples with primary AML had higher BM engraftment level in NSG-SGM3<sup>[4]</sup>. Similar results regarding variable engraftment potential in immunodeficient mice strains were obtained by Klco et al.<sup>[8]</sup> They injected blasts from six AML samples in tail vein of non-irradiated NSG and NSG-SGM3. The results showed that four samples had higher BM engraftment and CD34+ expression level in NSG-SGM3 than NSG mice.

Finally, we can conclude that next-generation humanized mouse models are able to support myeloid blast development and differentiation.

**XENOGRAFT MOUSE MODELS USED FOR ACUTE MYELOID LEUKEMIA**

**Cell line derived xenografts**

AML represents a heterogeneous disease including several subtypes which are characterized by specific fusion oncogenes as a result of chromosome abnormalities. The fusion oncogenes in AML are associated with different clinical and laboratory characteristics, highlighting the different ways of malignant transformation in this disease. A study focused on the evaluation of four important AML fusion oncogenes reported that MLL-AF9 and NUP98-HOXA9 had very similar effects in vitro on primary human CD34+ cells, resulting in erythroid hyperplasia and an obvious blockage in erythroid and myeloid maturation while AML1-ETO and PML-RARA produced only modest effects on myeloid and erythroid differentiation. Moreover, MLL-AF9, NUP98-HOXA9 and AML1-ETO fusion oncogenes generated a significant increase in long-term proliferation and self-renewal of CD34+ cells. The characterization of gene profiles determined by AML fusion oncogenes can be considered an important tool for the discovery of new potential drug targets. In this study, two different time patterns of gene deregulation as result of fusion of these oncogenes were observed: MLL-AF9 and NUP98-HOXA9, caused gene deregulation 3 d after transduction, while gene deregulation by AML1-ETO and PML-RARA appeared within 6 h. Interestingly, p53 inhibitor MDM2 was upregulated by AML1-ETO at 6 h suggesting that MDM2 upregulation was involved in cell transformation, being related to AML1-ETO<sup>[51]</sup>.

Wei et al<sup>[50]</sup> evaluated the in vitro and in vivo effects of MLL-AF9 gene fusion in human CD34+ cord blood cells using retroviral vectors. Thus, MA9 transduced cells became immortal and doubled in number every 2-3 d. The expression of CD33, CD11b, CD13, CD14 and CD15 suggested a myelomonocytic lineage. Moreover, long-term cultured MA9 cells failed to differentiate towards the erythroid or B lymphoid lineages, remaining cytokine and FLT3L dependent for growth. In non-obese diabetic/severe combined immunodeficient (NOD/SCID (NS)), NS-j2M-/- (NS-B2M) and NS mice, MA9 cells induced acute myeloid, lymphoid, or mixed-lineage leukemia with blast cells present in the peripheral blood, BM, spleen and liver. Gene expression profile of MLL-AF9 transduced cells was similar to human AML with 11q23 translocations, Rac signaling pathway being the most affected pathway and a promising therapeutic target in MLL-rearranged AML<sup>[52]</sup>. Another AML subtype with a particularly poor outcome is characterized by the t(6;9)(p22;q34) chromosome rearrangement which generates DEK-NUP214 chimeric gene. Qin et al<sup>[53]</sup> developed an AML model harboring DEK-NUP214, using CD34+ human hematopoietic progenitor cells and MO7e cell lines xenografted into immunocompromised mice that expressed human myeloid cell growth factors. The MO7e human megakaryoblastic leukemia cell line was strictly dependent on either IL-3 or GM-CSF for survival; retroviral expression of this fusion gene in IL-3 dependent MO7e cell line induced a cytokine independence and increased colony formation ability in soft-agar. DEK-NUP214 expression also modified the differentiation of human cord blood CD34+ progenitor
cells, which expressed myeloid lineage markers (CD13+), with small subsets showing T- (CD3+) and B- (CD19+) cell lineage markers. The obtained results suggested that DEK-NUP214 was involved in leukemic transformation and differentiation of myeloid cells. In this study, CD34+ progenitor cells obtained from three different umbilical cord blood samples and transduced with chimeric DEK-NUP214 were engrafted in NSG-SGM3 mice strain. Interestingly, two months after transplantation, almost 20% of peripheral blood cells from the transplanted mice displayed a human-specific CD45 immuno-phenotype with CD45+CD13+CD34+CD38+ cells. The analysis of peripheral blood smears also showed the typical human AML cell morphology with a larger nucleus and reduced cytoplasmic ratio. Therefore, the study demonstrated that DEK-NUP214 could transform human CD34+ progenitor cells and induced human AML in vivo. Gene profiling of this model revealed that several genes of HOX family (HOXA9, 10, B3, B4 and PBX3) were highly upregulated. In this AML model pathways involving KRAS, BRCA1 and ALK were significantly deregulated.

Similar results were obtained in case of t(8;13)- (p11;q12) chromosome translocation which led to ZMYM2-FGFR1 chimeric kinase, characteristic for another AML subtype. Human CD34+ cells harboring ZMYM2-FGFR1 transplanted into immune-compromised mice developed myeloproliferative disease that progressed to AML. Mice displayed hepatosplenomegaly, hypercellular BM and a CD45 + CD34 + CD13+ immunophenotype.[53]

Preclinical cancer research remains essential for the discovery and the development of new therapies in case of the most advanced cancers. Various cancer cell lines have been developed and used for the study of cancer but with a great disadvantage that they do not really reflect the behavior of the original cancer cells, due to the artificial nature of their culture conditions.

**Patient derived xenografts - patient stem-cells derived xenografts**

PDX models established by transplanting patient cancer cells into immunocompromised mice represent an important tool in cancer research. They have a great potential to offer important information on cancer biology and to guide the therapeutic approach. Unlike cell lines derived from primary tumors that might have lost their original characteristics due to a prolonged *in vitro* growth, PDX mouse models seemed to be able to overcome this issue.[55,56] Many studies demonstrated that PDX models kept the most important features of the original tumor including histology, genomic pattern, cellular heterogeneity, and more important, drug responsiveness or personalized drug selection.[57]

The development of PDX models of AML allows us to monitor *in vivo* the progression of the disease and to evaluate the efficacy of an experimental treatment on tumor growth using imaging techniques.[58]

A first full study on the engraftment ability of a large cohort of AML samples in immunodeficient animal models was published by Kennedy et al[59] who transplanted BM or peripheral blood cells from 307 AML patients intra-femorally into sublethally irradiated NOD. SCID mice pre-treated with an anti-CD122 antibody. AML xenografts were obtained in 44% of cases, leukemic engraftment being associated with a higher white cell count in peripheral blood (mean of 92 x 10⁹/L in engrafters vs 67 x 10⁹/L in non-engrafters, P = 0.01). Moreover, results showed that complete remission was achieved in only 51% of patients whose diagnostic samples established AML xenografts, compared to 80% of non-engrafting samples (P < 0.0001). As a conclusion, AML xenografting was successful when using samples from AML patients with aggressive disease and with a poor response to standard induction therapy.

**OVERCOMING CHALLENGES IN PATIENT-DERIVED XENOGRAFTS OF AML**

Recent experiments are trying to improve mouse experimental models of AML, aiming to engraf with a higher success rate even less aggressive leukemia samples. Most of them are based on immunodeficient mice with humanized microenvironment created by injection of human mesenchymal stem cell (MSC) that provide a better niche for leukemic blast engraftment.

An interesting approach was that of Reinisch et al[60], who used human MSC grafts injected subcutaneously in NSG mice to form a humanized microenvironment named “ossicles”, in which they subsequently injected (8 wk later) human HSCs and AML blasts. The final aim was to analyze the engraftment capacity the resulting niche. After 6-10 wk, the “ossicles” showed human BM-like functions and morphology and allowed enhanced engraftment of primary patient-derived AML.

A further attempt employed humanized niches based on genetically modified MSCs to express huIL-3 and TPO. Carretta et al[61] implanted subcutaneously these human MSCs in ceramic scaffolds or Matrigel in NGS mice, and 6 to 8 wk later transplanted CD34+ enriched AML blasts in the ectopically engineered BM niches. The engraftment capacity was then compared with the one from non-engineered MSC niches. The results showed that leukemic blasts efficiently engrafted in both models with no significant differences. An unexpected result was that CD33+-sorted myeloid clones from the animal model failed to self-renew in secondary recipients, probably due to overexpressed IL-3 and TPO cytokines from modified microenvironment that might have affected a proper self-renewal of myeloid blasts.

An important challenge for obtaining mouse models valuable as preclinical models is the capability of PDX cells to authentically mimic the heterogeneity of the initial disease. The xenograft mouse model of AML has been used mainly to study primary transplantation and further serial experiments were performed to
verify self-renewal competence or stability of gene expression profiles of engrafted cells. However, this model was rarely employed to investigate deeply AML biology or therapy\[62\]. Most of the published results revealed that PDX cells resembled the primary samples in terms of gene expression profiles but sub-clonal profiles were often not reflecting the primary sample. Another important drawback was the inability of the most proposed models to sensitively and repetitively monitor disease progression or drug effects. These were determined at single time points by invasive procedures or post mortem. However, researchers tried to overcome these challenges by proposing a better control of PDX cells. This control aimed to check the pattern of alterations in mutational or antigen expression possibly occurring during engraftment. For better monitoring disease progression or drug effects, recombinant luciferase enabled bioluminescence in vivo imaging has been proposed to facilitate in vivo monitoring of PDX AML cells as a quantitative, sensitive, reliable method for quantifying leukemia initiating cells\[62\].

CONCLUSION
Mouse models were of tremendous importance for understanding the molecular etiology of leukemia, proven to be valuable tools to facilitate preclinical in vivo studies.

Most of the studies verified that PDX models kept the most important features of the original tumor. However, mouse models should be controlled more carefully before and after xenotransplantation, especially in serial transplantation experiments, in order to ensure that the heterogeneity of the original sample is conserved and genetic drift is not modifying genetic, phenotypic or functional characteristics of the original disease.

Prospectively, advancements allowing repetitive, reliable, sensitive and fast studies, able to evaluate the efficacy of an experimental treatment in well genetically defined and heterogeneous subgroups of AML, will represent valuable tools to improve the individualized xenograft mouse model of AML and drastically reduce the number of mice to be used in these kinds of experiments.

REFERENCES
1 Bowman RL, Busque L, Levine RL. Clonal Hematopoiesis and Evolution to Hematopoietic Malignancies. Cell Stem Cell 2018; 22: 157-170 [PMID: 29395053 DOI: 10.1016/j.stem.2018.01.011]
2 Papaenmannui E, Döhner H, Campbell PJ. Genomic Classification in Acute Myeloid Leukemia. N Engl J Med 2016; 375: 900-901 [PMID: 27579651 DOI: 10.1056/NEJMct1608739]
3 Walsh NC, Kenney LL, Jangalwe S, Aryee KE, Greiner DL, Brehm MA, Shaltiz LD. Humanized Mouse Models of Clinical Disease. Annu Rev Pathol 2017; 12: 187-215 [PMID: 27959627 DOI: 10.1146/annurev-pathol-052016-100332]
4 Wunderlich M, Chou FS, Link KA, Mizukawa B, Perry RL, Carroll M, Mulloy JC. AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. Leukemia 2010; 24: 1785-1788 [PMID: 20686503 DOI: 10.1038/leu.2010.158]
5 Estey EH. Acute myeloid leukemia: 2014 update on risk-stratification and management. Am J Hematol 2014; 89: 1063-1081 [PMID: 25318680 DOI: 10.1002/ajh.23834]
6 Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. N Engl J Med 2015; 373: 1136-1152 [PMID: 26376137 DOI: 10.1056/NEJMr1406184]
7 Lindesay RC, Mar BG, Mazolla E, Grauman PV, Shareef S, Allen SL, Pigneeux A, Wotzler M, Stuart RK, Erba HP, Damon LE, Powell BL, Lindeman N, Steensma DP, Wadleigh M, DeAngelo DJ, Neuberg D, Stone RM, Ebert BL. Acute myeloid leukemia ontology is defined by distinct somatic mutations. Blood 2015; 125: 1367-1376 [PMID: 25550361 DOI: 10.1182/blood-2014-11-610543]
8 Stahl M, Kim TK, Zaidan AM. Update on acute myeloid leukemia stem cells: New discoveries and therapeutic opportunities. World J Stem Cells 2016; 8: 316-331 [PMID: 27822239 DOI: 10.4252/wjsc.v8.i10.316]
9 Ding Y, Gao H, Zhang Q. The biomarkers of leukemia stem cells in acute myeloid leukemia. Stem Cell Investig 2017; 4: 19 [PMID: 28447034 DOI: 10.21037/sci.2017.02.10]
10 Polleya DA, Gutman JA, Gore L, Smith CA, Jordan CT. Targeting acute myeloid leukemia stem cells: a review and principles for the development of clinical trials. Haematologica 2014; 99: 1277-1284 [PMID: 25082785 DOI: 10.3324/haematol.2013.085209]
11 Wang X, Huang S, Chen JL. Understanding of leukemia stem cells and their clinical implications. Mol Cancer 2017; 16: 2 [PMID: 28137304 DOI: 10.1186/s12934-016-0574-7]
12 Thomas D, Majeti R. Biology and relevance of human acute myeloid leukemia stem cells. Blood 2017; 129: 1577-1585 [PMID: 28159741 DOI: 10.1182/blood-2016-10-696054]
13 Jung N, Dai B, Gentles AJ, Majeti R, Feinberg AP. An LSC epigenetic signature is largely mutation independent and implicates the HOXA cluster in AML pathogenesis. Nat Commun 2015; 6: 8489 [PMID: 26444494 DOI: 10.1038/ncomms9489]
14 Mitchell GH. A vaccine for ovine cysticercosis. Vaccine 1989; 7: 379 [PMID: 28159741 DOI: 10.1016/0264-410X(89)90146-1]
15 Corces MR, Chang HY, Majeti R. Preleukemic Hematopoietic Stem Cells in Human Acute Myeloid Leukemia. Front Oncol 2017; 7: 263 [PMID: 29164062 DOI: 10.3389/fonc.2017.00263]
16 Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A 2014; 111: 2548-2553 [PMID: 24552081 DOI: 10.1073/pnas.1324297111]
17 Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, McLeod JL, Doedens M, Medeiros JJ, Marke R, Kim HJ, Lee K, McPherson JD, Hudson TJ. HALT Pan-Leukemia Gene Panel Consortium, Brown AM, Yousif F, Trinh QM, Stein LD, Minden MD, Wang JC, Dick JE. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 2014; 506: 328-333 [PMID: 24522528 DOI: 10.1038/nature13038]
18 Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730-737 [PMID: 9212098 DOI: 10.1038/nn0797-730]
19 Blair A, Hogge DE, Sutherland HJ. Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34(+)/CD71(-)/HLA-DR-. Blood 1998; 92: 4325-4335 [PMID: 9834239]
20 Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci U S A 1997; 94: 5320-5325 [PMID: 9144235 DOI: 10.1073/pnas.94.10.5320]
21 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105-111 [PMID: 11689955 DOI: 10.1038/35102167]
22 Sary D, Murphy K, Perry R, Sanchez PV, Srecoo A, Keef er C, Swider CR, Strzielecki AC, Cavelier C, Récher C, Mansat-Desmas V, Delabesse E, Danet-Desnoyers G, Carroll M. Human acute myelogenous leukemia stem cells are rare and heterogeneous when
assayed in NOD/SCID/IL2Rγc-deficient mice. *J Clin Invest* 2011; 121: 384-395 [PMID: 21157036 DOI: 10.1172/JCI41495]

23 **Tassoug DJ**, Vargaffig J, Mirakl-Moul F, Griessing E, Sharrock K, Luke T, Lillington D, Oskervyve H, Cavenagh J, Agrawal SG, Lister TA, Garzon R, Boultar D. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. *Blood* 2010; 115: 1976-1984 [PMID: 20053758 DOI: 10.1182/blood-2009-02-206565]

24 **Campos L**, Guyotat D, Archimbau E, Devaux Y, Treille D, Larose A, Maupas J, Gentilhomme O, Ebensam A, Fiere D. Surface marker expression in adult acute myeloid leukemia: correlations with initial characteristics, morphology and response to therapy. *Br J Haematol* 1989; 72: 161-166 [PMID: 2757962 DOI: 10.1111/j.1365-2141.1989.860767.x]

25 **Ouyang J**, Gowsami M, Tang G, Peng J, Rovandi F, Daver N, Routbort M, Konoplev S, Lin P, Medeiros LJ, Jongerson JL, Wang SA. The clinical significance of negative flow cytometry immunophenotypic results in a morphologically scored positive bone marrow in patients following treatment for acute myeloid leukemia. *Am J Hematol* 2015; 90: 504-510 [PMID: 25732229 DOI: 10.1002/ajh.23988]

26 **Sautz JN**, Garzon R. Acute Myeloid Leukemia: A Concise Review. *J Clin Med* 2016; 5: pii: E33 [PMID: 26959069 DOI: 10.3390/jcm5030033]

27 **Wolach O**, Stone RM. How I treat mixed-phenotype acute leukemia. *Blood* 2015; 125: 2477-2485 [PMID: 25605373 DOI: 10.1182/blood-2014-10-551465]

28 **Ho TC**, LaMere M, Stevens BM, Ashton JM, Myers JR, O'Dwyer TP. A natural killer cell marker in human acute myeloid leukemia. *Krensky AM*, Weissman IL. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukaemias. *Blood* 2003; 102: 421-436 [PMID: 12537891 DOI: 10.1182/blood-2002-09-263097]

29 **Ossenkoppele GJ**, van de Loosdrecht AA, Schuurhuis GJ. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. *Br J Haematol* 2011; 153: 421-436 [PMID: 21385170 DOI: 10.1111/j.1365-2141.2011.08395.x]

30 **Grimsdade D**, Freeman SD. Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for “prime time”? *Blood* 2014; 124: 3345-3355 [PMID: 25049280 DOI: 10.1182/blood-2014-05-757593]

31 **Kress A**, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell* 2014; 14: 275-291 [PMID: 24607403 DOI: 10.1016/j.stem.2014.02.006]

32 **Buccisano ST**, Rossi FM, Venditti A, Del Poeta G, Cox MC, Abbruzzese E, Rupolo M, Berretta M, Degan M, Russo S, Tamburini L, Buccisano F, Majeti R, Gielchens JG, Bucis G. The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. *Blood* 2007; 110: 2659-2666 [PMID: 17690428 DOI: 10.1182/blood-2007-03-083048]

33 **Zhou J**, Cheng WJ. Identification and targeting leukemia stem cells: The path to the cure for acute myeloid leukemia. *World J Stem Cells* 2014; 6: 473-484 [PMID: 25255669 DOI: 10.4122/wjasc.v6.i44.473]

34 **Coulombel L**. Identification of hematopoietic stem/progenitor cell markers: strengths and drawbacks of functional assays. Oncogene 2004; 23: 7210-7222 [PMID: 15378801 DOI: 10.1038/sj.onc.1207941]

35 **Bock TA**. Assays systems for hematopoietic stem and progenitor cells. *Stem Cells* 1997; 15 Suppl 1: 185-195 [PMID: 9368340 DOI: 10.1002/stem.5530150824]

36 **Frish BJ**, Calvi LM. Hematopoietic stem cell cultures and assays. *Methods Mol Biol* 2014; 1310: 315-324 [PMID: 24482184 DOI: 10.1007/978-1-62703-095-5_24]

37 **Clarke CJ**, Holyoake TL. Preclinical approaches in chronic myeloid leukemia: from cells to systems. *Exp Hematol* 2017; 47: 13-23 [PMID: 28017647 DOI: 10.1016/j.exphem.2016.11.005]

38 **Ramos CA**, Venezuela TA, Camargo FA, Goodall MA. Techniques for the study of adult stem cells: be fruitful and multiply. *Biotechniques* 2003; 34: 572-578, 580-584, 586-591 [PMID: 12661162]

39 **Kamel-Reid S**, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. *Science* 1988; 242: 1706-1709 [PMID: 2904073 DOI: 10.1126/science.2904073]

40 **Rongvaux A**, Willinger T, Takizawa H, Rathinam C, Auerbach W, Murphy AJ, Valenzuela DM, Yancopoulos GD, Eynon EE, Stevens S, Manz MG, Flavell RA. Human thrombopoietin knockin mouse efficiently support human hematopoiesis in vivo. *Proc Natl Acad Sci USA* 2011; 108: 2378-2383 [PMID: 21268227 DOI: 10.1073/pnas.1019524108]

41 **Rongvaux A**, Willinger T, Martinek J, Strowig T, Gearty SV, Teichmann LL, Saito Y, Marches F, Halene S, Palacka AK, Manz MG, Flavell RA. Development and function of human innate immune cells in a humanized mouse model. *Nat Biotechnol* 2014; 32: 364-372 [PMID: 24633240 DOI: 10.1038/nbt.2858]

42 **Shultz LD**, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol* 2007; 7: 118-130 [PMID: 17259968 DOI: 10.1038/nri2017]

43 **Shultz LD**, Lyons BL, Burzynski LM, Gott B, Chen X, Challef S, Korb M, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hematopoietic stem cells. *J Immunol* 2005; 174: 6477-6489 [PMID: 15879151 DOI: 10.4049/jimmunol.174.10.6477]

44 **Mcintosh BE**, Brown ME, Duffin BM, Mauffort JP, Vereide DT, Slukvin II, Thomson JA. Nonirradiated NOD,B6.SCID il2rg−/−Kit(W41/W41) (NSG) mice support multilineage engraftment of human hematopoietic cells. *Stem Cell Reports* 2015; 4: 171-180 [PMID: 25601207 DOI: 10.1016/j.stemcr.2014.12.005]

45 **Kleo JM**, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O’ Laughlin M, Fronick C, Magrini V, Demeter RT, Fulton RS, Eades WC, Link DC, Graubert TA, Walter MJ, Mardis ER, Dipersio JF, Wilson RK, Ley TJ. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell* 2014; 25: 379-392 [PMID: 24759668 DOI: 10.1016/j.ccr.2014.01.031]

46 **Abdul-Nabi AM**, Yassin ER, Varghese N, Deshmukh H, Yaseen NR. In vitro transformation of primary human CD34+ cells by AML fusion oncogenes: early gene expression profiling reveals possible drug target in AML. *PLoS One* 2010; 5: e12464 [PMID: 20805592 DOI: 10.1371/journal.pone.0012464]

47 **Wei J**, Wunderlich M, Fox C, Alvarez S, Cigudosa JC, Wilhelm JS, Zheng Y, Cancelas JA, Gu Y, Jansen M, Dimartino JF, Mulloy JC. Microenvironment determines lineage fate in a human model...
of MLL-AF9 leukemia. Cancer Cell 2008; 13: 483-495 [PMID: 18538732 DOI: 10.1016/j.ccr.2008.04.020]

53 Qin H, Malek S, Cowell JK, Ren M. Transformation of human CD34+ hematopoietic progenitor cells with DEK-NUP214 induces AML in an immunocompromised mouse model. Oncogene 2016; 35: 5686-5691 [PMID: 27065320 DOI: 10.1038/onc.2016.118]

54 Ren M, Qin H, Wu Q, Savage NM, George TI, Cowell JK. Development of ZMYM2-FGFR1 driven AML in human CD34+ cells in immunocompromised mice. Int J Cancer 2016; 139: 836-840 [PMID: 27005999 DOI: 10.1002/ijc.30100]

55 Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer 2015; 15: 311-316 [PMID: 25907221 DOI: 10.1038/nrc3944]

56 Yada E, Wada S, Yoshida S, Sasada T. Use of patient-derived xenograft mouse models in cancer research and treatment. Future Sci OA 2017; 4: FSO271 [PMID: 29568561 DOI: 10.4155/fs-oa-2017-0136]

57 Cho SY, Kang W, Han JY, Min S, Kang J, Lee A, Kwon JY, Lee C, Park H. An Integrative Approach to Precision Cancer Medicine Using Patient-Derived Xenografts. Mol Cells 2016; 39: 77-86 [PMID: 26831452 DOI: 10.14348/molcells.2016.2350]

58 Gelebart P, Popa M, McCormack E. Xenograft Models of Primary Acute Myeloid Leukemia for the Development of Imaging Strategies and Evaluation of Novel Targeted Therapies. Curr Pharm Biotechnol 2016; 17: 42-51 [PMID: 26278528 DOI: 10.2174/138920101666150817095703]

59 Mitchell A, Chen WC, McLeod J, Popescu AC, Arruda A, Minden MD, Dick JE, Wang JCY. Leukemic Engraftment In NOD/SCID Mice Is Correlated With Clinical Parameters and Predicts Outcome In Human AML. Blood 2013; 122: 50

60 Reinisch A, Hernandez DC, Schallmoser K, Majeti R. Generation and use of a humanized bone-marrow-ossicle niche for hematopoietic xenotransplantation into mice. Nat Protoc 2017; 12: 2169-2188 [PMID: 28933777 DOI: 10.1038/nprot.2017.088]

61 Carretta M, de Boer B, Jaques J, Antonelli A, Horton SJ, Yuan H, de Bruijn JD, Groen RWJ, Vellenga E, Schuringa JJ. Genetically engineered mesenchymal stromal cells produce IL-3 and TPO to further improve human scaffold-based xenograft models. Exp Hematol 2017; 51: 36-46 [PMID: 28456746 DOI: 10.1016/j.exphem.2017.04.008]

62 Vick B, Rothenberg M, Sandhöfer N, Carlet M, Finkenzeller C, Krukpa C, Grunert M, Trump M, Corbacioglu S, Ebinger M, Andre MC, Hiddemann W, Schneider S, Subklewe M, Metzeler KH, Spiekermann K, Jeremias I. An advanced preclinical mouse model for acute myeloid leukemia using patients’ cells of various genetic subgroups and in vivo bioluminescence imaging. PLoS One 2015; 10: e0120925 [PMID: 25793878 DOI: 10.1371/journal.pone.0120925]

P- Reviewer: Kiselev SL, Liu L, Scarfi S, Song YH
S- Editor: Wang JL
L- Editor: A
E- Editor: Tan WW
