

# The Impact of the Cellular Origin in Acute Myeloid Leukemia: Learning From Mouse Models

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## Abstract

Acute myeloid leukemia (AML) is a genetically heterogeneous disease driven by a limited number of cooperating mutations. There is a long-standing debate as to whether AML driver mutations occur in hematopoietic stem or in more committed progenitor cells. Here, we review how different mouse models, despite their inherent limitations, have functionally demonstrated that cellular origin plays a critical role in the biology of the disease, influencing clinical outcome. AML driven by potent oncogenes such as mixed lineage leukemia fusions often seem to emerge from committed myeloid progenitors whereas AML without any major cytogenetic abnormalities seem to develop from a combination of preleukemic initiating events arising in the hematopoietic stem cell pool. More refined mouse models may serve as experimental platforms to identify and validate novel targeted therapeutic strategies.

## Introduction

Acute myeloid leukemia (AML) is characterized by an accumulation of poorly differentiated myeloid cells and functional insufficiency of the hematopoietic system. Despite continuous advances in treatment, the majority of the patients still relapse and ultimately die of the disease.<sup>1</sup> AML is a clinically and genetically heterogeneous disease driven by functional cooperation of a relatively small number of mutations.<sup>2</sup> In addition to genetics and other factors, such as the patient's age and health status, the observed heterogeneity may also be the consequence of different cellular origins. It was the shift from a purely stochastic model toward a more hierarchical organization model of leukemia driven by a small population of cells, also referred as leukemia-initiating cells (LIC) or leukemic stem cells (LSC) that particularly raised interest in the role of cellular origin in the biology and clinical course of AML. Studies in genetically modified mice and xenografts of patient-derived cells (PDX) in immune deficient

mice led to the hypothesis that AML is the product of cooperating genetic alterations in the hematopoietic stem cell (HSC) pool. The combination of improved multicolor flow cytometry with high-throughput “next-generation” sequencing (NGS) technologies revealed a complex interplay of genomic and epigenetic alterations that seem to be necessary to transform normal hematopoietic stem and progenitor cells (HSPC) into preleukemic states that may ultimately progress to AML. More recent studies in transgenic mouse strains and PDX models combined with cross-species transcriptomics suggested that AML in mice and humans in most cases originates from a continuum of early multipotent to more differentiated hematopoietic progenitor cells. However, there is increasing evidence that in about 10% to 20% of patients, AML may originate from more immature cells that are most likely part of cell pool that we call today long-term HSC (LT-HSC). Modeling of HSC-derived AML driven by a strong oncogene in mice has revealed a particularly invasive and drug-resistant phenotype associated with a genetic signature that also characterizes human AML with poor outcome. However, in AML lacking any predominant oncogenic driver mutations developing from clonal hematopoiesis and/or myelodysplasia (MDS) with one or several preleukemic mutations in cells from the HSC compartment, the definition of the cellular origin remains a challenge. Here, we summarize some of the key contributions that illustrate how mouse models have provided critical insights into the role of the cellular origin of AML (Table 1). Collectively many of these studies underline the importance of the cellular origin of AML not only for prognosis but also for personalized therapeutic strategies, particularly in AML subtypes that are driven by very potent oncogenes. However, several studies have also identified important limitations to consider when modeling the cellular origin of AML arising from multiple preleukemic mutations in which the ultimate driver is difficult to define.

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**Table 1**

**Modeling the Cellular Origin of AML in Mice**

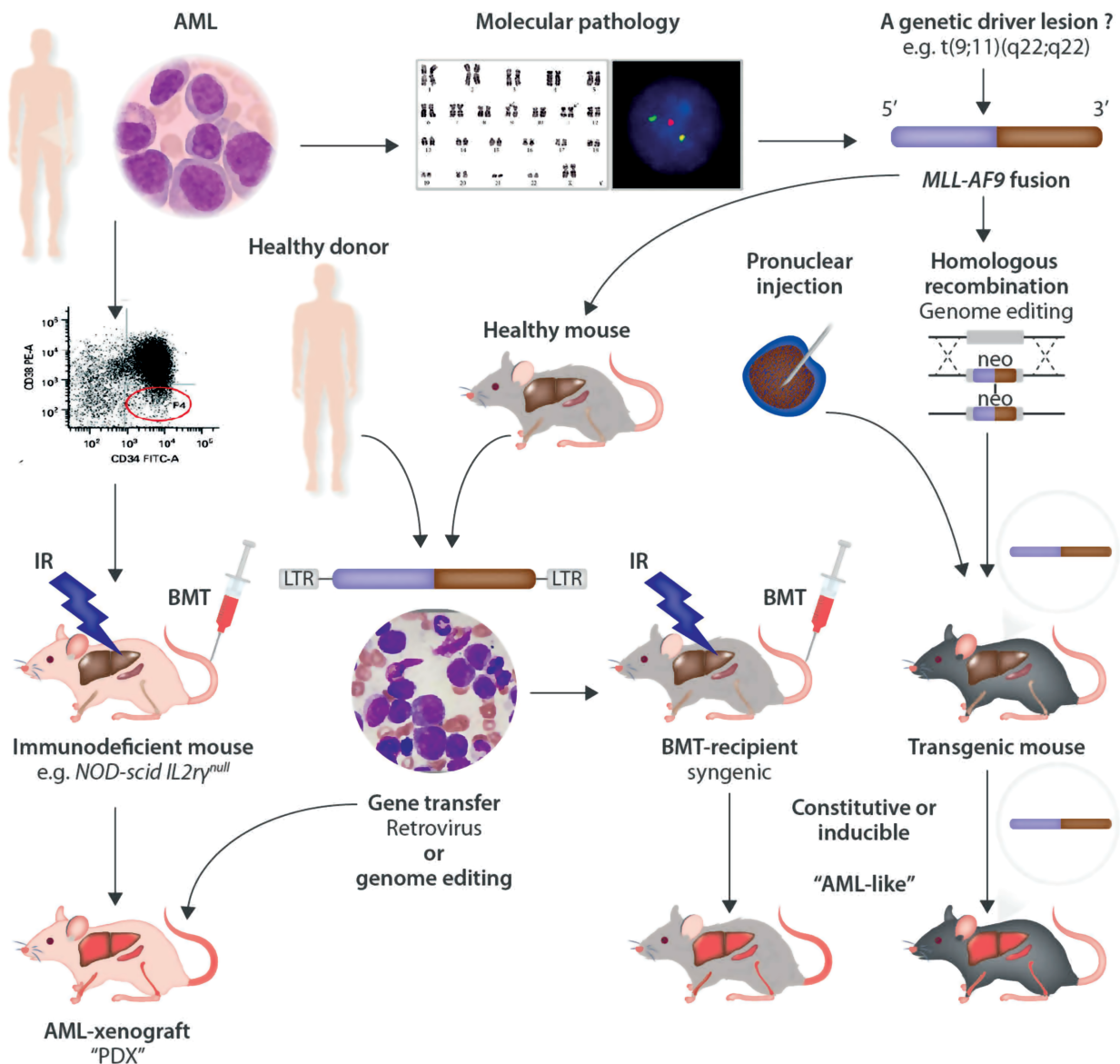
Year	Genetic Lesion	Strategy	Promoter	Inducer	Compartment (Expression/Propagation)	Phenotype (Median Latency)	Major Findings	References
<b>(A) Transplantation of cells that retrovirally overexpress an leukemogenic oncogene</b>								
1997	<i>MLL-ENL</i>	<i>pMSCV-pgk-neo</i>	<i>MSCV-LTR</i>		Thy-1 <sup>hi</sup> Scd-1 <sup>hi</sup> 2k <sup>hi</sup> day 2 post-5-FU BM GFP-sorted LSK	AML (BL6: 210 d, SCD: 70 d)	MLL-ENL induced increased clonogenic activity in vitro and AML in vivo	Laxau <i>et al</i> <sup>22</sup>
2002	<i>RUNX1-RUNX1T1</i>	<i>pMSCV-IRES-GFP</i>	<i>MSCV-LTR</i>		LSK, CMP, GMP	Myeloid hyperplasia (10 mo)	Increased BM HSC numbers in vivo and increased myeloid CFU in vitro, but not leukemia	de Guzman <i>et al</i> <sup>54</sup>
2003	<i>MLL-ENL</i>	<i>pMSCV-pgk-neo</i>	<i>MSCV-LTR</i>		Lin <sup>-</sup> , c-kit <sup>+</sup> HSPC	AML (90–100 d)	Similar latency to AML after transplanting transduced LSK, CMP, or GMP. No disease upon transplanting transduced MEP	Cozzio <i>et al</i> <sup>20</sup>
2003	<i>MLL-GAS7</i>	<i>pMSCV-pgk-neo</i>	<i>MSCV-LTR</i>		Lin <sup>-</sup> , c-kit <sup>+</sup> HSPC	AML (115 d), ALL (90 d), ABL (100 d)	Expression of the fusion resulted in lymphoid, myeloid, and mixed lineage leukemia phenotypes	So <i>et al</i> <sup>26</sup>
2004	<i>MOZ-TIF2, BCR-ABL</i>	<i>pMSCV-IRES-GFP</i>	<i>MSCV-LTR</i>		CMP, GMP	AML (103–162 d)	MOZ-TIF2, but not BCR-ABL, transduced CMP and GMP could be serially replated in MC and resulted in a transplantable AML in vivo	Huntly <i>et al</i> <sup>27</sup>
2006	<i>MLL-AF9</i>	<i>pMSCV-IRES-GFP</i>	<i>MSCV-LTR</i>		GMP	AML (80 d)	MLL-AF9 transduction gave a stem-like phenotype. Transplantation of transduced cells plated in MC resulted in AML	Krivtsov <i>et al</i> <sup>28</sup>
2009	<i>MLL-ENL</i>	<i>pMSCV-neo-TRE</i>	<i>TRE</i>	<i>rTA (MSCV)</i>	Lin <sup>-</sup> HSPC	AML (1°: 75 d, 2°: 25 d)	Conditional expression of MLL-ENL in hematopoietic progenitors leads to transplantable AML that remains fully dependent on the fusion	Horton <i>et al</i> <sup>40</sup>
2011	<i>MN1 (OA)</i>	<i>pMSCV-pgk-neo</i>	<i>MSCV-LTR</i>		CMP, GMP	AML (1°: 40 d, 2°: 25 d)	MN1 expression in CMP but not GMP induces AML, coexpression of MEIS1 makes GMP susceptible for transformation by MN1	Heuser <i>et al</i> <sup>70</sup>
2012	<i>MLL-AF9</i>	<i>pMSCV-pgk-puro</i>	<i>MSCV-LTR</i>		Evi1 <sup>+</sup> MLL-AF9 transduced cells	AML (>150 d)	MLL-AF9 maintains <i>Evi1</i> expression in HSC, but not in GMP. Knockdown of <i>Evi1</i> delayed leukemia induction in vivo	Bindelset <i>et al</i> <sup>38</sup>
2013	<i>MLL-AF9</i>	<i>pMSCV-IRES-GFP</i>	<i>MSCV-LTR</i>		HSC, GMP	AML (LSKs: 68 d, GMPs: 100 d)	Transduced HSC-induced AML earlier with fewer cells transplanted than GMP. HSC-derived disease is also more chemoresistant	Krivtsov <i>et al</i> <sup>30</sup>
<b>(B) Transgenic mouse lines</b>								
2004	<i>RUNX1-RUNX1T1</i>	Cond TG (+IRES-GFP)	<i>Lys6A (Scat1)</i>		Myeloid cells (BM, periphery)	MPD (12 mo)	MPD after a long latency, no AML. Not transplantable in NOD-SCID mice	Fenske <i>et al</i> <sup>65</sup>
2006	<i>CBFβ-SMMHC</i>	Cond KI	<i>Cbfβ</i>	<i>Mx-Cre</i>	BM (LSK; transplantable (+ rPLA2))	AML (18 wk)	Impaired competitive repopulation of LSK, aberrant myeloid progenitors, blocked megakaryocytic differentiation; spontaneous AML	Kuo <i>et al</i> <sup>63</sup>
2008	<i>MLL-AF9</i>	KI	<i>Mll</i>		LSK, CLP, CMP, (GMP: no disease)	AML (LSK: 165 d, CLP: 137 d, CMP: 181 d)	Leukemia-inducing potential highest in CLP and LSK, lowest in CMP; correlation with transgene expression	Chen <i>et al</i> <sup>37</sup>
2012	<i>MLL-ENL</i>	KI (ERTm-fusion)	<i>Mll</i>	ER-tfusion (TX)	BM cells (c-kit <sup>+</sup> )	MPD (19 mo)	Tamoxifen-regulated ENL-ERTm fusion in <i>Mll</i> locus. Symptomatic MPD after long latency; leukemia after blocking DDR (62 d)	Takacova <i>et al</i> <sup>43</sup>
2013	<i>RUNX1-RUNX1T1</i>	Cond TG (TET-ON)	<i>TRE</i>	<i>rTA (Rosaz26)</i>	LSK, GMP	Incident MPD (16–18 mo)	Impaired HSC differentiation and selective expansion of the myeloid compartment. HSCs and GMP acquired disease-inducing potential	Cabezas-Wallscheid <i>et al</i> <sup>36</sup>
2014	<i>MLL-ENL</i>	Cond TG (TET-ON)	<i>TRE (Coit1a1)</i>	<i>rTA (Rosaz26)</i>	GMP, CLP, some MPP	AML (1° BMT: 10–15 wk, 2° BMT: 5–10 wk)	Leukemia inducing potential was limited to GMP, CLP, and MPP: HSC seemed “protected”	Ugale <i>et al</i> <sup>44</sup>
2016	<i>MLL-AF9</i>	Cond TG (TET-ON)	<i>TRE (Hprt)</i>	<i>rTA (Rosaz26)</i>	LT-HSC, ST-HSC, CMP, GMP	AML (1° BMT: 73 d, 2° BMT: 32 d)	Transplanting LT-HSC induced an aggressive, drug-resistant disease with expression of EMT-related genes	Stavropoulou <i>et al</i> <sup>89</sup>
2018	<i>MLL-ENL</i>	Cond TG (TET-ON)	<i>TRE (Hprt)</i>	<i>rTA (Rosaz26)</i>	LT-HSC, LMPP, CMP	AML (72 d)	The transforming potential of iMLL-ENL was more pronounced in HSC, LMPP, and CMP, and when expression of <i>MLL-ENL</i> mRNA > <i>Mll</i>	Vaia Stavropoulou <i>et al</i> <sup>65</sup>
<b>(C) Transplantation of patient-derived primary AML cells into immune-deficient mice</b>								
1994	AML (FAB-M1, 2, 4)	1–4 × 10 <sup>7</sup> cells/mouse		SCID (400 c5y)		70/80 engrafted (10–100%, 30–45 d)	AML-initiating cells were CD34 <sup>+</sup> , CD38 <sup>-</sup> ; no disease propagation with CD34 <sup>+</sup> or CD34 <sup>+</sup> CD38 <sup>+</sup> cells suggesting a hierarchical organization in AML	Lapidot <i>et al</i> <sup>74</sup>
1997	AML (FAB-M1, 4, 5)	1–2 × 10 <sup>7</sup> cells/mouse		SCD, NOD-SCID (400 c5y)		5/18 engrafted (5–100%, 4–8 wk)	CD34 <sup>+</sup> , CD38 <sup>-</sup> AML cells are at the top of the leukemia-propagating hierarchy	Bonnet and Dick <sup>75</sup>
2011	AML (M0-M5)	1–100 × 10 <sup>5</sup> cells/mouse		NOD-SCID, NSG (2 × 100–125 c5y)	Grafting (10–12 wk)		Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD90 <sup>+</sup> CD45RA <sup>+</sup> and Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD123 <sup>+</sup> CD110 <sup>-</sup> CD45RA <sup>+</sup> (GMP-like) cells with LIC activity suggesting that AML derives mostly from progenitor cells	Goardon <i>et al</i> <sup>77</sup>
2014	AML	2.5–100 × 10 <sup>6</sup> , CD3-depleted		NSG (225 c5y)	Grafting (8–16 wk)		DNMT3A <sup>mut</sup> HSC/MPP capable of generating a long-term multilineage lymphomyeloid graft, confirming their designation as preleukemic HSC	Shlush <i>et al</i> <sup>88</sup>
2016	AML (CD34 <sup>+</sup> )	6–100 × 10 <sup>6</sup> CD3-depleted		NSG, NRG, or NSGS (2 × 100 c5y)	11/28 grafted (>0.1% hMNC) 12–28 wk		CD34 <sup>+</sup> AML LSC derives from committed GM precursors, as opposed to CD34 <sup>+</sup> LSCs which originate from progenitors	Quek <i>et al</i> <sup>78</sup>
2017	AML	6–100 × 10 <sup>6</sup> CD3-depleted		NSG or NSG-SGM3 (225 c5y)	Grafting (8–16 wk)		Release originated from either rare LSC with HSPC phenotype or from differentiated cells that retained stemness signatures	Shlush <i>et al</i> <sup>81</sup>

5-FU = fluorouracil, ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, BM = bone marrow, BMT = bone marrow transplant, CFU = colony-forming unit, CMP = common myeloid progenitors, CN = cytogenetically normal, cond = conditional, d = days, DDR = DNA-damage response, GMP = granulocyte/macrophage/lymphoid progenitor, GMP = hematopoietic stem cell, HSPC = hematopoietic stem and progenitor cells, KI = knock-in, LMPP = lymphoid-primed multipotent progenitor cells, LSC = leukemic stem cells, LT-HSC = long-term hematopoietic stem cells, LTR = long terminal repeat, MC = methylcellulose, MPD = megakaryocyte/erythroid progenitor, roliferative disorder, MPP = multipotent progenitors, MSCV = mouse stem cell virus, mo = months, NOD-SCID = NOD.Cg-Prkdcscid l2l2<sup>gmm</sup>Wjl/SzJ, or NSG (NOD.Cg-Prkdcscid l2l2<sup>gmm</sup>Wjl/SzJ), NSG = NOD.Cg-Prkdcscid l2l2<sup>gmm</sup>Wjl/SzJ, NSG = NOD.Cg-Prkdcscid l2l2<sup>gmm</sup>Wjl/SzJ, OE = overexpression, ST-HSC = short-term hematopoietic stem cells, rTA = reverse tet-responsive transactivator, TET = tetracycline, TG = transgene, TRE = tet-responsive element, wk = weeks.

## From clinical observations to transgenic mouse models

Pioneer studies by Phil Fialkow revealed that in chronic myeloid leukemia (CML) patients hematopoietic cells from multiple lineages carried the Philadelphia chromosome (the morphological correlate of the t(9;22)(q34;q11) translocation leading to expression of the BCR-ABL fusion) suggesting an origin high up in the hierarchy, most likely in stem cells. Expression of the same isotype of the polymorphic X-linked glucose-6-phosphate dehydrogenase in CML and AML cells led him to conclude that both malignancies may originate from multipotent cells within the HSC pool.<sup>3,4</sup> Later, flow cytometer-assisted cell sorting combined with fluorescent in situ hybridization made possible the

visualization of AML-associated cytogenetic aberrations in selected cells, which further supported a stem cell origin.<sup>5,6</sup> Improved molecular tools facilitated the cloning of a large number of genetic alterations from AML blasts such as fusion oncogenes that turned out to be hallmarks of biologically distinct AML subtypes.<sup>7</sup> The imminent question whether a given AML mutation might be a driver of the disease, initiated efforts to model AML, mostly in mice (Fig. 1). However, expression of AML-associated fusions as transgenes in the murine hematopoietic system by oocyte injections of randomly integrated expression cassettes turned out to be very challenging, as the regulatory elements of a given vector significantly influenced the resulting phenotype.<sup>8–11</sup> Homologous recombination strategies ultimately led to the establishment of mice that developed AML



**Figure 1. Strategies to model AML in mice.** There are 2 major approaches to model AML in mice. A particular AML-associated genetic mutation (eg, t(9;11) leading to expression of an *MLL-AF9* fusion gene) may be expressed in the hematopoietic system of the mouse either by retroviral transduction of BM cells followed by transplantation, or by engineering the mutation in the germline (either randomly integrated or targeted to the proper locus allowing constitutive or inducible expression, respectively). To model AML with primary patient cells, leukemic cells (or enriched fractions thereof) are transplanted into sublethally irradiated immunodeficient mice of which *NOD-scid-IL2 $\gamma$ <sup>null</sup>* (NSG) mice are currently the most frequently used strain. AML=acute myeloid leukemia, BM=bone marrow.

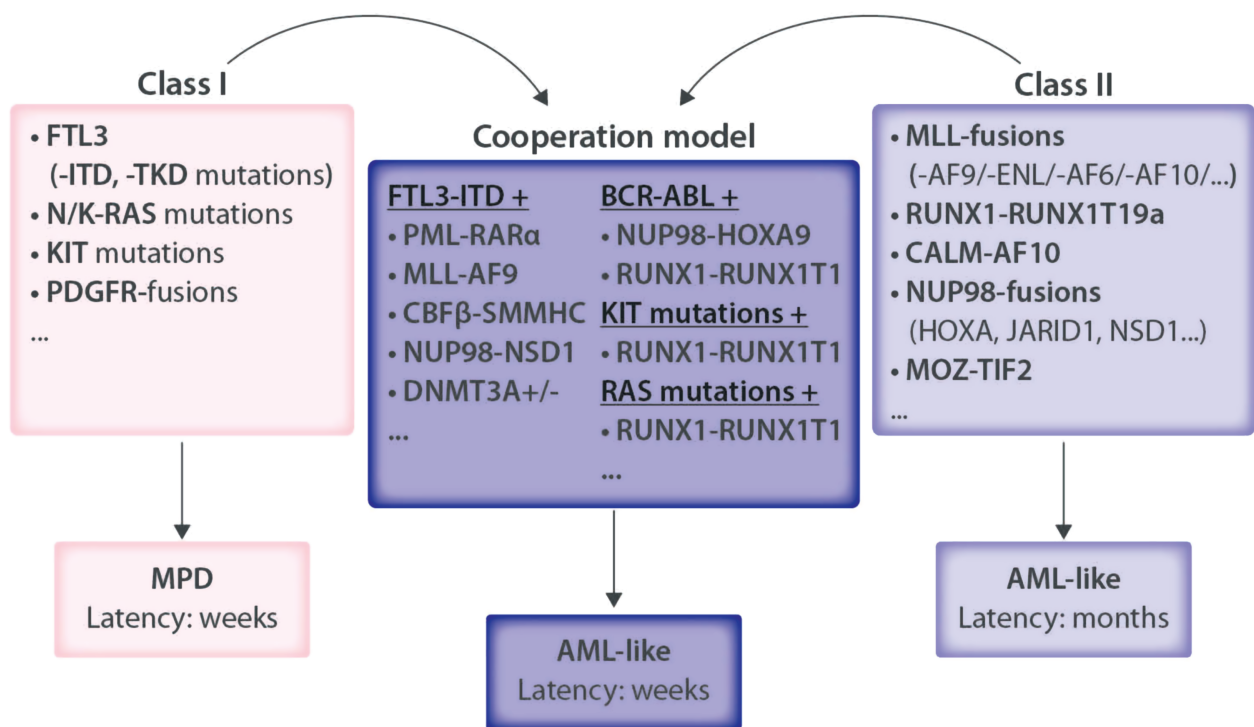
upon expression of the respective mutations from their natural promoters.<sup>12</sup>

To facilitate the expression of a potential driver of leukemogenesis, David Baltimore's lab developed a protocol for efficient retroviral transduction of bone marrow (BM) cells followed by transplantation into irradiated syngeneic recipients. This approach allowed them to demonstrate that expression of the *BCR-ABL* fusion gene induces a CML-like disease in mice.<sup>13</sup> A large number of subsequent studies revealed that retroviral expression of CML- and AML-associated mutations, which lead to constitutive activity of tyrosine kinases and related signaling mediators, induces a lethal myeloproliferative disorder (MPD) when retrovirally expressed in the mouse BM.<sup>14,15</sup> By contrast, expression of AML-associated fusion genes involving transcription factors or epigenetic regulators was often not sufficient to phenocopy the disease in mice, with the exception of some fusions including the those affecting the mixed lineage leukemia (MLL) gene that potentially induced AML, acute lymphoblastic leukemia (ALL), or MLL.<sup>16–23</sup> Identification of coexisting mutations initiated another wave of studies demonstrating that coexpression of AML-associated fusion oncogenes involving transcription factors (such as the core binding factor [CBF] composed of RUNX1 and CBFbeta, the retinoic acid receptor alpha (RARA), or the MLL histone methyltransferase) together with mutated constitutively active tyrosine kinases such as FLT3, JAK2, or c-KIT, or mutants of signaling mediators such as the RAS-GTPases were sufficient to rapidly induce AML with full penetrance. These findings led Gary Gilliland and colleagues to formulate a genetic

AML model based on the cooperation of 2 functional classes of mutations<sup>24</sup> (Fig. 2).

### Modeling the cellular origin of MLL fusion AML in mice

Multiple studies have demonstrated that expression of several (but not all) MLL fusion genes (hallmarks of infant acute leukemia and of some de novo and therapy-related adult ALL and AML) is able to induce a leukemic phenotype in mice.<sup>25,26</sup> To address whether activation of an MLL-fusion in stem or progenitor cells would affect the biology of the disease, researchers profited from improved cell enrichment strategies to retrovirally transduce defined cellular subpopulations of the hematopoietic hierarchy. Several groups found that expression of some of the most prevalent MLL fusions (eg, MLL-ENL, MLL-AF9) or other transcription factor fusions (eg, MOZ-TIF2) induced a similar AML phenotype in mice when expressed in lineage marker-depleted (Sca-1 and c-Kit-positive) BM stem and progenitors (LSK) cells, common myeloid progenitors (CMP), or granulocytic-macrophage progenitors (GMP).<sup>20,27,28</sup> In sharp contrast, expression of *BCR-ABL* induced a CML-like disease only when expressed in LSK but not in more committed progenitor cells.<sup>27</sup> Molecular characterization of CML progressing to blast crisis suggested the existence of leukemic GMP-like cells with alterations leading to aberrant self-renewal, indicating that cooperating mutations in progenitor cells may ultimately transform CML into AML.<sup>29</sup> Although retroviral expression of



**Figure 2.** Functional cooperation of AML-associated mutations as shown by the retroviral transduction transplantation model supported a model of AML as a product of 2 classes of mutations. Multiple studies have shown that transplantation of BM cells retrovirally expressing AML-associated mutations in tyrosine kinases and other signaling mediators that support proliferation and survival without altering differentiation (often referred as “class I mutations”) leads to lethal myeloproliferative diseases (MPD). By contrast, mutations in epigenetic regulators that mostly affect differentiation and/or self-renewal (“class II mutations”) may lead to development of an AML-like disease, normally after a rather long latency. Coexpression of a class I with a class II mutation achieved by retroviral cotransduction or by transduction of BM cells harboring a particular transgene generally leads to rapid development of a full penetrant AML-like disease in mice. AML = acute myeloid leukemia, BM = bone marrow.

the MLL-AF9 fusion in the LSK or GMP fraction resulted in a similar AML phenotype, some origin-related differences in disease latency, leukemia-initiating potential, and gene expression signatures were observed.<sup>30</sup> Collectively, these observations suggested that the cell of origin might influence the transforming capacity of particularly potent leukemogenic oncogenes.

Despite proven utility in the determination of the transforming potential of a leukemia-associated mutation, retroviral transduction of BM cells is associated with several important limitations.<sup>31,32</sup> First, efficient transduction depends on active cell proliferation; however, stimulation of BM cell growth with cytokines is inherently associated with cellular differentiation, resulting in a significant bias of the transduced cells. In addition, the number of LT-HSC that can be obtained from a single mouse is limited, and despite constant improvements in culture systems, it remains a challenge to expand these cells while maintaining their stem cell characteristics. Moreover, expression of the receptors that are necessary for viral transduction seems to depend on the differentiation status of the cells.<sup>33,34</sup> Second, retroviral transgene expression levels are unpredictable, and may result in overexpression far beyond the levels observed in primary leukemic cells. Third, retroviral integration can lead to activation or suppression of potentially cooperating oncogenes or tumor suppressor genes that, respectively, support clonal expansion. Transduction with the frequently used murine stem cell virus (MSCV)-based expression vector seems to be sufficient to partly in vitro immortalize primary murine BM cells.<sup>35,36</sup> Finally, transduction of the selected target cells might also be dependent on the titer of viral particles that, depending on the size and nature of the transgene, can be difficult to standardize.

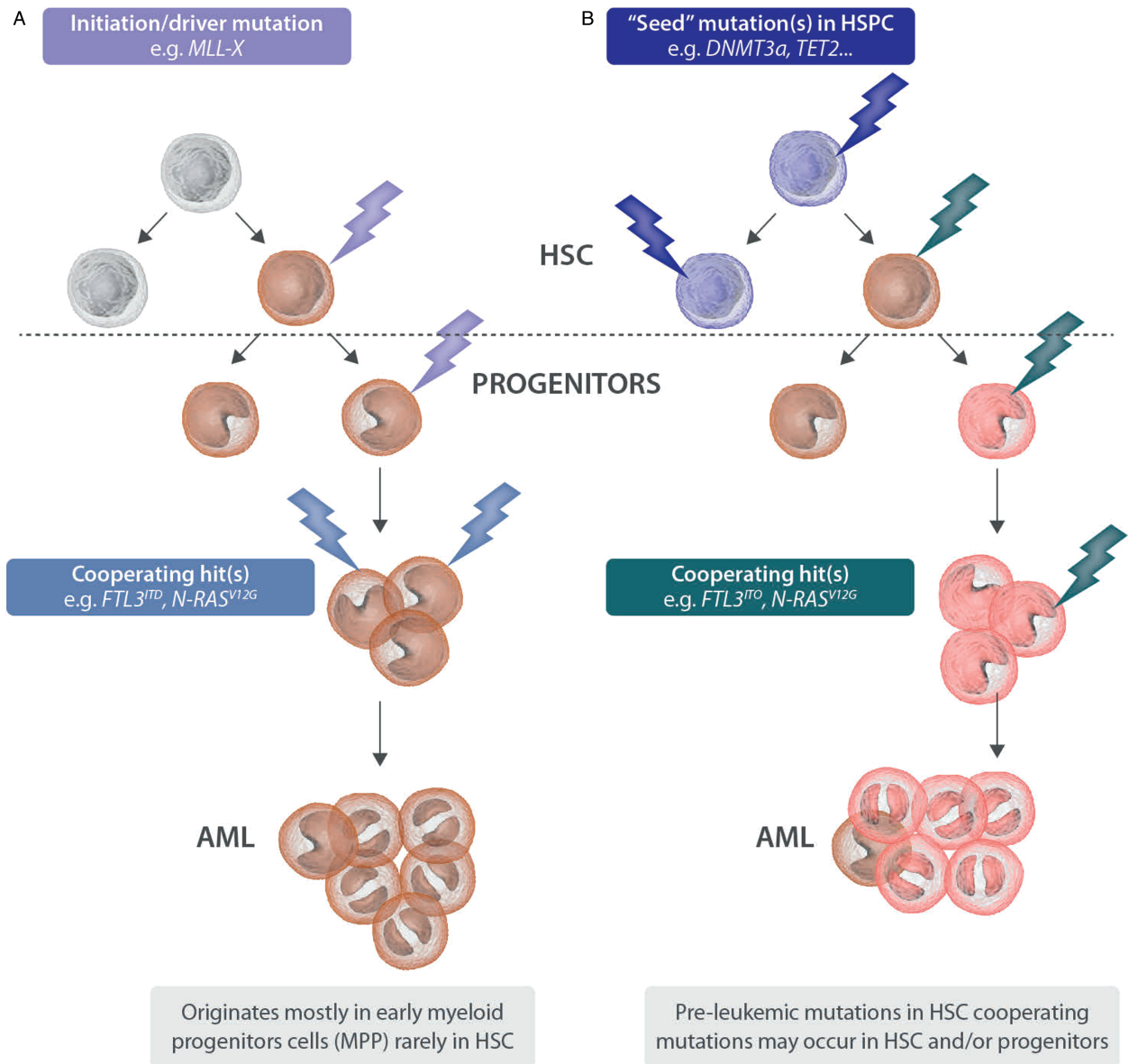
To overcome these limitations, researchers have generated a series of transgenic mouse models in which expression of a putative genetic AML driver is either controlled by the endogenous promoter or conditionally controlled from a heterologous promoter, for example, by a doxycycline (DOX)-inducible gene expression system. Rather surprisingly, transplantation of different subpopulations of the hematopoietic hierarchy from an *MLL-AF9* knock-in mouse strain suggested that, in contrast to the retroviral model, the disease was induced by transplanting CMP, LSK, and common lymphoid progenitors (CLP) but not GMP. Interestingly, the leukemia-initiation potential of the different BM cell subpopulations seemed to correlate with the dose of the *MLL-AF9* fusion, and the expression of the transcription factor *EVI1*, a putative direct downstream target.<sup>37</sup> Retroviral expression of *MLL-AF9* (“*rMLL-AF9*”) in murine LSK but not in more mature GMP was associated with high *Evi1* expression, which turned out to be a general marker of poor prognosis associated with LT-HSC multilineage repopulating potential.<sup>38</sup> The gene expression signatures of LSK-derived *rMLL-AF9* leukemia were enriched for poor prognosis genes in human AML carrying MLL fusions suggesting that the cell of origin is able to determine clinically relevant subtypes of MLL-rearranged AML.<sup>30</sup> To study the role of the cellular origin of MLL-AF9-mediated AML, we generated a DOX-inducible transgenic mouse (“*iMLL-AF9*”). DOX-induced *iMLL-AF9* expression was dose-dependent and reached levels that were about 10-fold higher than endogenous *Mll1* but 10 to 20 times lower than that achieved by *rMLL-AF9* and led to a fully penetrant and reversible AML phenotype.<sup>39</sup> Transplantation of naïve flow-sorted BM subpopulations into recipients on DOX revealed that the transfer of LT-HSC, short-term-HSC (ST-HSC), CMP, and GMP led to AML, whereas no disease was observed after transferring CLP, confirming earlier studies

indicating that MLL-AF9 is able to transform HSC and more committed myeloid progenitors.<sup>15–19</sup> Activation of the fusion in LT-HSC induced a more invasive disease than induction in ST-HSC or more committed downstream progenitor cells, further underlining that the cellular origin is a critical determinant for AML biology (Fig. 3A).

The MLL-ENL fusion that results from t(11;19)(q23;p13) is mostly associated with ALL but has also been found in AML.<sup>28</sup> Previous studies have shown that retroviral expression of *MLL-ENL* resulted in a very similar AML phenotype to that induced by the *MLL-AF9* fusion.<sup>20,23,40–42</sup> Further attempts modeling MLL-ENL-driven leukemia by knocking-in a tamoxifen (TX)-sensitive ENL-estrogen ligand binding domain (ER) fusion into the *Mll* locus resulted in an MPD: AML was only induced after chemically blocking the DNA damage response.<sup>43</sup> Later, Bryder and colleagues established a DOX-regulated transgenic mouse model where MLL-ENL was regulated by a *Tet* operon expressed from the *Col1a1* promoter.<sup>44</sup> As activation of the fusion in different BM subpopulations followed by transplantation did not induce a disease they concluded that HSC and multipotent progenitors are inherently protected from malignant transformation by MLL-ENL. However, only cells in which *MLL-ENL* expression levels were equal to, or higher than, endogenous *Mll1* were transformed by the fusion suggesting that the dose may be a critical denominator of the oncogenic fusion activity.<sup>44</sup> We also established a DOX-inducible *iMLL-ENL* mouse line in which similar to *iMLL-AF9* the *Rosa26*-controlled *rtTA* regulates the expression of the fusion integrated in the *Hprt* locus.<sup>45</sup> Transplantation of different hematopoietic BM cell populations revealed that, in contrast to *iMLL-AF9*, the disease was only induced by HSC and early progenitors, but not GMP. DOX-induced *iMLL-ENL* mRNA expression levels exceeded expression of endogenous *Mll1*. We observed MLL-ENL fusion protein levels equal or exceeding MLL1 not only in *iMLL-ENL*<sup>+</sup> leukemic cells, but also in t(11;19)+ human leukemic cell lines. Notably, we found that in primary leukemic cells from 5 out of 5 t(11;19)<sup>+</sup> patients, the expression of *MLL-ENL* mRNA also surpassed *Mll1*. These observations are in line with recent work suggesting that the ratio of chimeric to wild-type MLL protein might be a critical determinant for maintenance of the transformed phenotype.<sup>46</sup> This study also suggested that HSC or most likely bipotent lympho-myeloid progenitors might be the preferential targets of the MLL-ENL fusion. Notably transplantation of human cord blood HSPC retrovirally expressing *MLL-ENL* into NOD-SCID mice resulted in an acute leukemia-like phenotype with tumor cells expressing lymphoid markers without complete immunoglobulin gene rearrangements furthermore suggesting that HSC, CLP, or early B-cells are targets of MLL-ENL.<sup>47</sup>

### Modeling the cellular origin of non-MLL fusion AML in mice

Modeling AML driven by CBF rearrangements such as the *RUNX1-RUNX1T1* (initially referred to as *AML1-ETO*) or the *CBFbeta-MYH11* fusion genes turned out to be rather difficult. Conventional knock-in models were hampered by embryonic lethality due to the dominant-negative impact of the fusion to wild-type *RUNX1* and *CBFbeta*, both essential regulators of adult hematopoiesis.<sup>48–50</sup> Conditional knock-in mice demonstrated that whereas expression of the *CBFbeta-MYH11* fusion resulted in spontaneous AML after long latency, expression of *RUNX1-RUNX1T1* seemed insufficient to induce an AML



**Figure 3. Modeling the origin of AML.** (A) Observations in transgenic mouse models suggest that AML driven by strong oncogenes such as *MLL* fusions mostly originates in multipotent progenitors: few cooperating mutations are needed to develop a symptomatic disease. In a minor fraction, the disease might occur from the HSC compartment leading to a particularly invasive and highly resistant phenotype. (B) A significant fraction of AML seems to be the product of cooperation of multiple mutations: early lesions in the HSC compartment provide a clonal advantage leading to a "preleukemic," asymptomatic state; however, the gain of additional mutations (whether they occur in HSC or exclusively in more committed progenitor remains to be elucidated) is necessary to develop a symptomatic AML. AML=acute myeloid leukemia, BM=bone marrow, HSC=hematopoietic stem cell, *MLL*=mixed lineage leukemia.

phenotype.<sup>51–53</sup> The occurrence of tumors affecting different lineages including T-cells after chemical mutagenesis in *RUNX1-RUNX1T1* knock-in mice suggested the cellular origin within the HSC compartment. In addition, retroviral *RUNX1-RUNX1T1* overexpression resulted in a significantly increased number of LSK, further supporting the notion that this fusion might indeed affect the HSC compartment.<sup>54</sup> Expression of a *RUNX1-RUNX1T1* transgene under control of the *Ly6A* locus, which encodes the HSC marker Sca-1, resulted in a chronic MPD suggestive of some oncogenic activity in the HSC compartment.<sup>55</sup> More recently, a DOX-inducible mouse model was generated in which the *rtTA* was integrated in the *Rosa26* locus allowing *RUNX1-RUNX1T1* expression in various blood lineages including LT-HSC. Transplantation of BM cells expressing

*iRUNX1-RUNX1T1* induced an MPD-like disease after a long latency. Transgenic GMP were able to further propagate the disease suggesting acquired self-renewal capacity of GMP.<sup>56</sup> The presence of CBF fusions in perinatal blood spots on Guthrie cards from newborns, and the persistence of fusion-positive clones upon clinical remission suggest that these fusions might represent "preleukemic" mutations generally not sufficient to induce AML. Although cooperative mutations are necessary for development of the disease, AML blasts seem to be continuously dependent on these CBF fusions making them attractive targets for therapeutic intervention.<sup>57,58</sup>

The *PML-RARA* fusion gene is the product of *t(15;17)(q22;q21)* and the molecular hallmark of acute promyelocytic leukemia (APL).<sup>59</sup> Earlier attempts to model this disease in mice

showed that expression of a *PML-RARA* transgene controlled by myeloid promoters such as human *cathepsin G* (*CTSG*) or *S100A8/MRP8* was able to initiate a myeloproliferative disease with progression to an APL-like phenotype after long latency with incomplete penetrance.<sup>9,60,61</sup> Retroviral expression of potentially collaborating oncogenes such as *FLT3-ITD*, *PIM2*, or *mutDNMT3A* in BM cells from *CTSG-PML-RARA* transgenic mice reduced the latency and increased the penetrance of the APL phenotype.<sup>62–64</sup> However, the putative origin in myeloid progenitors was challenged by the expression of T-cell lineage associated transcripts, T-cell receptor (TCR) rearrangements as well as by frequent expression of lymphoid surface markers on t(15;17)<sup>+</sup> APL cells.<sup>65</sup> Interestingly, in about 20% of APL patients the blasts express CD34 and CD2 on their surface; however, whether these markers are indeed indicative of an HSC origin remains unclear.<sup>66,67</sup> More recently Ley and colleagues showed that expression of a *PML-RARA* transgene (controlled by the *Ctsg* or *Mrp8* promoter) might not be limited to myeloid progenitors but might also occur in HSC and early progenitor cells.<sup>68</sup> Relatively few studies have addressed the role of the cellular origin of aberrantly expressed or mutated genes in AML. Using a classical retroviral expression transplantation approach, Heuser and colleagues found that CMP but not GMP are susceptible to transformation by the *meningioma-1* (*MN1*) gene often found aberrantly highly expressed in AML.<sup>69</sup> Interestingly, overexpression of the HOX-interacting homeodomain transcription factor *MEIS1* rendered GMP susceptible to MN1-induced transformation by regulation of common target genes, which suggests that the cellular targets may change due to accumulation of functionally cooperating mutations.<sup>70</sup>

### Modeling the cellular origin by transplantation of human AML cells into immune-deficient mice

Pioneer work revealed that immune-deficient mice can be repopulated not only with normal human myeloid cells but also with primary leukemic blasts of the lymphoid and myeloid lineage.<sup>71–73</sup> Subsequent studies provided proof of concept that the leukemia-initiating capacity of primary human AML cells in immune deficient NOD-SCID mice could be attributed to the CD34<sup>+</sup>CD38<sup>−</sup> subpopulation containing HSC, suggesting a hierarchical organization.<sup>74,75</sup> Following the hypothesis that LIC are part of the HSC compartment, scientists studied RUNX1-RUNX1T1<sup>+</sup> AML. They found that *RUNX1-RUNX1T1* mRNA was expressed in mature monocytes and B cells as well as HSC, lymphoid progenitors, and myeloid progenitors during disease remission in patients strongly suggesting that RUNX1-RUNX1T1<sup>+</sup> HSC are capable of self-renewal and differentiate into mature blood cells in vivo.<sup>76</sup>

Another landmark study addressed the origin of AML by xenotransplanting a large number of primary human samples into NSG (*NOD-scid-IL2ry<sup>−/−</sup>*) mice.<sup>77</sup> Eighty percent of the CD34<sup>+</sup> AML cases contained 2 predominant cell populations with an immune phenotype of CD38<sup>−</sup>CD90<sup>−</sup>CD45RA<sup>+</sup> or CD38<sup>+</sup>CD110<sup>+</sup>CD45RA<sup>+</sup> both corresponding to normal early and more mature hematopoietic progenitor cells rather than HSC. Importantly, both cell populations had leukemia initiating potential upon serial transplantation. There seemed to be a hierarchical organization as CD38<sup>−</sup>CD45RA<sup>+</sup> cells with a higher number of LIC gave rise to leukemic GMP (with less LIC) but not vice versa. This work suggested that the majority of CD34<sup>+</sup> AML originate in hematopoietic progenitor cells rather than in the HSC

compartment. In AML composed of >98% CD34<sup>−</sup> cells, there seem to be multiple CD34<sup>−</sup> and CD34<sup>+</sup> LIC-containing populations. Even though the expression profiles of CD34<sup>−</sup> LIC seem very similar to those of normal CD34<sup>−</sup> GMP, these LIC express multiple normal stem cell transcriptional regulators, suggesting that the nature of the genetic/epigenetic driver events determine a disordered transcriptional program that results in LIC arrested in differentiation at either the progenitor or precursor stages of hematopoiesis.<sup>78</sup>

NGS studies of primary human AML cells led to the identification of several recurrent mutations mostly affecting metabolic regulators such as *IDH1/2*, *DNMT3A*, or *TET2*. Sequencing of 200 AML genomes revealed that, in addition to fusion oncogenes, most AML patients carry about 10 to 15 coding single nucleotide variants at diagnosis.<sup>79</sup> Earlier studies suggested that human HSPC naturally acquire about 5 to 10 potential mutations per year.<sup>80</sup> DNA sequencing of peripheral blood cells from a large number of individuals not selected for any cancer or other hematologic diseases revealed molecular evidence for clonal hematopoiesis with somatic mutations in up to 10% of people over 65 years of age, of which mutations of *DNMT3A*, *ASXL1*, and *TET2* were the most prevalent. Importantly, clonal hematopoiesis associated with distinct mutations turned out to be a strong risk factor with predictive power for the development of hematologic cancers.<sup>81–84</sup> Very recent work suggested that the risk to develop AML can be predicted in healthy individuals by screening the preleukemic mutations that are significantly different from the age-related mutations.<sup>85</sup> In fact, somatic mutations in *IDH1*, *IDH2*, *TP53*, *DNMT3A*, *TET2*, and spliceosome genes significantly increased the odds of developing AML.<sup>86</sup> In the same context, other AML-related mutations affecting *EZH2*, *CBP/EP300*, *CBL*, *JAK2*, but also fusion oncogenes such as *RUNX1-RUNX1T1* or *CBFbeta-MYH11* have been found as “preleukemic” mutations in healthy individuals.<sup>87</sup> Collectively these studies suggest that in a significant fraction of patients leukemogenesis can be considered as a continuum from normal to clonal hematopoiesis and to a preleukemic state from cells that carry particular epi/genetic variants initially conferring self-renewal but maintaining a normal differentiation potential. Upon the acquisition of additional alterations interfering with cellular differentiation progression to AML may occur (Fig. 3B). Such preleukemic states seem to represent a hematopoietic reservoir of mutant alleles that upon occurrence of the “matching” cooperating mutation will lead to the emergence of a dominant clone occurring not only in AML but also in a wide variety of hematologic malignancies such as MDS, MPD, CML, and lymphoid neoplasms.

Comparative sequencing of AML blasts at diagnosis and relapse using normal T-cells as surrogates for mutations originating from HSC allowed a better definition of preleukemic mutations. Comparing highly purified HSC, progenitor and mature cell populations, the Dick laboratory found that recurrent *DNMT3A* mutations exist at high allele frequency, but without coincident *NPM1* mutations present in AML blasts.<sup>88</sup> Therefore, the presence of *DNMT3A* mutations in both T-cells and AML cells, coupled with the lack of an *NPM1* mutation in T-cells, suggested that *DNMT3A* mutations may represent drivers in ancestral HSC that give rise to both lineages. In addition, comparison of allele frequencies at diagnosis, remission, and relapse revealed an increased prevalence of the mutated allele at remission and relapse, suggesting that these preleukemic mutations do indeed represent a genetic reservoir for disease recurrence. Similarly, another study found preleukemic mutations in HSPC that were associated with

long-term reconstitution in severely immune-compromised mice. They identified mutations in *IDH2*, *DNMT3A*, *ASXL1*, and *IKFZ1* in HSPC, leukemic blasts, and also in T- and/or B-cells.<sup>89,90</sup> Akin to Shlush *et al*, heterozygous *DNMT3A* and *IDH1/2* mutations were among the most prevalent preleukemic alterations and not eradicated by chemotherapy. Most relapses contained the preleukemic mutations, some acquired additional mutations and some seemed to originate from different clones. More recently, combined genetic and functional analysis of purified subpopulations and PDX from paired diagnostic/relapse samples allowed the Dick laboratory to show that therapy-resistant cells were indeed already present at diagnosis. Relapse originated either from rare LSC with a stem/progenitor phenotype or from larger subclones of immunophenotypically more committed cells that retained a strong stemness-related gene expression signature.<sup>91</sup>

These functional studies were mostly based on PDX of highly selected AML patients into immune-deficient mouse strains such as NOD-SCID or NSG. They showed that in contrast to AML expressing a strong oncogenic driver such as an MLL fusion, the cellular origin might be difficult to define, and seems more a moving rather than a static target. In a given individual, several preleukemic mutations may occur in different, or even the same HSC (Fig. 3B). Depending on its nature the mutation may be eliminated, tolerated, or even selected toward clonal hematopoiesis. Collaborator mutations most likely predominantly occur in the highly dividing compartment of early multipotent (eg, lymphoid-primed multipotent progenitor cells) or more lineage restricted (eg, GMP) compartments leading to rapid expansion of one of few clones resulting in a symptomatic disease. In the minority of the cases, the initiation and/or the cooperating mutations might occur in the HSC compartment, which, similar to MLL-driven AML, may result in a more aggressive disease. It will be important to determine the combination of preleukemic mutations with the highest risk for progressing into *de novo* AML or inducing MDS/MPN that ultimately progresses to AML. Likewise, one needs to determine which preleukemic mutations represent the highest risk for being positively selected by chemotherapy to provide a reservoir for disease relapse.

### Limitations of current AML mouse models

Apart from the obvious species-specific differences a major drawback of currently used mouse models is that the engineered genetic lesions only partially mimic those found in the leukemic blasts of a given patient, as even in the presence of leading cytogenetic lesions AML blasts often contain one or several potentially cooperating mutations.<sup>2</sup> The current tools generally limit expression to 2 to 3 putative oncogenic drivers in the same cell, either by viral cotransduction or by breeding-in different (conditional) transgenes. Improved genome editing strategies, for example, by using the CRISPR/Cas9 system in mouse zygotes will most likely facilitate the generation of more complex mouse models.<sup>92</sup> Another important limitation is the inability to ensure that a single cell at a particular stage of hematopoietic differentiation will undergo the initiating oncogenic mutation. In most models, even when conditionally regulated, the mutation occurs in a large population of genetically identical cells, failing to appropriately model the process of clonal selection starting from a single cell. Most *in vivo* human AML studies are based on PDX of patients into immune-deficient mouse strains. A major drawback of these models is the difference in the crosstalk between human hematopoietic cells and the murine stroma that are pivotal for a permissive microenvironment.<sup>93</sup> To overcome

these limitations, newer mouse strains are continuously being established that carry multiple transgenes expressing critical human hematopoietic factors, which have improved expansion of AML cells in mice.<sup>94,95</sup>

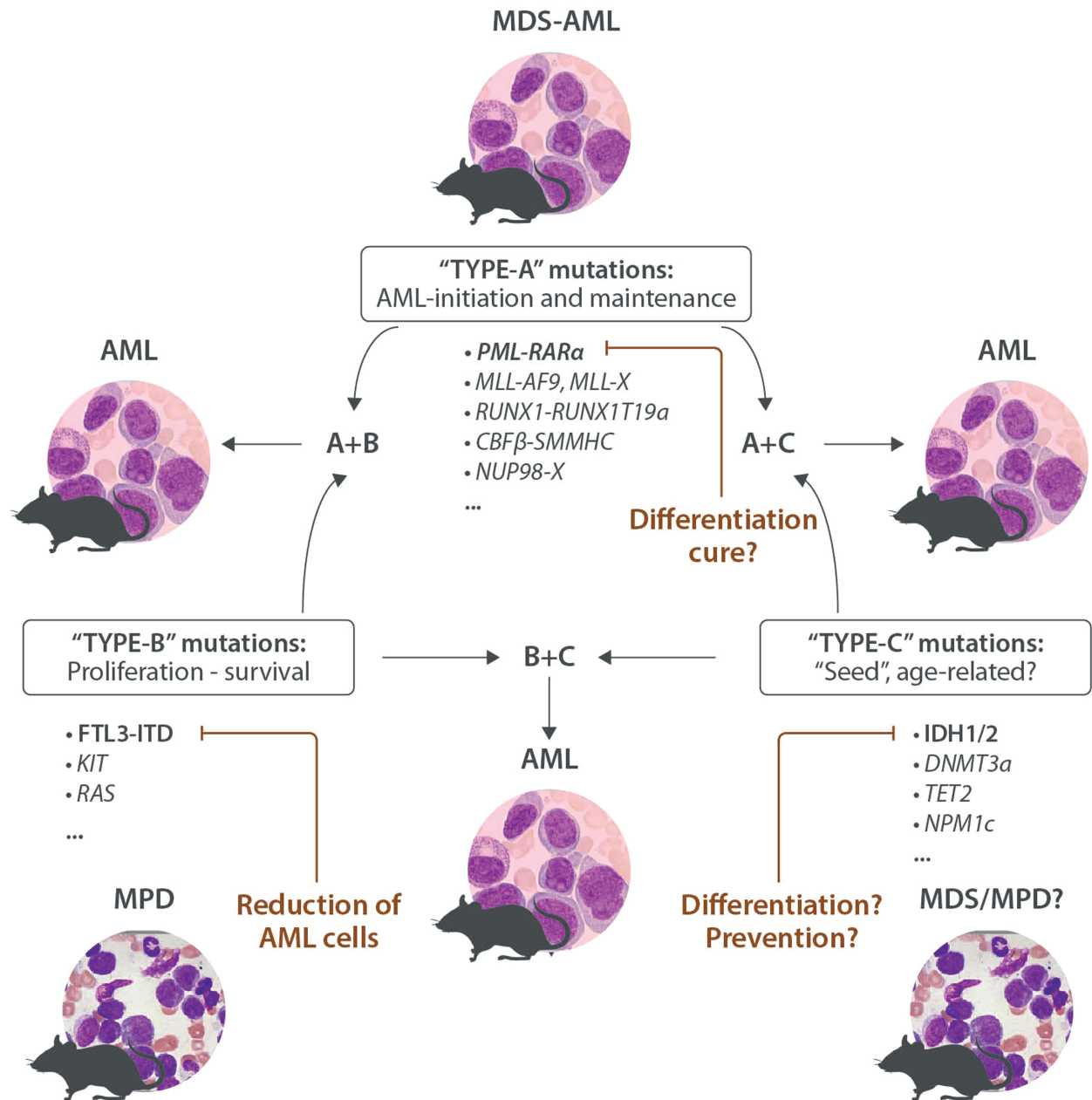
In addition, many mouse AML models are based on BM transplants into irradiated recipients. It was recently shown that total body irradiation of recipient mice permanently damages the BM stroma interfering with efficient engraftment and significantly affecting innate immunity.<sup>96</sup> However, there is increasing evidence that the immune system plays an essential role in expansion of leukemic clones in the BM. Using conditional transgenic mouse models, researchers showed that the absence of an intact immune system resulted in a 10- to 1000-fold reduction in the rate, extent, and duration of regression of mouse T-cell ALL upon inactivation of a potent driver oncogene.<sup>97</sup> The transplantation of rMLL-AF9 cells into an immune-competent mouse model revealed the potential role of the cytotoxic T-cell response in recognizing immunogenic antigens expressed on AML cells.<sup>98</sup> Importantly, it was reported that numerous empty niches exist in the mouse BM in steady-state hematopoiesis that allow low-level donor HSPC engraftment in nonconditioned recipients.<sup>99</sup> Several strategies are currently being explored to improve the transfer of hematopoietic cells without irradiation such as the application of blocking or toxin-conjugated antibodies targeting c-Kit or CD45, respectively, that selectively reduce the number HSC in the niche.<sup>100,101</sup> It remains open for debate whether and how such strategies may affect the long-term innate and adaptive immunity of the host and clonal expansion of AML cells.

In contrast to the majority of the patients, putative leukemogenic mutations are often expressed in the BM of young mice that most likely do not carry any additional mutations that define clonal hematopoiesis or a preleukemic state. Therefore, novel mouse strains are needed in which the animals carry 1, 2, or more mutated alleles to appropriately phenocopy this situation. These mice will be particularly useful to determine the potency of a given mutation for progression toward AML. In addition, it is of great importance to model how these mutations will allow the cells to escape immune surveillance, and to ascertain whether boosting immune recognition might facilitate the elimination of relapsing clones. It is thus essential to functionally dissect which alterations are “seed” mutations (capable of inducing a preleukemic state and ultimately lead to AML) from those that provide “soil” for other events supporting leukemogenesis. Conditional activation of mutant alleles will be essential to control the appropriate sequence of events, as some of the most prevalent mutations such as *FLT3-ITD* seem to occur rather late during leukemogenesis, whereas others such as *DNMT3A*, *IDH1/2*, or *TET2* mutations may primarily act as inducers of a preleukemic state. In fact, several studies have shown that expression of mutations associated with preleukemic states such as *NPM1*, *TET2*, or *DNMT3A* in HSPC carrying the *FLT3-ITD* mutation induce an AML phenotype in mice.<sup>102–107</sup> However, whether conditional *FLT3-ITD* expression in preleukemic cells with *DNMT3A* or *TET2* mutations will result in the same phenotype, remains to be shown. Recent studies suggest that the FLT3 protein is predominantly expressed in multipotent myeloid progenitor cells but only in a very small fraction of HSC.<sup>108</sup> It is also not clear how to appropriately model AML originating from HSC as this cell compartment seems to be rather heterogeneous.<sup>109</sup> In addition, expression of FLT3-ITD mutations may actively deplete the normal HSC reservoir suggesting that cooperating FLT3-ITD mutations arise in a more differentiated



GMP-like cells rather than in the HSC compartment harboring preleukemic mutations.<sup>108</sup> Several studies have shown that the nature of a given AML-associated mutation necessitates careful choice of approach in order to appropriately model its effect in different levels of the hematopoietic hierarchy. Inactivation of a gene that is recurrently targeted by loss-of-function mutations in AML will not necessarily result in an identical phenotype as

conditional activation of mutant knock-in allele driven by its native promoter, as illustrated by the divergent phenotypes of different approaches to model the biology of AML-associated *ASXL1* (*additional sex combs like 1*) gene mutations: while *Asxl1* deletion resulted in MDS, expression of a mutated knock-in allele resulted in increased susceptibility for leukemic transformation of the HSC compartment in mice.<sup>110–112</sup>



**Figure 4. Mouse models functionally classify AML-associated mutations.** “TYPE-A mutations” initiate and/or maintain a leukemic phenotype in the mouse. Targeted inactivation might have the potential to cure the disease, as illustrated by successful therapy of *PML-RARA*-driven APL by ATRA and arsenic. “TYPE-B mutations” support proliferation and survival of HSPCs; they induce a myeloproliferative disease (MPD) in mice. Targeting TYPE-B mutations has antileukemic potential but is probably insufficient to eradicate the disease as illustrated by the clinical efficacy of small molecule FLT3 inhibitors. “TYPE-C mutations” (often affecting epigenetic regulators of DNA methylation) are often found in older individuals with clonal hematopoiesis and patients with MDS, MPN, and AML. Mouse models suggest that they may affect differentiation and self-renewal, as well as proliferation, leading to myeloproliferation and/or dysplasia, but they seem not sufficient to induce AML. TYPE-C mutations also seem to be suitable therapeutic targets as illustrated by the potent clinical activity recently reported for small molecules that selectively target AML-associated *IDH2* mutations. Mouse models have demonstrated that the combination of A + B, A + C, and B + C TYPE mutations is sufficient to rapidly induce an AML-like disease. AML = acute myeloid leukemia, APL = acute promyelocytic leukemia, ATRA = all-trans retinoic acid, HSPCs = hematopoietic stem and progenitor cells, MDS = myelodysplasia, MPN = myeloproliferative neoplasm.

## What can one learn from mouse AML models?

Despite the inherent limitations, mouse models have proven to be important pillars for a better understanding of AML biology and for searching for and validating novel therapeutic strategies. As outlined above, AML is generally modeled by expression of distinct leukemia-associated mutations in particular cells of the hematopoietic system. Hereby, expression of AML-associated fusion genes involving transcription factors or other epigenetic regulators may result in a leukemic disease after a long latency (eg, some MLL fusions) or is not sufficient to induce any disease (eg, CBF or RARA fusions) (Fig. 4). However, functional studies have shown that these alterations are generally necessary for the maintenance of the transformed phenotype and might therefore be rational therapeutic targets. The PML-RARA fusion in APL is the archetype of this type of mutations (hereby called “TYPE-A mutations”): targeted degradation of the fusion protein by treatment with pharmacologic doses of all-trans retinoid acid (ATRA) and/or arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) is able to successfully treat patients without traditional chemotherapy.<sup>113</sup>

Alterations that constitutively activate kinases, such as fusions or mutants involving *ABL*, *PDGFR*, *KIT*, *FLT3*, or *JAK2* or related signaling mediators (such as signaling mediators of the RAS-MAPK pathway) primarily support proliferation/survival rather than affecting differentiation of HSPC. Transgenic expression of such mutations (hereby called “TYPE-B mutations”) in the hematopoietic system of the mouse generally leads to the development of a lethal MPD. Mouse models of tyrosine kinase-driven MPD helped to preclinically explore the *in vivo* efficacy of several highly potent and selective small molecules such as inhibitors blocking *JAK2* or *FLT3*.<sup>24,114–116</sup> Based on the coexistence of TYPE-A and TYPE-B mutations in some AML or CML blast crisis patients, a large number of mouse models were consequentially generated and used to show that coexpression of TYPE-A and TYPE-B mutations did indeed cooperate to rapidly induce AML-like phenotypes often after a short latency.<sup>62,117–121</sup>

The third type of genetic alterations classically characterizes clonal hematopoiesis and preleukemic states including point mutations in *IDH1/IDH2*, *DNMT3A*, *TET2*, *NPM1c*, and others. Based on their potential we call them “seed mutations” or “TYPE-C mutations.” Several studies have shown that expression of these lesions in the hematopoietic system of the mouse induce a wide spectrum of diseases including MPD, MDS, or both but are generally not sufficient to induce AML.<sup>105,122–125</sup> Several recent studies demonstrated that TYPE-C mutations functionally collaborate with TYPE-A or TYPE-B mutations resulting in AML with high penetrance in mice.<sup>106,107,126–129</sup>

All 3 types of mutations (A-B-C) are potential therapeutic targets. Targeting of TYPE-A mutations is most likely the best path to take to cure a fraction of AML in which the blasts remain fully addicted to the activity of the respective potent driver oncogene. Several critical protein-protein interactions have been identified and an increasing number of specific and potent small molecules are currently being tested that impair the transforming activity of these driver lesions. Following the success of the APL-associated PML-RARA fusion, most likely only targeted degradation below a yet-to-be-defined biological threshold will be able to efficiently eliminate the leukemic clones and ultimately cure the disease. Although targeting of TYPE-B mutations (eg, by small molecule inhibitors) clearly has clinical antileukemic activity it seems to be insufficient to eliminate the disease and therefore needs to be combined with traditional chemotherapy.<sup>130</sup> Recently compounds have been developed that selectively

block particular TYPE-C mutations such as mutant *IDH1* and *IDH2*. Emerging mouse model studies have demonstrated that these compounds are able to differentiate AML blasts with potent preclinical and promising clinical activity leading to fast FDA approval as antileukemic therapeutics.<sup>131–133</sup>

Collectively, mouse models represent the cornerstones to study AML biology and provide platforms to explore novel therapeutic strategies. Nardella and coworkers proposed that mouse models could be integrated more closely with clinical trials, the so-called “coclinical trial,”<sup>134</sup> where trials in mice would be operated contemporaneously with phase I/II clinical trials. Identification of key genetic and/or molecular factors that could affect patient outcome might be identified in appropriate mouse models allowing for better stratification of patient cohorts as well as the possibility of testing drug combinations to overcome acquired resistance.

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