Review Article

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Mouse models of MLL leukemia: recapitulating the human disease

Thomas A. Milne

Weatherall Institute of Molecular Medicine, Medical Research Council Molecular Haematology Unit, University of Oxford, Oxford, United Kingdom

Chromosome translocations involving the mixed lineage leukemia (*MLL*) gene fuse it in frame with multiple partner genes creating novel fusion proteins (MLL-FPs) that cause aggressive acute leukemias in humans. Animal models of human disease are important for the exploration of underlying disease mechanisms as well as for testing novel therapeutic approaches. Patients carrying MLL-FPs have very few cooperating mutations, making MLL-FP driven leukemias ideal for animal modeling. The fact that the MLL-FP is the main driver mutation has allowed for a wide range of different experimental model systems designed to explore different aspects of MLL-FP leukemogenesis. In addition, MLL-FP driven acute myeloid leukemia (AML) in mice is often used as a general model for AML. This review provides an overview of different MLL-FP mouse model systems and discusses how well they have recapitulated aspects of the human disease as well as highlights the biological insights each model has provided into MLL-FP leukemogenesis. Many promising new drugs fail in the early stages of clinical trials. Lessons learned from past and present MLL-FP models may serve as a paradigm for designing more flexible and dynamic preclinical models for these as well as other acute leukemias. (*Blood*. 2017; 129(16):2217-2223)

Introduction to *MLL* leukemias

Animal models of human disease are important for the exploration of underlying disease mechanisms as well as for testing novel therapeutic approaches. In order for this to be effective, the animal model should recapitulate if not the entire human disease phenotype, then at least the key attributes under study.

Rearrangements of the mixed lineage leukemia (*MLL*) gene cause aggressive acute lymphoblastic (ALL) and acute myeloid (AML) leukemias that generally respond poorly to treatment. The most common *MLL* rearrangements (MLLr) are chromosome translocations that fuse the *MLL* gene in frame to 1 of >121 different partner genes,[1] creating novel MLL fusion proteins (MLL-FPs). *MLL* fusions with the *AF4* (ALL-1 fused gene from chromosome 4), *AF9* (ALL-1 fused gene from chromosome 9), *ENL* (eleven-nineteen leukemia), *AF10* (ALL-1 fused gene from chromosome 10), *ELL* (eleven-nineteen lysine-rich leukemia), or *AF6* (ALL-1 fused gene from chromosome 6) genes represent ~85% of all *MLL* translocations, making this group the most common fusion partners.[1]

MLL translocations can potentially generate 2 fusion protein products in the same patient. For example, the t(4;11)(q21;q23) translocation (or t(4;11) for short) can produce both an *MLL-AF4* (5′-*MLL* translocation) and an *AF4-MLL* (3′-*MLL* translocation) fusion, both of which can produce functional proteins.[2] In many MLLr patients, the 3′-*MLL* translocation either is not in frame[1,3] or does not produce a detectable transcript,[3-5] whereas transcripts from the 5′-*MLL* translocation are detected in 100% of patients.[3-7] Although 3′-*MLL* translocations can have oncogenic potential,[7,8] the 5′-*MLL* translocation is usually considered to be the main driver of *MLL* leukemogenesis.

Relative to other acute leukemias, MLL-FP–driven leukemias generally have very few cooperating mutations.[3,9] The lack of cooperating mutations is especially true for infant MLLr ALLs that have a silent mutational landscape, except for rare activating mutations in *RAS* or *FLT3* that are often subclonal.[3,10] Conversely, in MLLr AML, the presence of a cooperating mutation can be detected in as many as 40% to 50% of cases,[11,12] and *RAS* and *FLT3* mutations have both been shown to accelerate leukemogenesis in MLL-FP mouse models.[11,13] Even in AML, however, MLL-FPs stand out as having a relatively low number of additional mutations.[9] Thus, the relative genetic simplicity of MLLr leukemias makes them potentially ideal for in vivo modeling and for testing the balance between the instructive role of strong oncogenes versus the importance of the cell of origin. For these reasons, it is worth revisiting how well different MLL-FP models have managed to recapitulate the human disease and what key principles this work reveals.

Key aspects of MLL-FP disease phenotypes in patients

As a group, MLLr leukemias are associated with a poor prognosis in both AML and ALL. However, recent work has suggested that prognosis varies with the specific translocation. For example, MLL-AF6 and MLL-AF10 are individually predictive of a significantly worse overall survival,[14-16] whereas MLL-AF9, MLL-ELL, and MLL-ENL are all associated with an intermediate to favorable prognosis in AML.[14-16] MLL-AF4 has a very poor prognosis in infant ALL as well as pediatric and adult ALL and AML,[15,16] whereas MLL-ENL displays a favorable prognosis in T-cell ALL.[16]

Specific MLL-FPs are also associated with distinct disease phenotypes. For instance, MLL-AF10, MLL-ELL, and MLL-AF6 are all predominantly associated with AML rather than ALL.[1] MLL-AF4 is predominantly associated with pro-B-ALL[1,10] and much more rarely with AML.[1,16] The pro-B phenotype of MLL-AF4 B-cell ALL (B-ALL) is consistent with the observation that MLLr B-ALLs usually lack expression of the pre-B-cell receptor and are generally resistant to BCR pathway kinase inhibitors.[17] MLL-AF9 is found in both B-ALL and AML in infants and children, but is predominantly associated with AML in adults,[1] whereas MLL-ENL is more commonly found in B-ALL than AML.[1] MLL-ENL is also the only MLL-FP that is also occasionally associated with T-cell ALL.[16]

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Further complicating matters, there are molecular subdivisions among MLL-FP patients. Gene expression profiles clearly show that MLLr infant ALL is a distinct entity as a group, but it is also possible to subdivide MLLr infant ALL into *MLL-ENL/ELL*, *MLL-AF9*, and *MLL-AF4* subsets.[18] MLL-AF4 infant ALL can be further subdivided into patients that express *HOXA* cluster genes,[18,19] and a second group that is *HOXA* negative and correlates with a worse prognosis.[18,19] MLL-AF4 ALL samples can also be subdivided on the basis of *FLT3* gene expression levels, with high expression of *FLT3* associated with a worse prognosis.[20]

Although MLLr leukemias are often classified as either predominantly AML or ALL, B-cell markers are often coexpressed with specific myeloid markers in MLLr ALL.[21] Gene expression profiling also indicates that MLLr ALL is a distinct entity on a molecular level from both conventional ALL and AML.[22,23] The importance of the mixed nature of MLLr ALL is highlighted by the observation that MLLr B-ALL can escape CD19 chimeric antigen receptor–T-cell therapy by relapsing as a $CD19^-$ AML.[24] The complexity of the patient data underscores the importance of having robust experimental models that can be used to test hypotheses generated from clinical observations.

General aspects of MLL-FP models in mice

In this review, the term "mouse models" will be used in a broad sense to also include a discussion of human cells when used in combination with xenotransplant assays. There are 4 general categories of models: (1) viral transduction of mouse cells followed by engraftment into recipient mice; (2) MLL-FP expression from the endogenous *Mll* locus; (3) viral transduction and/or genetic engineering of human cells combined with xenografts; or (4) xenografts of patient-derived cell lines or primary patient samples. Variations on each approach will be discussed in greater detail below, but each has different strengths and weaknesses. It is also worth noting that although murine hematopoiesis is similar to humans, there are key differences in physiology. For example, peripheral blood is highly neutrophil rich in humans but is instead lymphocyte rich in mice.[25] These differences could have a profound influence on the behavior of leukemic cells in mouse models relative to patients. Figure 1 outlines the publication timeline for the main models discussed in this review, which will mainly focus on the 3 most common MLL-FP models: MLL-AF4, MLL-AF9, and MLL-ENL.

Modeling MLL-AF9 leukemias in mice

One of the first MLL-FP models was the creation of an *Mll-AF9* knock-in.[26] One potential difficulty with this model is that heterozygosity for *Mll* in all cells throughout development also produces developmental defects.[27] However, despite expression in other tissues, after an early myeloproliferative phase, *Mll-AF9* mice primarily succumbed to AML, and also in rare cases to ALL,[28,29] a distribution that is reminiscent of adult MLL-AF9 leukemias.[1]

To more accurately model leukemogenesis, the Rabbitts laboratory developed an elegant in vivo translocator model with an ingenious use of the *Cre-loxP* system.[30] Mice were engineered with *loxP* sites inserted into the introns of the endogenous *Mll* and *Af9* genes corresponding to the most common translocation breakpoints in patients.[30] As with a similar *Mll-Enl* translocator model,[31] they were then able to use *Cre* expression driven by lineage-specific promoters to induce *Mll-Af9* translocations in vivo.[32] One surprising result was that *Mll-Af9* translocations in primitive progenitor cells (driven by *Lmo2-Cre*) produced an AML, whereas translocations in T cells (driven by *Lck-Cre*) were detectable but had no ability to initiate leukemia.[32] This result contrasts with the *Mll-Enl* translocator model where *Lmo2-Cre* produced AML and *Lck-Cre* produced both lymphoid and myeloid tumors,[32] leading to 3 key conclusions: (1) the Af9 and Enl partner proteins are functionally distinct, (2) the phenotype of the leukemia is dependent on both the fusion partner as well as the target cell type, and (3) *Mll-Enl* can potentially cause lineage switching.

One disadvantage of the translocator model is that it is dependent on restricting *Cre* expression to specific cell lineages, something that can be difficult to control precisely. In an attempt to more directly target specific cell types, viral transduction of *MLL-AF9* into highly purified granulocyte/macrophage progenitors (GMPs) was used to produce a transplantable AML that is phenotypically downstream from a normal GMP.[33,34] Earlier work had already established that highly purified $lineage^-$, $Sca1^+$, $c\text{-}kit^-$ (LSK) cells (a mixed population of hematopoietic stem cells [HSCs] and early progenitors), common myeloid progenitors (CMPs), or GMPs could all be transduced by *MLL-ENL* to produce a similar AML in mice.[35] Thus, it was concluded that both *MLL-AF9* and *MLL-ENL* could target more committed progenitors to produce AML. However, more recent work suggests that *MLL-AF9* retroviral transduction of LSK cells produces a much more aggressive leukemia than *MLL-AF9* transduction of GMPs.[36] MLL-AF9-LSK cells also have a gene expression signature that correlates with a poor prognosis in patients,[36] indicating that the target cell type might have significance for the human disease.

Two major problems with retroviral transduction studies are that the transgene may be expressed at nonphysiological levels, and the selection process of retroviral transduction could theoretically enrich for a rare cell impurity. To address this, Chen et al[37] used the *Mll-AF9* knock-in mouse to purify LSKs, CMPs, GMPs, and common lymphoid progenitors (CLPs) for transplantation into secondary recipients. They found that *Mll-AF9* knock-in LSK cells were more efficient at transplanting AML than CMPs or CLPs, while GMPs were entirely unable to induce AML in secondary recipients.[37] The difference between these results and the retroviral work was attributed to artificially high expression levels in retroviral transduction assays,[37] but because knock-in *Mll-AF9* is expressed throughout development and throughout the body, these experiments did not address leukemia initiation limited to specific target cell types.

A doxycycline (DOX) -inducible *MLL-AF9* mouse model was used in an attempt to directly address the target cell type question by purifying different cell populations such as long-term HSCs (LT-HSCs, Lin^- $CD34^-$ $Sca\text{-}1^-$ $c\text{-}Kit^+$ $CD150^+$ $CD48^-$) and GMPs.[38] Induced *MLL-AF9* expression in LT-HSCs (MLL-AF9-LT-HSC) produced a highly aggressive short latency leukemia that was resistant to chemotherapy, whereas MLL-AF9-GMP leukemias had a longer latency and were more responsive to treatment.[38] Similar to previous work,[36] MLL-AF9-LT-HSC AML also has a gene expression profile that correlates with a worse prognosis in patients than MLL-AF9-GMP AML,[38] again suggesting that the target cell type in patients could potentially be a key aspect of determining response to therapy.

None of the MLL-AF9 models discussed so far consistently produced MLL-AF9 ALL in mice, something that is commonly found in infants and children.[1] Transplantation of *Mll-AF9* knock-in fetal liver cells into secondary recipients produced a long-latency leukemia with lymphoid potential,[39] suggesting that the target cell type could be key for producing *MLL-AF9* ALL in mice. More strikingly, transduction of human cord blood (CB) cells with *MLL-AF9* produced both AML and B-ALL[40] as well as some MLLs[41] in xenografts, with secondary transplants replicating the phenotype of the primary recipients. Engraftment of *MLL-AF9* cells into NS-SGM3 mice that provide an

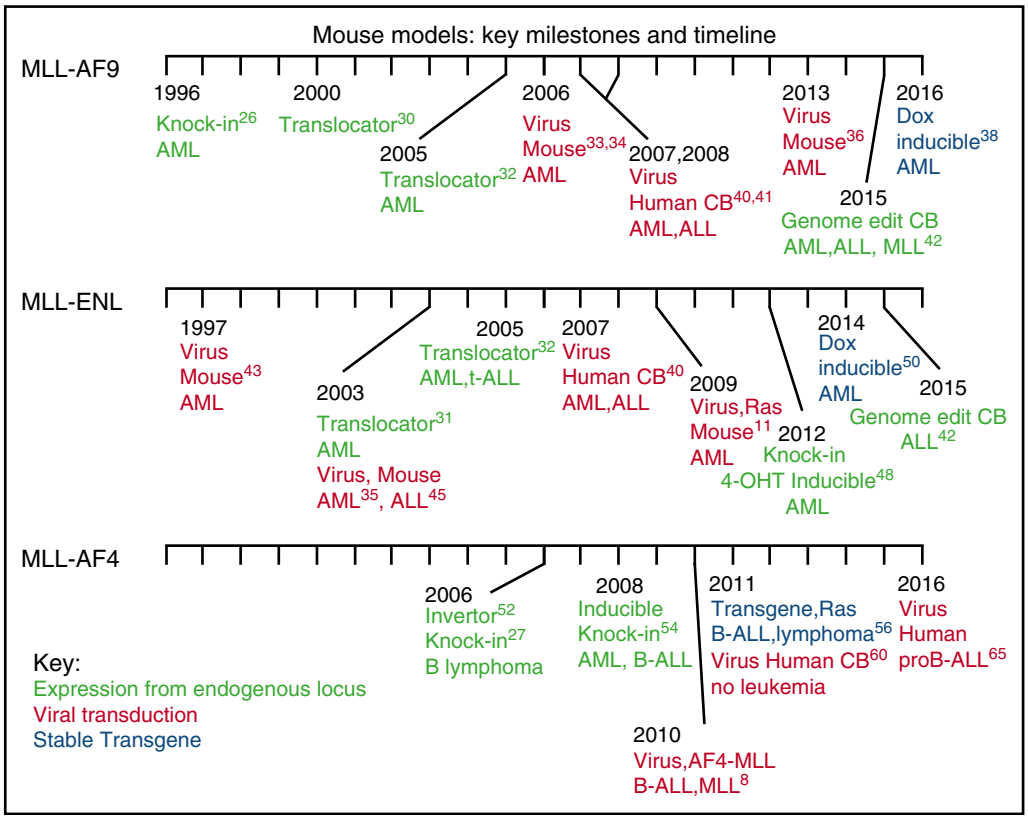

Figure 1. Mouse models: key milestones and timeline. A schematic of publication dates and the disease phenotype for different model systems. Green, expression from the endogenous locus; red, viral transduction models; blue, stable transgene models. MLL-AF9 models: 1996,[26] 2000,[30] 2005,[32]2006,[33,34] 2007,[40]2008,[41]2013,[36]2015,[42] 2016.[38] MLL-ENL models: 1997,[43] 2003,[31,35,45] 2005,[32] 2007,[40] 2009,[11] 2012,[48] 2014,[50]2015.[42] MLL-AF4 models: 2006,[27,52] 2008,[54] 2010,[8] 2011,[56,60] 2016.[65] OHT, 4-hydroxytamoxifen; Ras, rat sarcoma mutation; t-ALL, T-cell acute lymphoblastic leukemia.

in vivo cocktail of human cytokines (stem cell factor, granulocyte-macrophage colony-stimulating factor, and interleukin-3) resulted in primarily AML,[41] indicating that the microenvironment may have an influence on leukemia phenotype. More recent work where the *AF9* complementary DNA (cDNA) was directly engineered into the endogenous MLL locus of $CD34^+$CB cells also produced ALL, AML, and MLLs in xenotransplants.[42] These results will also be discussed further below in the context of *MLL-ENL*, but together they suggest that using human cells as a target may be a key aspect of recapitulating the human disease.

The main conclusion from all this work is that replicating the MLL-AF9 human phenotype is a complex combination of the specific fusion partner, the target cell, the species of the target cell (ie, mouse versus human), the developmental stage of the target cell, as well as the influence of the microenvironment.

Modeling MLL-ENL leukemias in mice

The earliest *MLL-ENL* mouse model used retroviral transduction into primitive bone marrow cells to produce AML in engrafted mice.[43] There are several advantages to this approach, including the possibility of parallel extensive in vitro characterization of transformed cells.[43,44] As previously mentioned, *MLL-ENL* could transform LSKs, CMPs, or GMPs, and all produced an AML in mice.[35] Using an in vitro B-cell differentiation system, retroviral transduction of *MLL-ENL* could also produce a leukemia reminiscent of human MLL-ENL ALL.[45]

To extend the functionality of the retroviral transduction approach, an inducible model was developed where MLL-ENL was fused to ERTm (the ligand-binding domain of the estrogen receptor modified to specifically recognize synthetic but not endogenous estrogens), allowing for 4-hydroxytamoxifen–inducible activation of the protein in transformed cells.[46] The MLL-ENL-ERtm model was instrumental in showing that MLL-ENL could induce a reversible myeloid differentiation block maintained by *HOXA9* and *MEIS1* expression[46] and showed that MLL-ENL binds directly to target genes such as *HOXA9* and *MEIS1* and activates them by promoting increased histone H3 lysine 79 dimethylation (H3K79me2) levels.[47] In an attempt to develop a more physiologically relevant in vivo model, MLL-ENL-ERTm was inserted into the endogenous mouse *Mll* locus so that MLL-ENL was both inducible and expressed at physiological levels.[48] Administering Tamoxifen (and activating the MLL-ENL-ERTm fusion protein) resulted in a long latency myeloproliferative disorder that only progressed to a highly transplantable AML through inhibition of the DNA damage response with caffeine.[48] Interestingly, this low level of MLL-ENL aggressiveness was somewhat reminiscent of earlier work using the *Mll-Enl* translocator model.[31]

As already briefly discussed above, the Rabbitts laboratory developed an elegant in vivo *Mll-Enl* translocator model using *Lmo2-Cre* to produce a "myeloid-like leukemia" that also had aspects of a myeloproliferative disorder.[31] It was also a nontransplantable leukemia[31] unless cultured in vitro first,[32] suggesting that it was a much less aggressive leukemia than that observed using retroviral transduction. One possibility is that MLL-ENL is dependent on acquiring additional mutational "hits" during this myeloproliferative phase. MLLr AML patient samples are associated with *NRAS* mutations (24%), and this

was used to construct an *MLL-ENL* retroviral construct that also carried an $Nras^{G12D}$ mutant as an *IRES* fusion.[11] Retroviral transduction of this construct produced an AML that was much more aggressive and rapid than with MLL-ENL alone,[11] showing that MLL-ENL can cooperate with additional oncogenic hits. Similar additional mutations may be required for MLL-ENL leukemia progression in humans.

As already mentioned, *Mll-Enl* has the surprising capability of inducing both myeloid and lymphoid leukemias when expressed in the T-cell compartment using *Lck-Cre*.[32] Using *Rag-Cre* (B- and T-cell precursors) and *CD19-Cre* (mainly B cells) to drive *Mll-Enl* translocation events, it was also found that although *Rag-Cre* produced similar myeloid-like leukemias as before, *CD19-Cre* failed to induce leukemogenesis even though the *Mll-Enl* translocation was clearly present.[49] Thus, *Mll-Enl* could not promote leukemogenesis in B cells in this model even though MLL-ENL is found in B-ALL in humans.

To more precisely define possible target cell types for MLL-ENL in AML, Ugale et al created an MLL-ENL DOX-inducible system.[50] Induction of MLL-ENL in precursor GMPs or CLPs produced a transplantable AML, but GMPs were relatively unresponsive,[50] contrasting with earlier retroviral transduction results.[35] In addition, unlike the *Mll-Enl* translocator model,[32] this DOX-inducible MLL-ENL produced only AML when expressed in T-cell progenitors.[51] More surprisingly, when they subfractionated the LSK compartment into granulocyte-monocyte-lymphoid progenitors, multipotent progenitors, and $CD150^{+}CD48^{-}$ HSCs, only granulocyte-monocyte-lymphoid progenitors produced a transplantable AML.[50] *MLL-ENL* expressing HSCs not only failed to induce AML, they also displayed severely compromised reconstitution potential.[50] These results sharply contrast with those using DOX-inducible *MLL-AF9* where expression in HSCs produced a much more aggressive AML.[38] One possible explanation for this discrepancy is that ENL and AF9 are functionally distinct on the molecular level, which could also explain previous results, indicating that ENL and AF9 had differing abilities to transform T cells.[32]

MLL-ENL is often associated with ALL in adults and children, and yet it took extensive in vitro treatment to produce a lymphoid leukemia in mouse cells.[45] Barabé et al were able to use stem- and progenitor-enriched human CB cells infected with *MLL-ENL* to produce a $CD19^{+}$ ALL in xenografts.[40] This propensity to produce B-ALL could be shifted toward mixed AML and B-ALL, or just AML if the *MLL-ENL*–transduced cells were first cultured in myeloid-promoting suspension cultures for 50 or 70 days, respectively, before engraftment.[40] When *MLL-ENL* B-ALL leukemias were harvested from mice and cultured in vitro, they were also sometimes able to switch to a myeloid blast culture and even back again to B-lineage cells.[40] This ability to switch between both AML and ALL is reminiscent of the *Mll-Enl* translocator results, although with T-ALL.[32] In support of these experiments, more recent work using transcription activator-like effector nucleases in $CD34^{+}$ CB cells to insert either the *AF9* or the *ENL* cDNA directly into the endogenous *MLL* locus produced a similar phenotypic distribution in xenotransplants. *MLL-ENL* predominantly produced ALL (unless first cultured in vitro under myeloid growth conditions prior to transplantation), whereas *MLL-AF9* could produce AML, ALL, as well as MLLs.[42] Of the models discussed so far, xenografts with transformed human CB are best able to replicate the B-ALL phenotype observed in patients, for both MLL-AF9 and MLL-ENL.

Modeling MLL-AF4 leukemias in mice

Although MLL-AF4 is the most common MLL-FP in patients[1] and is associated with a very aggressive leukemia, it has been the most difficult to model in mice. The reasons for this are not yet completely understood and are likely due to a range of different factors.[10] Broadly speaking, infant ALL caused by MLL-AF4 is considered to be a distinct entity from childhood or adult MLL-AF4 ALL,[10] so several MLL-AF4 mouse models may be required.

Building on the success of *Mll-Af9* and *Mll-Enl* in vivo models, *Mll-AF4* models were generated using both a knock-in approach[27] and a *Cre*-inducible invertor model.[52] For the invertor model, an *AF4* cDNA flanked with *loxP* sites inserted in reverse orientation into the endogenous *Mll* locus could be induced with *Cre* expression to flip into the proper orientation.[52] Both models produced a similar diffuse, large B-cell lymphoma rather than the more immature acute leukemias seen in humans.[27,52] Targeting more primitive cell types with *Lmo2-Cre* caused embryonic lethality,[52] indicating that the MLL-AF4 target cell for transformation is not likely to be an HSC or an early progenitor cell. Instead, the invertor model was able to produce B-cell lymphomas by targeting B- or T-cell progenitors with *Rag-Cre*, *Lck-Cre*, or *CD19-Cre*, suggesting that AF4 has the capability of inducing lineage switching, and that lymphoid progenitors may be the target cell type for MLL-AF4. This observation is supported by work showing that MLL-AF4 leukemia initiating clones from patients could originate from very early progenitors, but the founder clone was more often a cell that had already undergone immunoglobulin/T-cell receptor rearrangement.[53]

In an attempt to produce a different *Cre*-inducible mouse model, a *LoxP* flanked stop cassette was inserted before an endogenous *Mll-AF4* knock-in.[54] Using either a polyinosinic/polycytidylic acid–inducible *Mx Cre* driver or an in vitro transduction with a *Cre*-expressing virus, *Mll-AF4*-expressing mice developed both AML and pre-B-ALL as well as a few MLLs. No pro-B-ALLs were observed, and this model was slightly biased toward producing AML rather than B-ALL, both of which differ from the disease phenotype observed in humans, especially infant ALL.[10] However, this study was a very important step forward, and it established a crucial connection between MLL-AF4 activity and increased H3K79me2 levels at gene targets.[54]

As discussed briefly in the introduction, MLL-AF4 is considered to be the main driver of t(4;11) leukemias, but the difficulty in establishing a robust model raises the possibility that MLL-AF4 either needs to target the right cell type or may require cooperating mutations. For infant ALL, MLL-AF4 may target a fetal cell that is intrinsically more susceptible to transformation, thus producing rapid leukemia growth. To address this possibility, the invertor model[52] and *VE-Cadherin Cre* were used to target *Mll-AF4* expression to early hematopoietic precursor cells during mouse embryonic development.[55] Expression of *Mll-AF4* in these early precursor cells conferred an enhanced lymphoid potential on lymphoid-primed multipotential progenitor fetal cells within a developmental window of days E12 to E14, but despite this increase in lymphoid potential during embryogenesis, the mice still only displayed a long latency B-cell lymphoma.[55]

To test cooperativity with other mutations, a transgenic mouse model that overexpressed both *MLL-AF4* and a *KRAS* mutation produced both B-cell lymphomas and B-ALL at an accelerated rate.[56] However, in patients, *RAS* mutations are only rarely present, are subclonal, and are often lost upon relapse.[10] Conversely, *AF4-MLL* transcripts are found in 50% to 80% of patients.[3,4,6] To test the importance of *AF4-MLL*, Bursen et al retrovirally transduced *MLL-AF4* and *AF4-MLL* into $Lin^{-}/Sca1^{+}$ mouse cells.[8] *AF4-MLL* alone can initiate leukemogenesis, whereas both together produced pro-B-ALL and biphenotypic-ALL, a phenotype that came closest to replicating the human disease phenotype.[8,10] The importance of this result remains controversial due to the lack of *AF4-MLL* expression in some patients,[3,4,6] the fact that MLL-AF4 knockdowns alone can attenuate leukemic growth,[57] and the observation that AF4-MLL–specific small interfering RNAs have no effect on cell growth in

vitro.[58] However, a lack of expression in some patients could simply be explained by the possibility that AF4-MLL contributes to leukemic initiation rather than maintenance. Also, even when *AF4-MLL* is not in frame or expression is missing, a functional but truncated MLL C-terminal protein can be expressed from a cryptic site, which may itself have transformation capabilities.[1,6] Finally, although the AF4-MLL small interfering RNAs were highly transcript specific,[58] they failed to reduce AF4-MLL protein levels,[2] potentially due to AF4-MLL protein stability.[59]

In an attempt to replicate past success using human cells as a target, *MLL-AF4* was transduced into $CD34^+$ CB cells. This approach produced enhanced proliferation but no overt leukemia,[60] even when combined with activating mutations in *FLT3*[61] or *KRAS*.[62] Interestingly, *FLT3* and *MLL-AF4* cooperatively block hematopoietic differentiation in a human embryonic stem cell in vitro culture system, but fail to immortalize human embryonic stem cells.[63,64] However, recent work suggests that an important problem could be that the human *AF4* sequence interferes with virus production.[65] Using the mouse *Af4* cDNA sequence instead, an *MLL-Af4* hybrid virus efficiently transformed human $CD34^+$ cells and produced a pro-B-ALL (with myeloid markers such as CD15) in xenografts.[65] *MLL-Af4* cells expressed *RUNX1* but not *HOXA9*,[65] suggesting that this model captures a specific subset of *MLL-AF4* leukemias that do not display *HOXA9* expression.[18,19] By comparing *MLL-Af4*- and *MLL-AF9*-transformed human $CD34^+$ cells, Lin et al also showed that *MLL-Af4* was much more likely to produce ALL rather than AML, and *MLL-AF9* ALL samples were blocked at a pre-B rather than a pro-B stage. This work again suggests that although the lineage of the leukemia is dependent on the target cell type, it is also strongly influenced by the fusion partner.

In the past, the most common approach for studying MLL-AF4 B-ALL has been to use patient cell lines or primary cells in xenograft experiments.[66-68] Despite the recent exciting advancement in modeling *MLL-Af4* leukemias,[65] there are differences between MLL-AF4 infant ALL, pediatric ALL, and adult ALL,[10] so more work is still needed to understand what is potentially multiple diseases.

Final conclusions and future directions

Mouse knock-in models would seem to be the most physiologically relevant model for study. However, many of the knock-in models failed to reproduce the leukemia phenotype observed in patients. The main argument against using viral transduction methods is the possibility of producing an artificial leukemia (for instance, from GMP cells) due to an overexpression artifact. It is possible however that viral transduction simply increases the probability of transforming difficult cell types, rather than producing a nonbiologically relevant leukemia. The idea that viral transduction produces clinically relevant models is supported by the observation that MLL-FP lines established in HSCs and GMPs have differential aggressiveness that correlates with gene expression profiles and prognostic outcomes in humans.[36,38] In addition, MLL-FP overexpression models can mimic the chemotherapy resistance observed in patients[11] and have also been successfully used to screen for and test novel targets and inhibitors.[69,70]

Human cells xenotransplanted into mice would seem to have a distinct disadvantage as a model—the lack of an appropriate microenvironment or niche for human cells in the mouse, the limited ability to genetically manipulate primary human cells, as well as the difficulty of obtaining certain human cell types. However, *MLL-AF9* and *MLL-ENL* human CB models have done a better job of replicating the human disease phenotype than any of the mouse cell models, suggesting that the intrinsic nature of the target cell type may be crucial. The importance of the target cell type may be particularly true for MLL-AF4, where a human fetal progenitor cell would perhaps be needed to properly model aspects of the infant disease. Recent work has also begun to address the disadvantages of xenograft models, including creating human microenvironments in mice,[71] as well as using genome editing to create endogenous MLL-FPs in human cells.[42] One major goal of animal disease modeling is to provide effective preclinical models for testing new therapies before moving to the expense and complexity of clinical trials. An emerging paradigm is to use statistically powered randomized trials in mice with large banks of patient-derived xenografts.[72] With the broader application of whole genome sequencing of patients and the relative ease of current genome editing techniques using CRISPR/Cas9 technology, it may not be long before we are able to customize models with patient-specific translocation events and any additional accompanying mutations. Such patient-specific models could some day be used as a way to effectively prescreen drug response and direct personalized therapies.

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ORCID profiles: T.A.M., 0000-0002-0413-4271.

Correspondence: Thomas A. Milne, Medical Research Council Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DS, United Kingdom; e-mail: thomas.milne@imm.ox.ac.uk.

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