MINI-REVIEW

How to Establish Acute Myeloid Leukemia Xenograft Models Using Immunodeficient Mice

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Abstract

The discovery of the immunodeficient mice has provided a tool for establishing animal models as hosts for *in vivo* analysis of AML. Various model systems have been established in the last few decades, and it is essential that murine AML models are developed to exploit more specific, targeted therapeutics. In this review, we concentrate on the models of AML and discuss the development of immunodeficiency models for understanding of leukemogenesis, describe those now available and their values and document the methods used for establishing and identifying AML mice models, as well as factors influencing engraftment of human AML in immunodeficient mice. Thus, the function of this article is to provide clinicians and experimentalists with a chronological, comprehensive appraisal of all AML model systems.

Keywords: SCID - NOD/SCID - acute myeloid leukemia (AML) - model

Introduction

Acute myeloid leukemia (AML) is the most common hematological malignancy in adults, characterized by anomalous proliferation and differentiation of malignant myeloid progenitors. The resultant progeny, or blasts exhibit early differential blocks and proliferate uncontrollably, resulting in a relentless accumulation of immature, abnormal hematopoietic cells in the bone marrow and peripheral blood (Li et al., 2012), and frequently lead to fatal infection, bleeding, or organ infiltration, with or without leukocytosis (Lowenberg et al., 1999; Estey and Döhner, 2006; Fritsche-Polanz et al., 2010). The annual incidence of AML was approximately 1.8 per 100 000 and progressively increased with age to a maximum prevalence of 17.2 per 100 000 adults of 65+ years of age (Ries et al., 2008). Despite modern advances in supportive care and therapeutics (Appelbaum et al., 2001; Trail et al., 2003) and improvement in the diagnosis of AML subtypes (Bennett et al., 1985; Bennett et al., 1989; Cheson et al., 1990; Casasnovas et al., 1998; Lowenberg et al., 1999), the rate of long-term survivors was greatly low.

Our understanding of leukemogenic disease pathogenesis has to a large extent been derived from several decades of research on human subjects (Appelbaum et al., 2001). Primary human AML cells could be isolated and studied in vitro, but many experimental questions could only be addressed using in vivo models. Thus the use of mice models of leukemia served the function of making the disease accessible to experimentation impossible in human

patients. Despite intensive effort from many laboratories around the world, none of the existing models was ideal. Considerable progress has been made by using a variety of complementary approaches. This review will emphasize the advantages and drawbacks of the human AML models established by using immunodeficient mice.
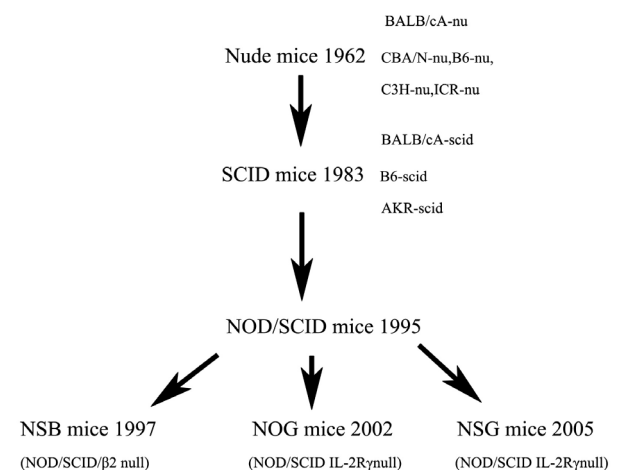

Figure 1. The History of the Development on Immunodeficient Mice for Establishing AML Mice Model. In retrospect, nude mice or SCID mice were the first immunodeficient strains. Subsequently, their strains were generated to improve engraftment capacities. NOD/SCID mice established in 1995 have been a milestone in this field. In early 2000s, NSB, NOG and NSG mice were established. Due to the complete loss of murine immune systems, these strains showed extremely high engraftment rates

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Immunodeficient mice

The history of the development on immunodeficient mice for establishing AML model was shown in Figure 1. The discoveries of nude and severe combined immunodeficiency (SCID) mice were key advances in the development of immunodeficient mice for xenotransplantation (Bosma et al., 1983; Pearce et al., 2006). SCID mice provided an attractive humanized model for research in AML. However, engraftment rates were not high. The development of non-obese diabetic NOD/SCID (NS) mice also contributed to the generation of humanized mice (Shultz et al., 1995; Koyanagi et al., 1997). NOD/Cg -Prkdc^{scid}B2m^{tm1Unc}/J (NSB) was subsequently derived from NS mice (Christianson et al., 1997). Compared to NS, absence of β2 microglobulin in NSB mice lead to a lack of MHC class I expression and decreased NK-cell function (Feuring-Buske et al., 2003; Kawano et al., 2005; Shultz et al., 2007). Since the early 2000s, a series of immunodeficient mice were developed by combining the IL-2Rcnull gene with conventional SCID mice. These strains showed extremely high engraftment rates, resulting in remarkable advances in the development of AML mice models. These strains included the NOD/Shi-Prkdc^{scid}Il2rγ^{tm1Sug}/Jic (NOG) mice reported in 2002 (Ninomiya et al., 2006); and NOD/LtSz-Prkdc^{scid} Il2rγ^{tm1Wjl}/J (NSG) mice reported in 2005 (Shultz et al., 2005). Compared to NS, the IL-2Rg-chain deficiency in NSG mice impaired the signaling through multiple cytokine receptors blocking NK development and resulted in additional defects in innate immunity (Shultz et al., 2005; Shultz et al., 2007). Data from Agliano et al suggested that NOG and NSG mice were the best recipients for cell engraftment (Agliano et al., 2008; Brehm et al., 2010). Today, SCID and NOD/SCID strains have been commonly used for establishing AML models, especially the NOD/SCID strains. Nude mice were rarely used in recent years.

Xenograft Models

Nude models

Nude mice were first described in 1962 (Kim et al., 2004). Initial attempts to xenograft human (hu) AML focussed on subcutaneous transplantation of bone marrow into heavily immunocompromised mice (Palu et al., 1979) and athymic nude (nu) mice lacking functional T and B cells (Flanagan, 1966). However, transplantation of leukemic haematopoietic tissues proved difficult to propagate in these models (Nara and Miyamoto, 1982; Caretto et al., 1989). Even with heavy immunosuppression of nu mice with radiation (Watanabe et al., 1980; Nara and Miyamoto, 1982; Caretto et al., 1989), drugs (Fingert et al., 1987), antibodies (Caretto et al., 1989) and in some cases splenectomy (SI) (Watanabe et al., 1980; Caretto et al., 1989), xenografting of myeloid leukaemias proved inconsistent and unreliable. Subsequent studies showed that greater success was achieved (YAMADA et al., 1983; Machado et al., 1984; Potter et al., 1984; Janssen et al., 1987; Nguyen et al., 1993; Thacker and Hogge, 1994; Kiser et al., 2001; Ozpolat et al., 2003). The major

limitation of this initial model was reproducible growth only as localised myelosarcomas, little evidence of bone marrow engraftment (Nilsson et al., 1977). Efforts to develop further immunodeficient models resulted in the generation of mice which were beige (Lystbg, and henceforth bg) and X-linked immunodeficiency (Btkxid, and henceforth xid) (Kamel-Reid and Dick, 1988; Dick et al., 1991a; Mulé et al., 1992). Although engraftment of human myeloid tissues using bg/nu/xid mice suggested seeding, proliferation and differentiation of human stem cells (Kamel-Reid and Dick, 1988), later efforts to engraft human peripheral blood lymphocytes (hu-PBLs) proved difficult (Mosier et al., 1991; Pollock et al., 1994). Thus a further immunodeficient model should be generated through our efforts.

SCID models

In 1983 Bosma et al. reported a SCID mice which was deficient in mature B and T lymphocytes (Bosma et al., 1983). Germ line DNA segments encoding immunoglobulin (Ig) and T lymphocyte antigen receptor molecules fail to undergo rearrangement in the SCID mouse, resulting in lacking functional antigen receptors (Fell and Preston, 1993). Several studies demonstrated that the SCID model had advantages over the nude model (Kawata et al., 1994; Paine-Murrieta et al., 1997). SCID mice had been proposed as a useful model for studying normal and neoplastic hematopoiesis in vivo (Dick et al., 1991b). The first successful report of engraftment of hu-AML into SCID recipients was reported in 1991 (De Lord et al., 1991). Because of innate host resistance and lacking species cross reactivity in cytokines, consequent studies (De Lord et al., 1991; Cesano et al., 1992; Ratajczak et al., 1992; Namikawa et al., 1993; Cesano et al., 1994; Chelstrom et al., 1994; Lapidot et al., 1994; Pirruccello et al., 1994; Yan et al., 1996) were eclipsed by major practical and limiting considerations. While giving exogenous human growth factors, including interleukin 3 (IL-3) (Goan et al., 1995; Cashman et al., 1997), IL-6 (Goan et al., 1995), granulocyte-macrophage-colony stimulating factor (GM-CSF) (Goan et al., 1995; Cashman et al., 1997), a fusion protein of IL-3 and GM-CSF (PIXY321) (Lapidot et al., 1992; Lapidot et al., 1994), steel factor (SF) (Cashman et al., 1997), mast-cell growth factor (hMGF) (Lapidot et al., 1994) and erythropoietin (EPO) (Cashman et al., 1997) to these recipients, resulted in enhanced engraftment and differentiation of human cells in SCID recipients. However, the SCID mice might occasionally express mature B and T lymphocytes indicating "leakiness" for the SCID defect (Bosma et al., 1988; Carroll et al., 1989). The defect did not affect myeloid, antigen-presenting and natural killer (NK) cells (Shultz, 1991). All these factors abrogated xenograft potential in this model. Although enhanced engraftment resulted from purging NK cells from SCID recipients through the pretreatment with radiation and/or anti-asialo-GM1 antibody (Kudo et al., 1993; Shpitz et al., 1994; Sandhu et al., 1996a; Sandhu et al., 1996b), which only increased short-term engraftment but shortened survival of the recipient. Nevertheless, further development of this immunodeficient mice led to generation of the NOD/SCID strains.

NOD/SCID models

NOD/SCID mice, which were created by back-crossing the SCID gene onto the NOD background, exhibited multiple defects in adaptive and innate immunologic function. These mice were not only lack of functional T and B lymphocytes but also had impaired NK and antigen-presenting cell function. Additionally, NOD/SCID mice were both insulitis- and diabetes-free throughout life (Shultz et al., 1995). Subsequent transplant of NOD/SCID mice resulted in appreciably higher engraftment rates using fewer cells (Dick, 1996a; Bonnet and Dick, 1997), with ensuing xenografts preserving observed morphological, phenotypical, genotypical and biological characteristics of the donors AML cells (Dick, 1996b; Blair et al., 1998; Ailles et al., 1999; Rombouts et al., 2000a; Lumkul et al., 2002; Marx, 2003). Cells from patients with poor prognostic markers (e.g. high WBC, poor risk cytogenetics, FLT3-ITD positive, high CD34 expression) engrafted more efficiently in NOD/SCID mice than general AML cases (Ailles et al., 1999; Kondo et al., 1999; Rombouts et al., 2000a; Rombouts et al., 2000b; Lumkul et al., 2002; Pearce et al., 2006). However, there was a report showed that high WBC and FLT3-ITD were not predictors of engraftment success (Pearce et al., 2006). In their study, nucleophosmin mutational status also had no apparent impact on the success of engraftment (Pearce et al., 2006).

Despite the obvious advantages of NOD/SCID model over all other immunodeficient models, there were still some inherent obstacles. The disadvantages of NOD/SCID mice included the frequent occurrence of thymic lymphoma and discernable NK cell activity (Shultz et al., 1995), which hindered execution of enduring engraftment. While various strategies had been evaluated co-transplantation of growth, only 70% of all AML samples exhibited detectible engraftment in NOD/SCID mice (Terpstra et al., 1997; Ailles et al., 1999; Bonnet et al., 1999; Rombouts et al., 2000b; Nitsche et al., 2003). The shortened life span of this strain (mean 8.5 months) also limited the duration of tumor watches (Ishikawa et al., 2007). However, NOG and NSG mice showed no leakiness or spontaneous thymic lymphoma, but prolonged the life span of mice (Ishikawa et al., 2007; Kato et al., 2009; Katano et al., 2011; Woiterski et al., 2013). This might be attributed to inactivation of IL-2Rc. Some researchs (Agliano et al., 2008; Brehm et al., 2010; Woiterski et al., 2013) indicated that NOG and NSG mice were better recipients of AML cells.

Xenogeneic Transplantation and Assessment of Engraftment

All animals used for xenogeneic transplantation had some impairment in their immune system and might succumb to infections not affecting normal mice. Thus mice should be kept in a specific pathogen-free environment within barrier systems to protect them from intercurrent infections.

Before cells were inoculated, SCID and NOD/SCID strains were usually pretreated by cyclophosphamide (CTX) or irradiationg to improve engraftment rates. Conditioning with CTX had often been used in doses 100mg/kg (Rombouts et al., 2000b; Tan et al., 2012), and it had also been used in doses 20mg/kg (Zhang et al., 2012a). The dose of total body irradiation (TBI) depended on the mouse strains and also on the source and irradiator. It usually varied from 2 Gy to 4Gy (Chelstrom et al., 1994; Tavor et al., 2004; Liu et al., 2007; Sanchez et al., 2009). Mice should be kept in a specific pathogen-free environment with acidified water at least one week before receiving pretreatment. Transplantation of AML cells was done at lest 24h later after the pretreatment (Liu et al., 2007; Zhang et al., 2012b). In a typical transplant, $5–40 \times 10^6$ cells of AML were often injected into mice (Fortier and Graubert, 2010). Woiterski et al reported that the engraftment levels itself did not correlate with the number of injected blasts (Woiterski et al., 2013). Diverse routes of administration of cells had been employed, including subcutaneous (s.c.), intraperitoneal (i.p.) and intravenous (i.v.) routes (Kiser et al., 2001; Zhang et al., 2012a; Zhang et al., 2012b). In recent years, Wang et al (Wang et al., 2012) reported a successful engraftment of human acute lymphoblastic leukemia cells in NOD/SCID mice via intrasplenic inoculation. In addition, Chen et al (Chen et al., 2012) highlighted the potential of a novel in vivo model of human BM microenvironment that could be genetically modified.

Mice should be monitored daily after transplantation of the AML cells, and we could observe the changes of body weight and the number of WBC and leukemia cells among peripheral blood every week. Mice might be culled if they showed signs of ill health, such as piloerection, hunched posture, inactivity or inappetence for a period of 48 h. In addition, any animal that lost 20% body weight or that developed more serious clinical signs, such as diarrhoea or dyspnoea should also be sacrificed (Bonnet, 2009). Peripheral blood and tissues including bone marrow, solid tumors, spleen, liver and kidney were collected from the mice. Peripheral blood samples were then smeared onto glass microscope slides and stained using Wright's stain or analysed by flow cytometry to monitor the percentages of immature granular leukocytes and monocytes (Na et al., 2000; Henschler et al., 2005). Tissues were fixed in 4% formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin or using immunohistochemistry to check for the presence or absence of tumor cells under the microscope (Liu et al., 2007; Agliano et al., 2008; Sanchez et al., 2009). To determine the quantitative level of engraftment, tissues could be also flushed in PBS and made into single cell suspensions for flow cytometry analysis (Sanchez et al., 2009). The percentage of engraftment was determined as the number of AML cells over the total number of viable cells harvested per mouse (Sanchez et al., 2009). However, flow cytometry could only detect human leukemia cells as 0.1–0.5% of the cells. Thus we could further confirm human engraftment by PCR (Agliano et al., 2008). Furthermore, we could select fluorescent in situ hybridization (FISH) to detect the presence of cytogenetic abnormalities characteristic of the leukemic cells (Ailles et al., 1997).

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Table 1. The Factors Influencing Engraftment of Human AML in the Immunodeficient Mice

Factors	Research results
Mice strains	NSG or NOG>NSB>NS>SCID
FAB subtypes	M3 or M2<other subtypes; M0>M2 or M4 or M5
Routes of cell administration	i.v.>i.p.
Prognostic	Poorer prognosis engraft better, such as high WBC, FLT3-ITD positive, high CD34 expression and relapse
CXCR4	Not critical for AML CD34+ cells
Sex of mice	Male recipients>Female recipients

i.p, intraperitoneal ; i.v, intravenous

Factors influencing Engraftment of Human AML in Immunodeficient Mice

There were several factors influencing engraftment of human AML in the immunodeficient mice (Table 1). Firstly, engraftment of human AML was linked with the characteristic of immunodeficient mice. Data accumulated to date suggested that NOG and NSG mice were the best recipients for humanized tissue and human cell engraftment occurred in the following order: NSG or NOG>NSB>NS>SCID (Greiner et al., 1995; Lapidot et al., 1997; Feuring-Buske et al., 2003; Agliano et al., 2008; Snchez et al., 2009). Although NOD/SCID strains had been widely used in recent years, we also considered the SCID mice to be a valuable system for the evaluation of new therapies for AML (Tan et al., 2012; Zhang et al., 2012a). The second factor determining the engraftment potential of human AML was FAB subtypes. Cells with FAB subtypes M3 and, to a lesser extent, M2, engrafted more poorly than those from other subtypes (Ailles et al., 1999). Rombouts et al reported that a significantly higher engraftment was observed in the FAB class M0 than in FAB classes M2, M4 and M5 (Rombouts et al., 2000b). However, there was also a research reporting that engraftment did not correlate with FAB subtype (Sanchez et al., 2009). Thirdly, engraftment potential of AML was associated with routes of administration of the cells. The i.v. route generally resulted in higher levels of engraftment than the i.p. route (Terpstra et al., 1995; Lumkul et al., 2002). Another factor influencing engraftment potential of AML was a poorer prognosis of AML patients. Samples from patients with a poorer prognosis tended to engraft better (Ailles et al., 1999; Rombouts et al., 2000b; Yan et al., 2009). Adverse prognostic features, such as high WBC, FLT3-ITD positive, high CD34 expression, relapse (Giavazzi et al., 1995; Rombouts et al., 2000b; Lumkul et al., 2002; Sanchez et al., 2009), were also associated with an enhanced engraftment potential in AML model. However, high WBC, relapse and FLT3-ITD were not predictors of engraftment success in other studies (Ailles et al., 1999; Pearce et al., 2006). Pearce et al reported that the ability of AML to engraft in the NOD/SCID assay seemed to be an inherent property of AML cells, independent on homing, conditioning, or cell frequency/source (Pearce et al., 2006). Further studies were needed to investigate whether these adverse prognostic features were linked with enhanced engraftment. Additionally, CXCR4 also influenced engraftment of human AML in the immunodeficient mice. CXCR4 played a important role in the control of human AML cell trafficking and disease progression, and CXCR4 antibodies or CXCR4 inhibitors blocked AML cells homing into the BM and spleen of transplanted mice (Tavor et al., 2004; Zhang et al., 2012b). Another study demonstrated that CXCR4 was not critical for the engraftment of AML CD34+ cells in NOD/SCID mice (Monaco et al., 2004). The last factor influencing engraftment potential of AML was the sex of mice. There were two studies showing that male recipients conferred significantly higher engraftment levels than female recipients (Martin-Padura et al., 2010; Woiterski et al., 2013).

Conclusions

There have been significant advances in the understanding of AML models. Contemporary xenographic murine model systems have been critical to our comprehension and treatment of AML. Over the last decades, remarkable progress has been achieved in AML mouse models. Various mouse models have been established and enabled direct research of human AML, which was previously impossible in immunocompetent mice. These models have certainly been fundamental to our current understanding of leukemogenesis. However, they may not be sufficient for the further development necessary in the evolution of more optimal AML models (Nicolini et al., 2003; Ahmed et al., 2004; Avigdor et al., 2004; Perez et al., 2004; Spiegel et al., 2004). With many recent failures of new therapeutic strategies, we must question the current application and validity of these model systems. Furthermore, these in vitro models could not precisely recapitulate the in vivo setting of human AML.

It is essential that current murine AML models are further developed by revolutions of molecular biological and genomic eras to exploit more specific, targeted therapeutics. Due to the failure of significant numbers of new drugs in late clinical trials, it is necessary to use of more salient, higher throughput, prognostic preclinical animal models. Combining the use of such models should also offer more pertinent preclinical data, thus reducing current expenditure on drug development and afford greater clinical success.

References

Agliano A, Martin-Padura I, Mancuso P, et al (2008). Human acute leukemia cells injected in NOD/LtSz-scid/IL-2Rγ null mice generate a faster and more efficient disease compared to other NOD/scid-related strains. *Int J Cancer*, **123**, 2222-7.

Ahmed F, Ings SJ, Pizzey AR, et al (2004). Impaired bone marrow homing of cytokine-activated CD34+ cells in the NOD/SCID model. *Blood*, **103**, 2079-87.

Ailles LE, Gerhard B, Hogge DE (1997). Detection and characterization of primitive malignant and normal progenitors in patients with acute myelogenous leukemia using long-term coculture with supportive feeder layers and cytokines. *Blood*, **90**, 2555-64.

Ailles LE, Gerhard B, Kawagoe H, et al (1999). Growth characteristics of acute myelogenous leukemia progenitors that initiate malignant hematopoiesis in nonobese diabetic/severe combined immunodeficient mice. *Blood*, **94**, 1761-72.

Appelbaum FR, Rowe JM, Radich J, et al (2001). Acute myeloid leukemia. *Hematol Am Soc Hematol Educ Program*, **2001**, 62-86.

Avigdor A, Goichberg P, Shivtiel S, et al (2004). CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. *Blood*, **103**, 2981-9.

Bennett J, Catovsky D, Daniel M, et al (1989). Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. French-American-British (FAB) Cooperative Group. *Am J Clin Pathol*, **42**, 567-84.

Bennett JM, Catovsky D, Daniel MT, et al (1985). Proposed revised criteria for the classification of acute myeloid leukemia A report of the French-American-British Cooperative Group. *Ann Intern Med*, **103**, 620-5.

Blair A, Hogge D, Sutherland H (1998). Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34+/CD71–/HLA-DR–. *Blood*, **92**, 4325-35.

Bonnet D. Humanized model to study leukemic stem cells. Leukemia, 2009. p. 247-62.

Bonnet D, Bhatia M, Wang J, et al (1999). Cytokine treatment or accessory cells are required to initiate engraftment of purified primitive human hematopoietic cells transplanted at limiting doses into NOD/SCID mice. *Bone Marrow Transplant*, **23**, 203.

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

Bosma GC, Custer RP, Bosma MJ (1983). A severe combined immunodeficiency mutation in the mouse. *Nature*, **301**, 527-30.

Bosma GC, Fried M, Custer RP, et al (1988). Evidence of functional lymphocytes in some (leaky) scid mice. *J Exp Med*, **167**, 1016-33.

Brehm MA, Cuthbert A, Yang C, et al (2010). Parameters for establishing humanized mouse models to study human immunity: Analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rγnull mutation. *Clin Immunol*, **135**, 84-98.

Caretto P, Forni M, d'Orazi G, et al (1989). Xenotransplantation in immunosuppressed nude mice of human solid tumors and acute leukemias directly from patients orin vitro cell lines. *Ric Clin Lab*, **19**, 231-43.

Carroll A, Hardy R, Bosma M (1989). Occurrence of mature B (IgM+, B220+) and T (CD3+) lymphocytes in scid mice. *J Immunol*, **143**, 1087-93.

Casasnovas R, Campos L, Mugneret F, et al (1998). Immunophenotypic patterns and cytogenetic anomalies in acute non-lymphoblastic leukemia subtypes: a prospective study of 432 patients. *Leukemia*, **12**, 34-43.

Cashman JD, Lapidot T, Wang JC, et al (1997). Kinetic evidence of the regeneration of multilineage hematopoiesis from primitive cells in normal human bone marrow transplanted into immunodeficient mice. *Blood*, **89**, 4307-16.

Cesano A, Hoxie J, Lange B, et al (1992). The severe combined immunodeficient (SCID) mouse as a model for human myeloid leukemias. *Oncogene*, **7**, 827.

Cesano A, Visonneau S, Cioe L, et al (1994). Reversal of acute myelogenous leukemia in humanized SCID mice using a novel adoptive transfer approach. *Eur J Clin Invest*, **94**, 1076.

Chelstrom LM, Gunther R, Simon J, et al (1994). Childhood acute myeloid leukemia in mice with severe combined immunodeficiency. *Blood*, **84**, 20-6.

Chen Y, Jacamo R, Shi Y-x, et al (2012). Human extramedullary bone marrow in mice: a novel in vivo model of genetically controlled hematopoietic microenvironment. *Blood*, **119**, 4971-80.

Cheson BD, Cassileth PA, Head DR, et al (1990). Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *J Clin Oncol*, **8**, 813-9.

Christianson SW, Greiner DL, Hesselton RA, et al (1997). Enhanced human CD4+ T cell engraftment in beta2-microglobulin-deficient NOD-scid mice. *J Immunol*, **158**, 3578-86.

De Lord C, Clutterbuck R, Titley J, et al (1991). Growth of primary human acute leukemia in severe combined immunodeficient mice. *Exp Hematol*, **19**, 991.

Dick JE (1996a). Human stem cell assays in immune-deficient mice. *Curr Opin Hematol*, **3**, 405-9.

Dick JE (1996). Normal and leukemic human stem cells assayed in SCID mice. *Semin Immunol*, **8**, 197-206.

Dick JE, Kamel-Reid S, Murdoch B, et al (1991a). Gene transfer into normal human hematopoietic cells using in vitro and in vivo assays. *Blood*, **78**, 624-34.

Dick JE, Lapidot T, Pflumio F (1991b). Transplantation of normal and leukemic human bone marrow into immune-deficient mice: development of animal models for human hematopoiesis. *Immunol Rev*, **124**, 25-43.

Estey E, Döhner H (2006). Acute myeloid leukaemia. *J Lancet*, **368**, 1894-907.

Fell A, Preston P (1993). Proliferation of Theileria annulata and Theileria parva macroschizont-infected bovine cells in scid mice. *Int J Parasitol*, **23**, 77-87.

Feuring-Buske M, Gerhard B, Cashman J, et al (2003). Improved engraftment of human acute myeloid leukemia progenitor cells in beta 2-microglobulin-deficient NOD/SCID mice and in NOD/SCID mice transgenic for human growth factors. *Leukemia*, **17**, 760-3.

Fingert HJ, Chen Z, Mizrahi N, et al (1987). Rapid growth of human cancer cells in a mouse model with fibrin clot subrenal capsule assay. *Cancer Res*, **47**, 3824-9.

Flanagan S (1966). 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genet Res*, **8**, 295-309.

Fortier JM, Graubert TA. Murine models of human acute myeloid leukemia. *Cancer Treat Res*, 2010. p. 183-96.

Fritsche-Polanz R, Fritz M, Huber A, et al (2010). High frequency of concomitant mastocytosis in patients with acute myeloid leukemia exhibiting the transforming KIT mutation D816V. *Mol Oncol*, **4**, 335-46.

Giavazzi R, Berardino CD, Garofalo A, et al (1995). Establishment of human acute myelogenous leukemia lines secreting interleukin-1β in SCID mice. *Int J Cancer*, **61**, 280-5.

Goan S-R, Fichtner I, Just U, et al (1995). The severe combined immunodeficient-human peripheral blood stem cell (SCID-huPBSC) mouse: a xenotransplant model for huPBSC-initiated hematopoiesis. *Blood*, **86**, 89-100.

Greiner DL, Shultz LD, Yates J, et al (1995). Improved engraftment of human spleen cells in NOD/LtSz-scid/scid mice as compared with CB-17-scid/scid mice. *Am J Pathol*,

146, 888-902.

Henschler R, Göttig S, Junghahn I, et al (2005). Transplantation of human acute myeloid leukemia (AML) cells in immunodeficient mice reveals altered cell surface phenotypes and expression of human endothelial markers. *Leuk Res*, **29**, 1191-9.

Ishikawa F, Yoshida S, Saito Y, et al (2007). Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol*, **25**, 1315-21.

Janssen J, Steenvoorden A, Losekoot M, et al (1987). Novel transforming sequences in human acute myelocytic leukemia cell lines. *Oncogene*, **1**, 175-9.

Kamel-Reid S, Dick JE (1988). Engraftment of immune-deficient mice with human hematopoietic stem cells. *Science*, **242**, 1706-9.

Katano I, Ito R, Eto T, et al (2011). Immunodeficient NOD-scid IL-2Rγ null Mice Do Not Display T and B Cell Leakiness. *Exp Anim*, **60**, 181-6.

Kato C, Fujii E, Chen YJ, et al (2009). Spontaneous thymic lymphomas in the non-obese diabetic/Shi-scid, IL-2Rγnull mouse. *Lab Anim*, **43**, 402-4.

Kawano N, Ishikawa F, Shimoda K, et al (2005). Efficient engraftment of primary adult T-cell leukemia cells in newborn NOD/SCID/β2-microglobulinnull mice. *Leukemia*, **19**, 1384-90.

Kawata A, Yoshida M, Okazaki M, et al (1994). Establishment of new SCID and nude mouse models of human B leukemia/lymphoma and effective therapy of the tumors with immunotoxin and monoclonal antibody: marked difference between the SCID and nude mouse models in the antitumor efficacy of monoclonal antibody. *Cancer Res*, **54**, 2688-94.

Kim JB, O Hare MJ, Stein R (2004). Models of breast cancer: is merging human and animal models the future? *Breast Cancer Res*, **6**, 22-30.

Kiser M, McCubrey J, Steelman L, et al (2001). Oncogene-dependent engraftment of human myeloid leukemia cells in immunosuppressed mice. *Leukemia*, **15**, 814-8.

Kondo M, Horibe K, Takahashi Y, et al (1999). Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. *Med Pediatr Oncol*, **33**, 525-9.

Koyanagi Y, Tanaka Y, Tanaka R, et al (1997). High levels of viremia in hu-PBL-NOD-scid mice with HIV-1 infection. *Leukemia*, **11**, 109-12.

Kudo T, Saijyo S, Saeki H, et al (1993). Production of a human monoclonal antibody to a synthetic peptide by active in vivo immunization using a SCID mouse grafted with human lymphocytes. *Tohoku J Exp Med*, **171**, 327.

Lapidot T, Fajerman Y, Kollet O (1997). Immune-deficient SCID and NOD/SCID mice models as functional assays for studying normal and malignant human hematopoiesis. *J Mol Med*, **75**, 664-73.

Lapidot T, Pflumio F, Doedens M, et al (1992). Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. *Science*, **255**, 1137-41.

Lapidot T, Sirard C, Vormoor J, et al (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*, **367**, 645-8.

Li P, Ji M, Park J, et al (2012). Th17 related cytokines in acute myeloid leukemia. *Front Biosci*, **17**, 2284.

Liu J, Li Y, Tang L, et al (2007). Treatment of lycorine on SCID mice model with human APL cells. *Biomed Pharmacother*, **61**, 229-34.

Lowenberg B, Downing JR, Burnett A (1999). Acute myeloid leukemia. *N Engl J Med*, **341**, 1051-62.

Lumkul R, Gorin N, Malehorn M, et al (2002). Human AML cells in NOD/SCID mice: engraftment potential and gene expression. *Leukemia*, **16**, 1818-26.

Machado EA, Gerard DA, Lozzio CB, et al (1984). Proliferation and differentiation of human myeloid leukemic cells in immunodeficient mice: electron microscopy and cytochemistry. *Blood*, **63**, 1015-22.

Martin-Padura I, Agliano A, Marighetti P, et al (2010). Sex-related efficiency in NSG mouse engraftment. *Blood*, **116**, 2616-7.

Marx J (2003). Mutant stem cells may seed cancer. *Science*, **301**, 1308-10.

Monaco G, Konopleva M, Munsell M, et al (2004). Engraftment of acute myeloid leukemia in NOD/SCID mice is independent of CXCR4 and predicts poor patient survival. *Stem Cells*, **22**, 188-201.

Mosier DE, Gulizia RJ, Torbett BE, et al (1991). Break for SCIDS. *Nature*, **353**, 509.

Mulé JJ, Jicha DL, Rosenberg SA (1992). The use of congenitally immunodeficient mice to study human tumor metastases and immunotherapy. *J Immunother*, **12**, 196-8.

Na X, He Q, Jiang D (2000). Effect of a novel aminosteroid on animal model of murine myelomonocytic leukemia]. *Hunan Yi Ke Da Xue Xue Bao*, **25**, 12.

Namikawa R, Ueda R, Kyoizumi S (1993). Growth of human myeloid leukemias in the human marrow environment of SCID-hu mice. *Blood*, **82**, 2526-36.

Nara N, Miyamoto T (1982). Direct and serial transplantation of human acute myeloid leukaemia into nude mice. *Br J Cancer*, **45**, 778.

Nguyen HQ, Hoffman-Liebermann B, Liebermann DA (1993). The zinc finger transcription factor Egr-1 is essential for and restricts differentiation along the macrophage lineage. *Cell*, **72**, 197-209.

Nicolini F, Cashman J, Hogge D, et al (2003). NOD/SCID mice engineered to express human IL-3, GM-CSF and Steel factor constitutively mobilize engrafted human progenitors and compromise human stem cell regeneration. *Leukemia*, **18**, 341-7.

Nilsson K, Giovanella BC, Stehlin JS, et al (1977). Tumorigenicity of human hematopoietic cell lines in athymic nude mice. *Int J Cancer*, **19**, 337-44.

Ninomiya M, Abe A, Katsumi A, et al (2006). Homing, proliferation and survival sites *of* human leukemia cells in vivo in immunodeficient mice. *Leukemia*, **21**, 136-42.

Nitsche A, Junghahn I, Thulke S, et al (2003). Interleukin-3 promotes proliferation and differentiation of human hematopoietic stem cells but reduces their repopulation potential in NOD/SCID mice. *Stem Cells*, **21**, 236-44.

Ozpolat B, Lopez-Berestein G, Adamson P, et al (2003). Pharmacokinetics of intravenously administered liposomal all-trans-retinoic acid (ATRA) and orally administered ATRA in healthy volunteers. *J Pharm Pharm Sci*, **6**, 292-301.

Paine-Murrieta GD, Taylor CW, Curtis RA, et al (1997). Human tumor models in the severe combined immune deficient (scid) mouse. *Cancer Chemother Pharmacol*, **40**, 209-14.

Palu G, Selby P, Powles R, et al (1979). Spontaneous regression of human acute myeloid leukaemia xenografts and phenotypic evidence for maturation. *Br J Cancer*, **40**, 731.

Pearce DJ, Taussig D, Zibara K, et al (2006). AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. *Blood*, **107**, 1166-73.

Perez LE, Alpdogan O, Shieh J-H, et al (2004). Increased plasma levels of stromal-derived factor-1 (SDF-1/CXCL12) enhance human thrombopoiesis and mobilize human colony-forming cells (CFC) in NOD/SCID mice. *Exp Hematol*, **32**, 300-7.

Pirruccello SJ, Jackson JD, Sharp JG (1994). The leukemic myeloid cell line OMA-AML-1: an in vitro model of

hematopoietic cell differentiation. *Leuk Lymphoma*, **13**, 169-78.

Pollock PL, Germolec DR, Comment CE, et al (1994). Development of human lymphocyte-engrafted SCID mice as a model for immunotoxicity assessment. *Toxicol Sci*, **22**, 130-8.

Potter G, Shen RN, Chiao J (1984). Nude mice as models for human leukemia studies. *Am J Pathol*, **114**, 360.

Ratajczak MZ, Kant JA, Luger SM, et al (1992). In vivo treatment of human leukemia in a scid mouse model with c-myb antisense oligodeoxynucleotides. *Proc Natl Acad Sci*, **89**, 11823-7.

Ries L, Melbert D, Krapcho M, et al (2008). SEER cancer statistics review, 1975-2005. *Natl Cancer Inst*, 1975-2005.

Rombouts W, Blokland I, Lowenberg B, et al (2000a). Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the Flt3 gene. *Leukemia*, **14**, 675-83.

Rombouts W, Martens A, Ploemacher R (2000b). Identification of variables determining the engraftment potential of human acute myeloid leukemia in the immunodeficient NOD/SCID human chimera model. *Leukemia*, **14**, 889-97.

Sanchez P, Perry R, Sarry J, et al (2009). A robust xenotransplantation model for acute myeloid leukemia. *Leukemia*, **23**, 2109-17.

Sandhu J, Boynton E, Gorczynski R, et al (1996a). The use of SCID mice in biotechnology and as a model for human disease. *Crit Rev Biotechnol*, **16**, 95-118.

Sandhu JS, Clark B, Boynton E, et al (1996b). Human hematopoiesis in SCID mice implanted with human adult cancellous bone. *Blood*, **88**, 1973-82.

Shpitz B, Chambers CA, Brij Singhal A, et al (1994). High level functional engraftment of severe combined immunodeficient mice with human peripheral blood lymphocytes following pretreatment with radiation and anti-asialo G< sub> M1</ sub>. *J Immunol Methods*, **169**, 1-15.

Shultz LD (1991). Immunological mutants of the mouse. *Am J Anat*, **191**, 303-11.

Shultz LD, Ishikawa F, Greiner DL (2007). Humanized mice in translational biomedical research. *Nat Rev Immunol*, **7**, 118-30.

Shultz LD, Lyons BL, Burzenski LM, et al (2005). Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2Rγnull mice engrafted with mobilized human hemopoietic stem cells. *J Immunol*, **174**, 6477-89.

Shultz LD, Schweitzer PA, Christianson SW, et al (1995). Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J Immunol*, **154**, 180-91.

Spiegel A, Kollet O, Peled A, et al (2004). Unique SDF-1– induced activation of human precursor-B ALL cells as a result of altered CXCR4 expression and signaling. *Blood*, **103**, 2900-7.

Tan K-B, Ling L-U, Bunte RM, et al (2012). < i> In vivo</i> efficacy of a novel liposomal formulation of safingol in the treatment of acute myeloid leukemia. *J Control Release*, **160**, 290-8.

Tavor S, Petit I, Porozov S, et al (2004). CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. *Cancer Res*, **64**, 2817-24.

Terpstra W, Leenen P, Van Den Bos C, et al (1997). Facilitated engraftment of human hematopoietic cells in severe combined immunodeficient mice following a single injection of Cl2MDP liposomes. *Leukemia*, **11**, 1049-54.

Terpstra W, Prins A, Visser T, et al (1995). Conditions for engraftment of human acute myeloid leukemia (AML) in SCID mice. *Leukemia*, **9**, 1573-7.

Thacker J, Hogge D (1994). Cytokine-dependent engraftment of human myeloid leukemic cell lines in immunosuppressed nude mice. *Leukemia*, **8**, 871-7.

Trail PA, King DH, Dubowchik GM (2003). Monoclonal antibody drug immunoconjugates for targeted treatment of cancer. *Cancer Immunol Immunother*, **52**, 328-37.

Wang N, Huang L, Wang D, et al (2012). Successful engraftment of human acute lymphoblastic leukemia cells in NOD/SCID mice via intrasplenic inoculation. *Cancer Biol Ther*, **13**, 1158-64.

Watanabe S, Shimosato Y, Kuroki M, et al (1980). Transplantability of human lymphoid cell line, lymphoma, and leukemia in splenectomized and/or irradiated nude mice. *Cancer Res*, **40**, 2588-95.

Woiterski J, Ebinger M, Witte KE, et al (2013). Engraftment of low numbers of pediatric acute lymphoid and myeloid leukemias into NOD/SCID/IL2Rcγnull mice reflects individual leukemogenecity and highly correlates with clinical outcome. *Int J Cancer*, **133**, 1547-56.

YAMADA M, Midori M, SUGIMURA T (1983). Myeloperoxidases of human myeloid leukemia cells HL-60 grown in culture and in nude mice. *J Biochem*, **93**, 1661-8.

Yan Y, Salomon O, McGuirk J, et al (1996). Growth pattern and clinical correlation of subcutaneously inoculated human primary acute leukemias in severe combined immunodeficiency mice. *Blood*, **88**, 3137-46.

Yan Y, Wieman EA, Guan X, et al (2009). Autonomous growth potential of leukemia blast cells is associated with poor prognosis in human acute leukemias. *J Hematol Oncol*, **2**, 51.

Zhang S, Zhang Y, Zhuang Y, et al (2012a). Matrine induces apoptosis in human acute myeloid leukemia cells via the mitochondrial pathway and Akt inactivation. *PloS One*, **7**, e46853.

Zhang Y, Patel S, Abdelouahab H, et al (2012b). CXCR4 inhibitors selectively eliminate CXCR4-expressing human acute myeloid leukemia cells in NOG mouse model. *Cell Death Dis*, **3**, e396.