Mixed lineage leukemia protein in normal and leukemic stem cells

Bon Ham Yip and Chi Wai Eric So

Leukemia and Stem Cell Biology Lab, Department of Haematological Medicine, King's College London, Denmark Hill, London SE5 9NU, UK
Corresponding author: Chi Wai Eric So. Email: eric.so@kcl.ac.uk

Abstract

Transcription factors critical for normal hematopoietic stem cell functions are frequently mutated in acute leukemia leading to an aberrant re-programming of normal hematopoietic progenitor/stem cells into leukemic stem cells. Among them, re-arrangements of the mixed lineage leukemia gene (*MLL*), including chimeric fusion, partial tandem duplication (PTD), amplification and internal exonic deletion, represent one of the most common recurring oncogenic events and associate with very poor prognosis in human leukemias. Extensive research on wild type MLL and MLL-fusions has significant advanced our knowledge about their functions in normal and malignant hematopoiesis, which also provides a framework for the underlying pathogenic role of *MLL* re-arrangements in human leukemias. In contrast, research progress on MLL-PTD, MLL amplification and internal exonic deletion remains stagnant, in particular for the last two abnormalities where mouse model is not yet available. In this article, we will review the key features of both wild-type and re-arranged MLL proteins with particular focuses on MLL-PTD and MLL amplification for their roles in normal and malignant hematopoiesis.

Keywords: mixed lineage leukemia, MLL, MLL-PTD, MLL amplification, MLL fusion, hematopoietic stem cells, Leukemic stem cells

Experimental Biology and Medicine 2013; **238**: 315–323. DOI: 10.1177/1535370213480717

Introduction

Hematopoiesis, the process of blood cell production, is based on the tight regulation of self-renewal, differentiation and apoptosis of hematopoietic stem cells (HSCs). In definitive hematopoiesis, HSCs are at the apex of the hierarchy, and they can be divided into long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs) (Figure 1). Immediately downstream of ST-HSCs is multipotential progenitors (MPPs) that has lost their self-renewal property.[1] This classical model of hematopoiesis infers that the first lineage commitment step of MPPs results in a strict separation of myeloid and lymphoid pathways, as supported by the identification of the common myeloid progenitors (CMPs)[2] and common lymphoid progenitors (CLPs).[3] However, more recent detailed analysis on the differentiation potential of $Flt3^+$ cells within the $Lin^-Sca\text{-}1^+ckit^+$ (LSK) population has revealed the existence of lymphoid primed multipotent progenitors (LMPPs) that sustains the lymphoid and myeloid differentiation potential but lacks the erythroid and megakaryocyte potentials.[4,5] Along differentiation pathway, MPPs can give rise to (1) CLPs and CMPs which can further differentiate into granulocyte/macrophage progenitors (GMPs) and megakaryocyte/erythroid progenitors; or (2) LMPPs, which can generate GMPs or CLPs (Figure 1). This process is regulated by specific transcriptional program governed by master transcription factors and epigenetic regulators. Among them, the mixed lineage leukemia gene (*MLL*) encoding a master transcription factor with a histone methyltransferase activity plays a key role in maintaining the normal functions of HSCs and its deregulation frequently leads to development of leukemia.

Normal MLL functions and hematopoiesis

MLL encodes a 3696-amino-acid protein with several distinct domains including N-terminal AT-hooks, a CXXC domain sharing homology to the DNA methyltransferase, 4 PHD fingers, a transactivation domain and a C-terminal SET domain which imparts a highly specific histone 3 lysine 4 trimethylation (H3K4me3) activity that marks for transcriptional activation (Figure 2).[6] To allow full execution of its function, MLL protein is proteolytically cleaved by taspase1 into the N-terminal fragment containing most of the DNA targeting sequence and the C-terminal fragment possessing transcriptional activation activity.[7,8] Following specific association between the N-terminal and the C-terminal fragments via the consensus interaction motifs,

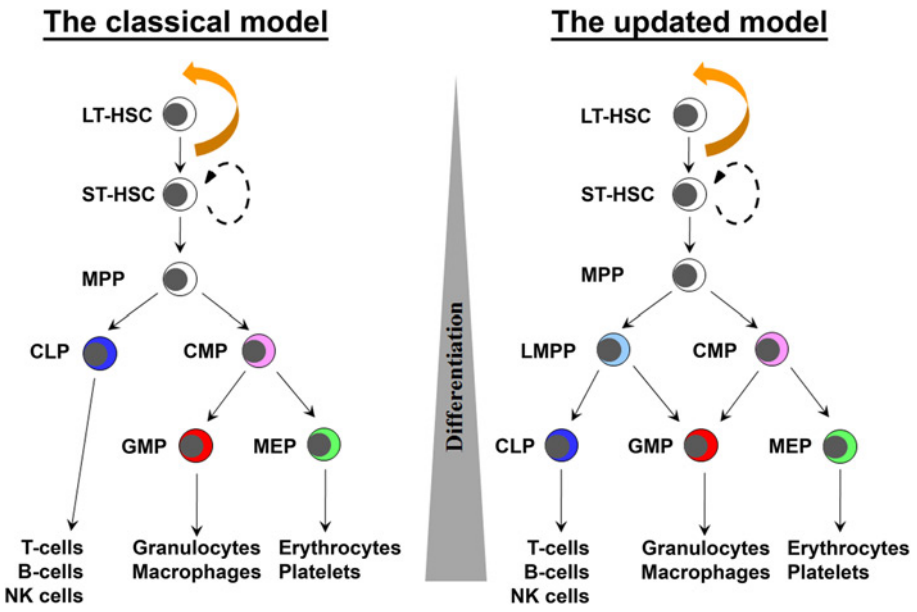

Figure 1 The classical and updated models of haematopoiesis. LT-HSC, long-term haematopoietic stem cell; ST-HSC, short-term haematopoietic stem cell; MPP, multipotential progenitor; LMPP, lymphoid primed multipotent progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte/erythroid progenitor; GMP, granulocyte/macrophage progenitor; CLP, common lymphoid progenitor; B, B-cell; T, T-cell; NK, NK cell. (A color version of this figure is available in the online journal)

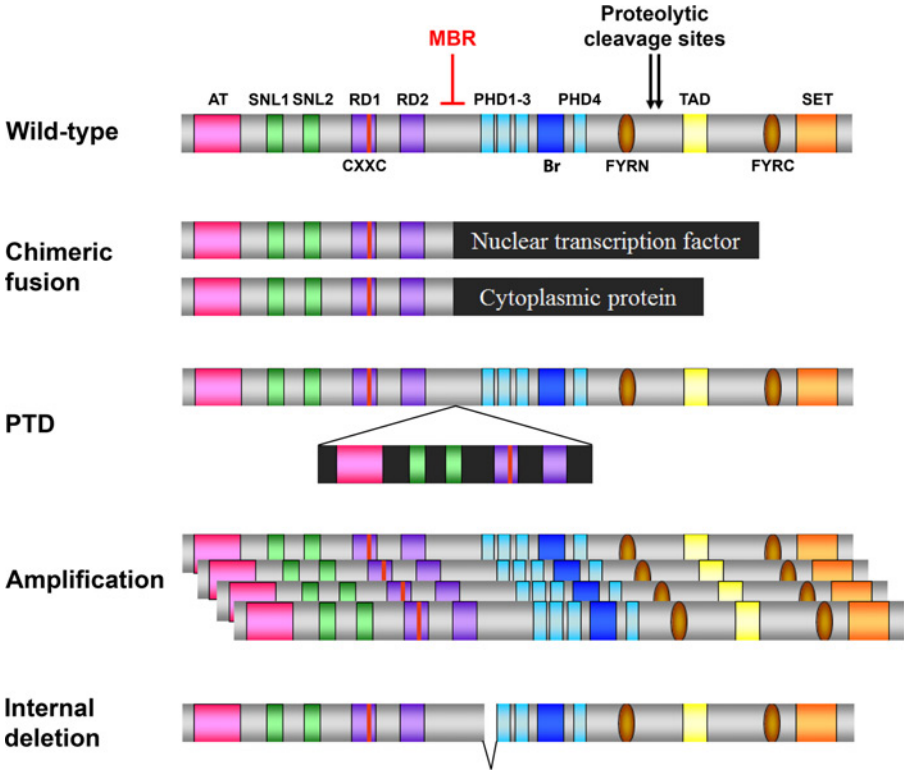

Figure 2 Schematic representation of different types of MLL re-arrangement in human leukemia. The wild-type MLL protein is included for comparison. Figures are not in scale. AT, AT hooks; SNL, subnuclear localization motif; RD, repression domain; CXXC, CXXC domain; MBR, major breakpoint region; PHD, plant homeodomain; Br, Bromo domain; FYRN, FYRN domain; TAD, transactivation domain; FYRC, FYRC domain; SET, SET domain. (A color version of this figure is available in the online journal)

they recruit and form a multicomponent supercomplex for transcriptional activation of target genes by mediating both chromatin modifications (methylation and acetylation) and chromatin re-modeling.[9]

The role of *Mll* in early development and definitive hematopoiesis was, at first, demonstrated in several studies using *Mll* complete knockout embryos.[10–12] By disrupting the endogenous *Mll* loci with a lacZ reporter, heterozygous

knockout mice ($Mll^{+/-}$) were found to have retarded growth and skeletal malformations while the homozygous mice ($Mll^{-/-}$) were embryonic lethal.[13] Analogy to the homolog of *Drosophila Trithorax*, *Mll* is essential for maintenance of *Hox* gene expression during embryonic development. Before E9, there was no difference in the pattern and level of *Hoxa7* expression between the wild-type and $Mll^{-/-}$ embryos. But at E9, in contrast to the wild-type and $Mll^{+/-}$ embryos, $Mll^{-/-}$ embryos failed to maintain the expression of *Hoxa7* in the somatic or neural tube.[14] The loss of late *Hox* expression in the absence of *Mll* indicated its function in maintenance instead of initiation for expression of *Hox* genes, which are critical for normal hematopoiesis. While $Mll^{-/-}$ embryo exhibited severe hematopoietic defects, characterization of *Mll* in adult hematopoiesis was made possible by the recent development of inducible *Mll* knockout models.[15,16] To this end, two distinctive conditional knockout models, using the Vav-Cre and MX1-Cre systems to achieve specific deletion of *Mll* exons 8 and 9 or exons 3 and 4, respectively, were generated.[15,16] Conditional deletion of *Mll* exon 3 and 4 using MX1-Cre induced rapid bone marrow failure and depletion of HSCs. On the other hand, deletion of *Mll* exon 8 and 9 by pan-hematopoietic and endothelial cell specific Vav-Cre did not grossly impair normal hematopoiesis in the mice, but HSCs derived from these knockout mice had severely compromised reconstitution ability and failed to maintain self-renewal upon transplantation/replicative stress. In spite of the different severity of the phenotypes, which might be in part due to different gene inactivation strategies, these results consistently show that *Mll* is required for maintenance of adult HSCs.

MLL re-arrangement in leukemia

Given the critical function of MLL protein in normal HSC functions, it is not difficult to envision that MLL is also a frequent target in human leukemia. *MLL* located on chromosome 11q23 is frequently re-arranged in acute leukemias, and accounts for more than 70% of infant leukemia.[17,18] The incidence of *MLL* re-arrangement is significantly higher in therapy-related acute myeloid leukemia (AML) (10%) than in *de novo* AML (5%).[17] The existence of *MLL* re-arrangements is strongly predictive of poor clinical outcome and short-term survival.[19–28] Leukemias carrying *MLL* re-arrangements also have increased drug resistance to both the standard and targeted therapies.[29,30] There are four different forms of *MLL* deregulation found in patients including *MLL* fusion, *MLL*-partial tandem duplication (PTD), *MLL* amplification and in rare cases of internal deletion of *MLL* exons encoding the first PHD finger (Figure 2). With the exception of gene amplification, all these re-arrangements lead to a different degree of structural alternation on the *MLL* sequence. Although both *MLL* fusion and *MLL*-PTD share the same breakpoint region located between the CXXC domain and PHD fingers, they lead to very different domain re-organizations. As a result of chimeric fusion, the MLL protein will lose its C-terminal sequence including the transactivation and SET domains but gains novel transcriptional effector domains or homo-dimerization domains from one of its fusion partners. In contrast, MLL-PTD involves an internal duplication of the MLL N-terminal sequence flanking the AT-hook and CXXC domain. More importantly, it will also retain the full-length MLL sequence including both transactivation and SET domains. Given the distinctive structural and functional organization of the resultant MLL proteins from these different gene re-arrangements, it is likely that they may also have different transformation mechanisms. In the rest of the review, we will summarize the molecular and cellular mechanisms for leukemia induction by MLL re-arrangement. While most of our current knowledge about the oncogenic function of MLL comes from studying of the fusion proteins, we will also discuss the recent progress and importance of understanding the other *MLL* alternations in leukemia.

MLL fusions in transcriptional deregulation and development of leukemic stem cells

In AML where leukemic stem cells (LSCs) have been functionally identified, MLL fusion as a result of a reciprocal translocation is one of the most common and well-characterized initiating mutations in LSCs. *MLL* translocations are observed in both *de novo* and therapy-related acute leukemia.[31] MLL fusions including MLL-ENL[32] and MLL-GAS7[33] were capable of transforming both HSCs and myeloid progenitors such as CMPs and GMPs for development of LSCs, demonstrating their ability to initiate aberrant self-renewal program in the targeted hematopoietic stem/progenitor cells.[34] Consistently, subsequent analyses of LSCs derived from MLL-AF9 leukemia mouse models revealed aberrant HSC[35] and embryonic stem cell[36] transcriptional programs in committed progenitor cells transformed by the MLL fusion. Therefore, one of the key functions of MLL fusion is to reinitiate/sustain specific expression program in committed progenitors/stem cells to confer aberrant self-renewal[37] (Figure 3), although the final transcriptional and transformation outcome may also depend on the level of expression of the fusions.[38]

To date, more than 60 partner genes have been identified, but six partner proteins (AF4, AF9, ENL, AF10, ELL and AF6) constitute more than 85% of all leukemia cases carrying *MLL* fusion.[39] Most of these frequent partner proteins (except for AF6) are nuclear proteins. Based on their functional properties, the fusion partners can be broadly classified into nuclear proteins with transcription effector domains and cytoplasmic proteins with dimerization domains.[40,41] There is evidence that some of the most common MLL nuclear fusion partners (including AF4, AF9, ENL and ELL) form a higher-order complex called AF4/ENL/P-TEFb complex (AEP)[42] or Super Elongation Complex (SEC).[43] AEP is normally recruited to the wild-type MLL target loci (such as *Hoxa9*) to facilitate transcription under physiological conditions.[42] However, when cells are transformed by MLL fusions carrying AF4 or ENL family protein, the AEP/SEC complex is constitutively recruited to the MLL-target loci for transcriptional activation and elongation.[42,43] Interestingly, the H3K79

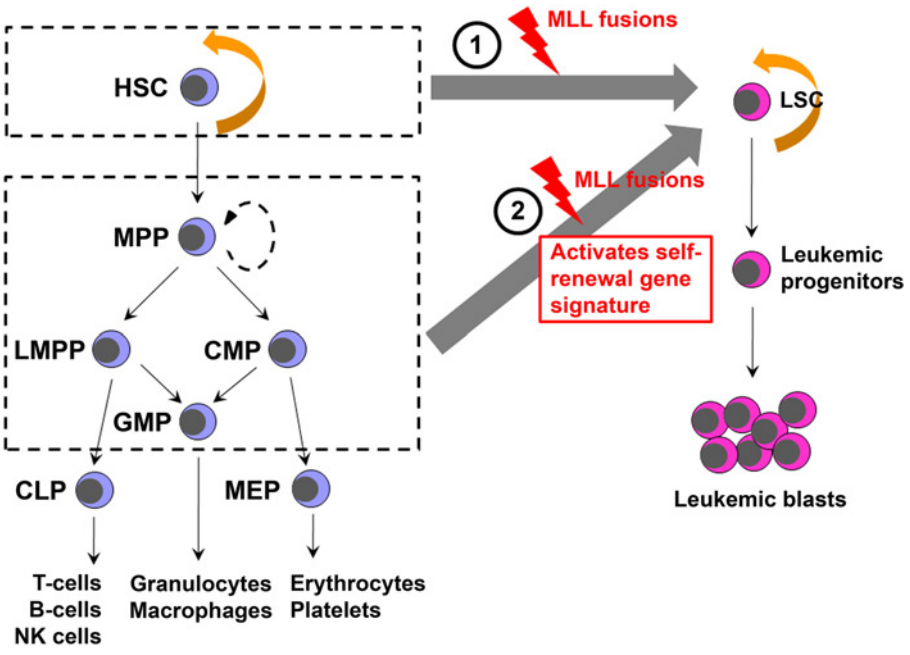

Figure 3 Transformation of HSCs and progenitors into LSCs by MLL fusions. MLL fusions not only can directly transform HSCs into LSCs but also can impart self-renewal ability to the committed progenitors and transform them into LSCs. HSC, haematopoietic stem cell; MPP, multipotential progentior; LMPP, lymphoid primed multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; GMP, granulocyte/macrophage progenitor; MEP, megakaryocyte/erythroid progenitor. (A color version of this figure is available in the online journal)

histone methyltransferase, DOT1L, previously identified to interact with AF10 and later with AF4/AF9/ENL is absent in the AEP/SEC.[42,43] While aberrant recruitment of DOT1L by MLL-AF10 is key for its aberrant transcriptional and transformation activity,[44] further biochemical analyses indicate that the interaction between AF9/ENL with AF4 and DOT1L is mutually exclusive, indicating that DOT1L may not have a direct interaction with other MLL fusions in spite of its requirement for survival of leukemic cells carrying various oncogenic fusions including MLL-AF6.[45–49] On the other hand, protein arginine methyltransferase 1 (PRMT1) that mediates asymmetric dimethylation of histone 4 arginine 3 (H4R3me2a) has been identified as a critical interacting protein for MLL-EEN-mediated leukemia. Suppression of Prmt1 inhibits *Hox* expression and transformation mediated by MLL-EEN.[50] Taken together, these results indicate that recruitment of transcriptional/elongation complexes and specific epigenetic modifying enzymes are critical for the oncogenic functions of MLL fusions. These findings also lead to current efforts in developing small molecule inhibitors targeting these major components, which have begun to show the promise.[48,51,52]

MLL-PTD in transcriptional deregulation and leukemia induction

MLL-PTD mutation is observed in approximately 8% (5–11% among studies) of AML patients with normal cytogenetics[19–21] and in 25–54% of AML patients with trisomy 11 as the only abnormality.[19,53] Although *MLL*-PTD RNA transcripts resulting from mis-splicing or exon scrambling can be detected in the bone marrow and peripheral blood of healthy individuals,[54–56] only duplication of exons 2–6, 2–7 and 2–8 (or exons 3–9, 3–10 and 3–11 in new nomenclature) detected at both transcriptional (by reverse transcriptase polymerase chain reaction [RT-PCR]) and genomic (by Southern blot or DNA PCR) levels have clinical significance.[19] *Alu*-repeat-mediated homologous recombination is the likely mechanism to generate this MLL mutant protein[57–59] containing a duplicated copy of the MLL N-terminus containing AT hooks and CXXC domain with a strong transactivation activity in luciferase reporter assay.[60] Interestingly, the CXXC domain of MLL was recently found to interact with the polymerase-associated factor complex (PAFc), which enhanced both the wild-type MLL- and MLL-AF9-mediated transcriptional activation and their recruitment to the target loci.[61,62] Knockdown of PAFc suppressed MLL-AF9-mediated immortalization, suggesting a critical function of CXXC/PAFc interaction in MLL pathogenesis. On the other hand, *MLL*-PTD mutation might suppress wild-type *MLL* expression in AML blasts.[63] Leukemic cell death was observed when wild-type *MLL* expression was restored,[63] suggesting that a tight balance of MLL activity is critical for normal and malignant hematopoiesis.

The knock-in model of *Mll*-PTD ($Mll^{PTD/WT}$) generated by Dorrance *et al.*[64] has provided unique insights for the role of MLL-PTD in acute leukemogenesis. In this model, *Mll*-PTD triggered aberrant *Hox* expression and altered hematopoiesis in part via epigenetic deregulation. An increase in both histone acetylation and H3K4 methylation but a decrease in H3K9 methylation were observed at the promoters of *Hoxa7* and *Hoxa9* genes in $Mll^{PTD/WT}$ mice. To further study the relationship between *MLL*-PTD mutation and the wild-type *MLL* allele, Dorrance *et al.*[65] generated mice that harbor *Mll*-PTD in one allele but lack the other wild-type

Mll allele in the germline ($Mll^{PTD/-}$) so as to recapitulate the situation in human AML blasts. By comparing the phenotypes of $Mll^{PTD/-}$ to $Mll^{PTD/WT}$ fetal liver cells, $Mll^{PTD/-}$ cells produced lower numbers of Burst forming unit erythroid (BFU-E) and Colony-Forming Unit-Granulocyte, Erythrocyte, Monocyte/macrophage, Megakaryocyte (CFU-GEMM) than $Mll^{PTD/WT}$ cells, however the early lethal phenotype precludes the studies in adult hematopoiesis.

Further insights into the role of MLL-PTD in hematopoietic development were obtained by recent extensive analyses of different hematopoietic stem and progenitor cell populations from $Mll^{PTD/WT}$ mice.[66] $Mll^{PTD/WT}$ hematopoietic stem and progenitor cells exhibited a proliferative advantage in colony replating assays, CFU-spleen assays, and competitive transplantation assays over the wild-type cells. Apart from LT-HSCs, $Mll^{PTD/WT}$ ST-HSCs, MPPs and even GMPs allow long-term reconstitution in bone marrow transplantation (compare with only LH-HSCs in the wild-type). These $Mll^{PTD/WT}$ cells in recipient mice exhibited normal lymphoid differentiation but had reduced myeloid differentiation, suggesting a blockade in myeloid differentiation. $Mll^{PTD/WT}$ hematopoietic stem and progenitor cells also appeared to have a survival advantage (increased proliferation and reduced apoptosis) under stress. Thus, *Mll*-PTD may facilitate leukemic transformation by enhancing self-renewal and blocking myeloid differentiation of hematopoietic stem and progenitors. However, none of the *Mll*-PTD mice developed frank leukemia within two years, suggesting the requirement of cooperative mutations. In line with this, Flt3-internal tandem duplication (ITD) is found in 25% MLL-PTD patients.[67] Consistently, a recent AML model was developed using the double knock-in model expressing both *Mll*-PTD and *Flt3*-ITD[68] This model recapitulates various molecular features of the corresponding human diseases including normal cytogenetics, reduced wild-type *Mll* expression and a loss of wild-type *Flt3* expression.[19–21,69–74] Taken together, these results indicate that *MLL*-PTD contributes to leukemogenesis by enhancing self-renewal of hematopoietic stem/progenitor cells, which requires secondary mutations for development of full-blown leukemia.

MLL amplification in leukemia

While the wild-type *MLL* has shown to be required for MLL-fusion-mediated transformation,[75] the direct evidence for its oncogenic function comes from the discovery of *MLL* amplification in approximately 1% of AML[76,77] and some cases of myelodysplastic syndrome (MDS)[24,78] as well as pre-B acute lymphoblastic leukemia (ALL).[22] Gene amplification is one of the mechanisms for activating proto-oncogene commonly observed in solid tumors. By fluorescence *in situ* hybridization (FISH), different forms of *MLL* amplification are observed including homogeneously staining regions (HSR; intrachromosomal amplification), double-minute chromosomes (dmin; extrachromosomal amplification) and, in rare cases, ring chromosomes.[77–79] *MLL* amplification can be found in both *de novo* and therapy-related AML or MDS patients.[77,78,80] In both cases, the presence of *MLL* amplification is associated with a poor prognosis[23,24] although the correlation between *MLL* copy number and clinical outcome of patients remains unclear.

While the size of 11q23 amplicon can be as big as 10 Mb, *MLL* was demonstrated to be the main target of amplification by cytogenetic[24,76–78,81] and expression analyses in patients.[82] Detailed molecular analyses on *MLL* and five other candidate oncogenes within the 11q23 amplicon (*DDX6*, *CBL*, *ETS1*, *PLZF* and *FLI1*) were carried out to correlate with the 11q23 copy number in patients, in which *MLL* was the strongest candidate concluded to be the target gene of 11q23 amplification.[82] Nevertheless, co-amplification of neighboring genes was also detected[83–85] and might cooperate with *MLL* amplification in leukemogenesis. Interestingly, over-expression of *HOXA9* and *MEIS1* is observed in both groups of patients with *MLL* amplification and *MLL* fusions,[82,86] suggesting that *MLL* fusions and amplification may share similar downstream targets for oncogenic transformation. Consistently, the AEP complex co-localizes with the wild-type MLL at the *HOXA9* and *MEIS1* loci,[42] suggesting that the amplified MLL protein can recruit the AEP complex for aberrant transcription elongation. Moreover, PAFc also interacts with the wild-type MLL through the sequences flanking the CXXC domain[62] to promote gene expression. In addition, the SET domain of the wild-type MLL will confer H3K4me3 marks for transcription activation. Therefore, over-expression of the wild-type *MLL* in cells carrying *MLL* amplification can lead to aberrant expression of downstream targets.

In contrast to *Mll* fusions and *Mll*-PTD, the lack of mouse model has significantly hampered the progress in understanding the role and underlying mechanisms of *MLL* amplification in disease progression. Generation of a mouse model with multiple copies of *Mll* to mimic the condition of *MLL* amplification in patients is therefore of upmost importance. However, given the size of the full-length *MLL* (12kb), it is not possible to model the disease using the classical retroviral transduction/transformation

Table 1 Coexisting mutations in AML/MDS patients with *MLL* amplification

Coexisting mutation	Chromosomal location	Frequency	References
MYC amplification	8q24	1/5 (20%)	78
GAB2 amplification	11q14	21/31 (68%)	85
AML1 amplification	21q22	1/29 (3.5%)	88
rRNA amplification	13p12	2/5 (40%)	78
Del(5q)	5q31	19/21 (90%); 24/29 (83%)	88,89
Del(7q)	7q	8/29 (27.6%)	88
TP53 mutations	17p13	7/8 (87.5%); 18/56 (32%)	81,90
MLL-PTD	11q23	3 patients	91,92

MYC, v-MYC avian myelocytomatosis viral oncogene homolog; GAB2, GRB2-associated binding protein 2; AML1, acute myeloid leukemia 1; rRNA, ribosomal RNA; p53, tumor protein p53

assay that has been successfully used for MLL fusion proteins.[87] It will also be difficult to target the endogenous *Mll* allele to achieve a significant level of whole locus amplification as seen in the patients. Conversely, transgenic approach may be a solution to model *MLL* amplification if an appropriate promoter is employed to drive the expression of the transgenes. In this case, the transgenes may scatter on different chromosomes and/or join together as a concatamer on a single chromosome, they will recapitulate the conditions in patients with extra-chromosomal amplification and HSR, respectively. Given the knowledge we have from MLL fusions and MLL-PTD, it is likely that *MLL* amplification is not sufficient to induce full-blown leukemia. Consistently, a number of genetic abnormalities are reported to coexist with *MLL* amplification in AML or MDS patients (Table 1), including amplification of *MYC*,[78] GRB-associated binding protein (*GAB2*),[85] *AML1/RUNX1*[88] and *rRNA*[78] gene; deletion of chromosome arm 5q[88,89] and 7q[88] and mutations of *TP53*.[81,90] Strikingly, *MLL*-PTD was also detected in few patients carrying *MLL* amplification.[91,92] The findings of co-existence of *MLL*-PTD and *MLL* amplification in leukemia patients are particularly interesting as this may suggest a potential collaboration and dosage effect of the wild-type MLL protein and MLL-PTD in leukemia induction.

Internal deletion of the first PHD finger of MLL

Among all the *MLL* re-arrangements, internal deletion of the first PHD finger of MLL is the least common one. To date, only three T-ALL patients are reported to have an internal deletion in one of the two alleles of the *MLL* gene that eliminates parts of introns 7 and 8, together with exon 8.[93] As the PHD fingers consistently lost in MLL fusion are reported to interact with transcriptional corepressors,[94] they may have a negative regulatory role in MLL function. This hypothesis is also supported by the recent findings that inclusion of additional PHD fingers (e.g. the 3rd PHD finger) in MLL fusion suppresses the expression of the *Hoxa9* locus and its transforming ability.[94,95] However, it is not clear if deletion of the first PHD finger may also alter the overall functions of the clustered PHD fingers resulting in aberrant transcription regulation.

Prospective

While we start to gather some basic concepts about the normal function of *MLL* and the oncogenic mechanisms mediated by their fusions, the progress in studying the role of other *MLL* alternations has significantly lagged behind. These are largely due to the lack of appropriate animal models that can closely mimic the corresponding human diseases, particularly the recurring *MLL*-PTD and *MLL* amplification, which likely have distinctive transformation mechanisms as compared with the *MLL* fusions. The recent development of *Mll*-PTD/*Flt3*-ITD AML model provides a useful platform to study the oncogenic function of *Mll*-PTD in this specific context. However, there is still no disease model available for *MLL* amplification, which would have provided unique insights into the role of normal MLL protein in oncogenesis. While the underlying transformation mechanisms remain largely unknown, the co-existence of *MLL*-PTD with *MLL* amplification in a small number of patients suggests a potential functional crosstalk between these two *MLL* aberrations, and provides a unique and pathologically relevant starting point to dissect their roles in development of LSCs. Thus, further development of relevant novel disease models and characterization of the underlying transformation mechanisms associated with full-length or mutated forms of MLL protein are desperately needed to improve our understanding of the associated diseases and to facilitate the design of more effective therapeutics.

Author contributions: BHY and CWES wrote the manuscript.

ACKNOWLEDGEMENTS

This work is supported by Leukemia and Lymphoma Research (LLR), Kay Kendall Leukemia Fund (KKLF), the Association for International Cancer Research (AICR) and Medical Research Council (MRC).

REFERENCES

1 Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL. Identification of a lineage of multipotent hematopoietic progenitors. *Development* 1997;**124**:1929–39
2 Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 2000;**404**:193–7
3 Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 1997;**91**:661–72
4 Adolfsson J, Borge OJ, Bryder D, Theilgaard-Monch K, Astrand-Grundstrom I, Sitnicka E, Sasaki Y, Jacobsen SE. Upregulation of Flt3 expression within the bone marrow Lin(−)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* 2001;**15**:659–69
5 Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, Bryder D, Yang L, Borge OJ, Thoren LA, Anderson K, Sitnicka E, Sasaki Y, Sigvardsson M, Jacobsen SE. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* 2005;**121**:295–306
6 Daser A, Rabbitts TH. Extending the repertoire of the mixed-lineage leukemia gene MLL in leukemogenesis. *Genes Dev* 2004;**18**:965–74
7 Hsieh JJ, Ernst P, Erdjument-Bromage H, Tempst P, Korsmeyer SJ. Proteolytic cleavage of MLL generates a complex of N- and C-terminal fragments that confers protein stability and subnuclear localization. *Mol Cell Biol* 2003;**23**:186–94
8 Yokoyama A, Kitabayashi I, Ayton PM, Cleary ML, Ohki M. Leukemia proto-oncoprotein MLL is proteolytically processed into 2 fragments with opposite transcriptional properties. *Blood* 2002;**100**:3710–8
9 Slany RK. The molecular biology of mixed lineage leukemia. *Haematologica* 2009;**94**:984–93
10 Yagi H, Deguchi K, Aono A, Tani Y, Kishimoto T, Komori T. Growth disturbance in fetal liver hematopoiesis of Mll-mutant mice. *Blood* 1998;**92**:108–17
11 Ernst P, Fisher JK, Avery W, Wade S, Foy D, Korsmeyer SJ. Definitive hematopoiesis requires the mixed-lineage leukemia gene. *Dev Cell* 2004;**6**:437–43
12 Hess JL, Yu BD, Li B, Hanson R, Korsmeyer SJ. Defects in yolk sac hematopoiesis in Mll-null embryos. *Blood* 1997;**90**:1799–806

13 Yu BD, Hess JL, Horning SE, Brown GA, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. *Nature* 1995;**378**:505–8

14 Yu BD, Hanson RD, Hess JL, Horning SE, Korsmeyer SJ. MLL, a mammalian trithorax-group gene, functions as a transcriptional maintenance factor in morphogenesis. *Proc Natl Acad Sci USA* 1998;**95**:10632–36

15 McMahon KA, Hiew SY, Hadjur S, Veiga-Fernandes H, Menzel U, Price AJ, Kioussis D, Williams O, Brady HJ. Mll has a critical role in fetal and adult hematopoietic stem cell self-renewal. *Cell Stem Cell* 2007;**1**:338–45

16 Jude CD, Climer L, Xu D, Artinger E, Fisher JK, Ernst P. Unique and independent roles for MLL in adult hematopoietic stem cells and progenitors. *Cell Stem Cell* 2007;**1**:324–37

17 Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood* 2003;**102**:2395–402

18 Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. *J Clin Oncol* 2005;**23**:6306–15

19 Schnittger S, Kinkelin U, Schoch C, Heinecke A, Haase D, Haferlach T, Buchner T, Wormann B, Hiddemann W, Griesinger F. Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. *Leukemia* 2000;**14**:796–804

20 Caligiuri MA, Strout MP, Lawrence D, Arthur DC, Baer MR, Yu F, Knuutila S, Mrozek K, Oberkircher AR, Marcucci G, de la Chapelle A, Elonen E, Block AW, Rao PN, Herzig GP, Powell BL, Ruutu T, Schiffer CA, Bloomfield CD. Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics. *Cancer Res* 1998;**58**:55–9

21 Dohner K, Tobis K, Ulrich R, Frohling S, Benner A, Schlenk RF, Dohner H. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. *J Clin Oncol* 2002;**20**:3254–61

22 Espinet B, Florensa L, Salido M, Sole F. MLL intrachromosomal amplification in a pre-B acute lymphoblastic leukemia. *Haematologica* 2003;**88**:EIM03

23 Maitta RW, Cannizzaro LA, Ramesh KH. Association of MLL amplification with poor outcome in acute myeloid leukemia. *Cancer Genet Cytogenet* 2009;**192**:40–3

24 Michaux L, Wlodarska I, Stul M, Dierlamm J, Mugneret F, Herens C, Beverloo B, Verhest A, Verellen-Dumoulin C, Verhoef G, Selleslag D, Madoe V, Lecomte M, Deprijck B, Ferrant A, Delannoy A, Marichal S, Duhem C, Dicato M, Hagemeijer A. MLL amplification in myeloid leukemias: a study of 14 cases with multiple copies of 11q23. *Genes Chromosomes Cancer* 2000;**29**:40–7

25 Pui CH, Behm FG, Downing JR, Hancock ML, Shurtleff SA, Ribeiro RC, Head DR, Mahmoud HH, Sandlund JT, Furman WL. 11q23/MLL rearrangement confers a poor prognosis in infants with acute lymphoblastic leukemia. *J Clin Oncol* 1994;**12**:909–15

26 Pui CH, Frankel LS, Carroll AJ, Raimondi SC, Shuster JJ, Head DR, Crist WM, Land VJ, Pullen DJ, Steuber CP. Clinical characteristics and treatment outcome of childhood acute lymphoblastic leukemia with the t(4;11)(q21;q23): a collaborative study of 40 cases. *Blood* 1991; **77**:440–7

27 Rubnitz JE, Link MP, Shuster JJ, Carroll AJ, Hakami N, Frankel LS, Pullen DJ, Cleary ML. Frequency and prognostic significance of HRX rearrangements in infant acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Blood* 1994;**84**:570–3

28 Shiah HS, Kuo YY, Tang JL, Huang SY, Yao M, Tsay W, Chen YC, Wang CH, Shen MC, Lin DT, Lin KH, Tien HF. Clinical and biological implications of partial tandem duplication of the MLL gene in acute myeloid leukemia without chromosomal abnormalities at 11q23. *Leukemia* 2002;**16**:196–202

29 Yeung J, Esposito MT, Gandillet A, Zeisig BB, Griessinger E, Bonnet D, So CW. β-catenin mediates the establishment and drug resistance of MLL leukemic stem cells. *Cancer Cell* 2010;**18**:606–618

30 Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer* 2007;**7**:823–33

31 Daser A, Rabbitts TH. The versatile mixed lineage leukaemia gene MLL and its many associations in leukaemogenesis. *Semin Cancer Biol* 2005;**15**:175–88

32 Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes Dev* 2003;**17**:3029–35

33 So CW, Karsunky H, Passegue E, Cozzio A, Weissman IL, Cleary ML. MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. *Cancer Cell* 2003;**3**:161–71

34 Zeisig BB, Kulasekararaj AG, Mufti GJ, So CW. Acute myeloid leukemia: Snapshot. *Cancer Cell* 2012;**22**:698

35 Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR, Armstrong SA. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* 2006;**442**:818–22

36 Somervaille TC, Matheny CJ, Spencer GJ, Iwasaki M, Rinn JL, Witten DM, Chang HY, Shurtleff SA, Downing JR, Cleary ML. Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. *Cell Stem Cell* 2009;**4**:129–40

37 Smith LL, Yeung J, Zeisig BB, Popov N, Huijbers I, Barnes J, Wilson AJ, Taskesen E, Delwel R, Gil J, Van Lohuizen M, So CW. Functional crosstalk between Bmi1 and MLL/Hoxa9 axis in establishment of normal hematopoietic and leukemic stem cells. *Cell Stem Cell* 2011;**8**:649–662

38 Chen W, Kumar AR, Hudson WA, Li Q, Wu B, Staggs RA, Lund EA, Sam TN, Kersey JH. Malignant transformation initiated by Mll-AF9: gene dosage and critical target cells. *Cancer Cell* 2008;**13**:432–40

39 Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, Ben Abdelali R, Macintyre E, De Braekeleer E, De Braekeleer M, Delabesse E, de Oliveira MP, Cave H, Clappier E, van Dongen JJ, Balgobind BV, van den Heuvel-Eibrink MM, Beverloo HB, Panzer-Grumayer R, Teigler-Schlegel A, Harbott J, Kjeldsen E, Schnittger S, Koehl U, Gruhn B, Heidenreich O, Chan LC, Yip SF, Krzywinski M, Eckert C, Moricke A, Schrappe M, Alonso CN, Schafer BW, Krauter J, Lee DA, Zur Stadt U, Te Kronnie G, Sutton R, Izraeli S, Trakhtenbrot L, Lo Nigro L, Tsaur G, Fechina L, Szczepanski T, Strehl S, Ilencikova D, Molkentin M, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. New insights to the MLL recombinome of acute leukemias. *Leukemia* 2009;**23**:1490–9

40 So CW, Lin M, Ayton PM, Chen EH, Cleary ML. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. *Cancer Cell* 2003;**4**:99–110

41 So CW, Cleary ML. Common mechanism for oncogenic activation of MLL by forkhead family proteins. *Blood* 2003;**101**:633–639

42 Yokoyama A, Lin M, Naresh A, Kitabayashi I, Cleary ML. A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription. *Cancer Cell* 2010;**17**:198–212

43 Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, Washburn MP, Conaway JW, Conaway RC, Shilatifard A. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol Cell* 2010;**37**:429–37

44 Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, Su L, Xu G, Zhang Y. hDOT1L links histone methylation to leukemogenesis. *Cell* 2005;**121**:167–78

45 Jo SY, Granowicz EM, Maillard I, Thomas D, Hess JL. Requirement for Dot1l in murine postnatal hematopoiesis and leukemogenesis by MLL translocation. *Blood* 2011;**117**:4759–68

46 Chang MJ, Wu H, Achille NJ, Reisenauer MR, Chou CW, Zeleznik-Le NJ, Hemenway CS, Zhang W. Histone H3 lysine 79 methyltransferase Dot1 is required for immortalization by MLL oncogenes. *Cancer Res* 2010;**70**:10234–42

47 Bernt KM, Zhu N, Sinha AU, Vempati S, Faber J, Krivtsov AV, Feng Z, Punt N, Daigle A, Bullinger L, Pollock RM, Richon VM, Kung AL, Armstrong SA. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer Cell* 2011;**20**:66–78

48 Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, Johnston LD, Scott MP, Smith JJ, Xiao Y, Jin L, Kuntz KW, Chesworth R, Moyer MP, Bernt KM, Tseng JC, Kung AL, Armstrong SA, Copeland RA, Richon VM, Pollock RM. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell* 2011; **20**:53–65

49 Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. *Blood* 2011;**117**:6912–22

50 Cheung N, Chan LC, Thompson A, Cleary ML, So CW. Protein arginine-methyltransferase-dependent oncogenesis. *Nat Cell Biol* 2007;**9**:1208–15

51 Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirguet O, Doehner K, Delwel R, Burnett AK, Jeffrey P, Drewes G, Lee K, Huntly BJ, Kouzarides T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 2011;**478**:529–33

52 Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* 2011;**478**:524–8

53 Rege-Cambrin G, Giugliano E, Michaux L, Stul M, Scaravaglio P, Serra A, Saglio G, Hagemeijer A. Trisomy 11 in myeloid malignancies is associated with internal tandem duplication of both MLL and FLT3 genes. *Haematologica* 2005;**90**:262–4

54 Caldas C, So CW, MacGregor A, Ford AM, McDonald B, Chan LC, Wiedemann LM. Exon scrambling of MLL transcripts occur commonly and mimic partial genomic duplication of the gene. *Gene* 1998;**208**:167–76

55 Marcucci G, Strout MP, Bloomfield CD, Caligiuri MA. Detection of unique ALL1 (MLL) fusion transcripts in normal human bone marrow and blood: distinct origin of normal versus leukemic ALL1 fusion transcripts. *Cancer Res* 1998;**58**:790–3

56 Schnittger S, Wormann B, Hiddemann W, Griesinger F. Partial tandem duplications of the MLL gene are detectable in peripheral blood and bone marrow of nearly all healthy donors. *Blood* 1998; **92**:1728–34

57 Strout MP, Marcucci G, Bloomfield CD, Caligiuri MA. The partial tandem duplication of ALL1 (MLL) is consistently generated by Alu-mediated homologous recombination in acute myeloid leukemia. *Proc Natl Acad Sci USA* 1998;**95**:2390–5

58 So CW, Ma ZG, Price CM, Dong S, Chen SJ, Gu LJ, So CK, Wiedemann LM, Chan LC. MLL self fusion mediated by Alu repeat homologous recombination and prognosis of AML-M4/M5 subtypes. *Cancer Res* 1997;**57**:117–22

59 Schichman SA, Caligiuri MA, Strout MP, Carter SL, Gu Y, Canaani E, Bloomfield CD, Croce CM. ALL-1 tandem duplication in acute myeloid leukemia with a normal karyotype involves homologous recombination between Alu elements. *Cancer Res* 1994;**54**:4277–80

60 Martin ME, Milne TA, Bloyer S, Galoian K, Shen W, Gibbs D, Brock HW, Slany R, Hess JL. Dimerization of MLL fusion proteins immortalizes hematopoietic cells. *Cancer Cell* 2003;**4**:197–207

61 Milne TA, Kim J, Wang GG, Stadler SC, Basrur V, Whitcomb SJ, Wang Z, Ruthenburg AJ, Elenitoba-Johnson KS, Roeder RG, Allis CD. Multiple interactions recruit MLL1 and MLL1 fusion proteins to the HOXA9 locus in leukemogenesis. *Mol Cell* 2010;**38**:853–63

62 Muntean AG, Tan J, Sitwala K, Huang Y, Bronstein J, Connelly JA, Basrur V, Elenitoba-Johnson KS, Hess JL. The PAF complex synergizes with MLL fusion proteins at HOX loci to promote leukemogenesis. *Cancer Cell* 2010;**17**:609–21

63 Whitman SP, Liu S, Vukosavljevic T, Rush LJ, Yu L, Liu C, Klisovic MI, Maharry K, Guimond M, Strout MP, Becknell B, Dorrance A, Klisovic RB, Plass C, Bloomfield CD, Marcucci G, Caligiuri MA. The MLL partial tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. *Blood* 2005;**106**:345–52

64 Dorrance AM, Liu S, Yuan W, Becknell B, Arnoczky KJ, Guimond M, Strout MP, Feng L, Nakamura T, Yu L, Rush LJ, Weinstein M, Leone G, Wu L, Ferketich A, Whitman SP, Marcucci G, Caligiuri MA. Mll partial tandem duplication induces aberrant Hox expression in vivo via specific epigenetic alterations. *J Clin Invest* 2006;**116**:2707–16

65 Dorrance AM, Liu S, Chong A, Pulley B, Nemer D, Guimond M, Yuan W, Chang D, Whitman SP, Marcucci G, Caligiuri MA. The Mll partial tandem duplication: differential, tissue-specific activity in the presence or absence of the wild-type allele. *Blood* 2008;**112**:2508–11

66 Zhang Y, Yan X, Sashida G, Zhao X, Rao Y, Goyama S, Whitman SP, Zorko N, Bernot K, Conway RM, Witte D, Wang QF, Tenen DG, Xiao Z, Marcucci G, Mulloy JC, Grimes HL, Caligiuri MA, Huang G. Stress hematopoiesis reveals abnormal control of self-renewal, lineage-bias and myeloid differentiation in Mll partial tandem duplication (Mll-PTD) hematopoietic stem/progenitor cells. *Blood* 2012

67 Whitman SP, Ruppert AS, Marcucci G, Mrozek K, Paschka P, Langer C, Baldus CD, Wen J, Vukosavljevic T, Powell BL, Carroll AJ, Kolitz JE, Larson RA, Caligiuri MA, Bloomfield CD. Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and MLL partial tandem duplication: a Cancer and Leukemia Group B study. *Blood* 2007;**109**:5164–7

68 Zorko NA, Bernot KM, Whitman SP, Siebenaler RF, Ahmed EH, Marcucci GG, Yanes DA, McConnell KK, Mao C, Kalu C, Zhang X, Jarjoura D, Dorrance AM, Heerema NA, Lee BH, Huang G, Marcucci G, Caligiuri MA. Mll-partial tandem duplication and Flt3-internal tandem duplication in a double knock-in mouse recapitulates features of counterpart human acute myeloid leukemias. *Blood* 2012

69 Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, Carroll AJ, Mrozek K, Vardiman JW, George SL, Kolitz JE, Larson RA, Bloomfield CD, Caligiuri MA. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. *Cancer Res* 2001;**61**:7233–9

70 Munoz L, Nomdedeu JF, Villamor N, Guardia R, Colomer D, Ribera JM, Torres JP, Berlanga JJ, Fernandez C, Llorente A, Queipo de Llano MP, Sanchez JM, Brunet S, Sierra J. Acute myeloid leukemia with MLL rearrangements: clinicobiological features, prognostic impact and value of flow cytometry in the detection of residual leukemic cells. *Leukemia* 2003;**17**:76–82

71 Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, Wermke M, Bornhauser M, Ritter M, Neubauer A, Ehninger G, Illmer T. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* 2002;**99**: 4326–35

72 Frohling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, Dohner H, Dohner K. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood* 2002;**100**:4372–80

73 Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, Goldstone AH Linch DC. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* 2001;**98**:1752–9

74 Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Loffler H, Sauerland CM, Serve H, Buchner T, Haferlach T, Hiddemann W. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood* 2002;**100**:59–66

75 Thiel AT, Blessington P, Zou T, Feather D, Wu X, Yan J, Zhang H, Liu Z, Ernst P, Koretzky GA, Hua X. MLL-AF9-induced leukemogenesis requires coexpression of the wild-type Mll allele. *Cancer Cell* 2010;**17**:148–59

76 Avet-Loiseau H, Godon C, Li JY, Daviet A, Mellerin MP, Talmant P, Harousseau JL, Bataille R. Amplification of the 11q23 region in acute myeloid leukemia. *Genes Chromosomes Cancer* 1999;**26**:166–70

77 Cuthbert G, Thompson K, McCullough S, Watmore A, Dickinson H, Telford N, Mugneret F, Harrison C, Griffiths M, Bown N. MLL amplification in acute leukaemia: a United Kingdom Cancer Cytogenetics Group (UKCCG) study. *Leukemia* 2000;**14**:1885–91

78 Streubel B, Valent P, Jager U, Edelhauser M, Wandt H, Wagner T, Buchner T, Lechner K, Fonatsch C. Amplification of the MLL gene on double minutes, a homogeneously staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. *Genes Chromosomes Cancer* 2000; **27**:380–6

79 Dolan M, McGlennen RC, Hirsch B. MLL amplification in myeloid malignancies: clinical, molecular, and cytogenetic findings. *Cancer Genet Cytogenet* 2002;**134**:93–101
80 Reddy KS, Parsons L, Mak L, Dighe P, Saphner T, Crow MK, Scott M. Segmental amplification of 11q23 region identified by fluorescence in situ hybridization in four patients with myeloid disorders: a review. *Cancer Genet Cytogenet* 2001;**126**:139–46
81 Andersen MK, Christiansen DH, Kirchhoff M, Pedersen-Bjergaard J. Duplication or amplification of chromosome band 11q23, including the unrearranged MLL gene, is a recurrent abnormality in therapy-related MDS and AML, and is closely related to mutation of the TP53 gene and to previous therapy with alkylating agents. *Genes Chromosomes Cancer* 2001;**31**:33–41
82 Poppe B, Vandesompele J, Schoch C, Lindvall C, Mrozek K, Bloomfield CD, Beverloo HB, Michaux L, Dastugue N, Herens C, Yigit N, De Paepe A, Hagemeijer A, Speleman F. Expression analyses identify MLL as a prominent target of 11q23 amplification and support an etiologic role for MLL gain of function in myeloid malignancies. *Blood* 2004;**103**:229–35
83 Zatkova A, Ullmann R, Rouillard JM, Lamb BJ, Kuick R, Hanash SM, Schnittger S, Schoch C, Fonatsch C, Wimmer K. Distinct sequences on 11q13.5 and 11q23–24 are frequently coamplified with MLL in complexly organized 11q amplicons in AML/MDS patients. *Genes Chromosomes Cancer* 2004;**39**:263–76
84 Lee AS, Lie DK, Tien SL, Rudduck-Sivaswaren C. Increased amplification proximal to the MLL gene in acute myeloid leukemia. *Int J Mol Med* 2004;**13**:63–7
85 Zatkova A, Schoch C, Speleman F, Poppe B, Mannhalter C, Fonatsch C, Wimmer K. GAB2 is a novel target of 11q amplification in AML/MDS. *Genes Chromosomes Cancer* 2006;**45**:798–807
86 Faber J, Krivtsov AV, Stubbs MC, Wright R, Davis TN, van den Heuvel-Eibrink M, Zwaan CM, Kung AL, Armstrong SA. HOXA9 is required for survival in human MLL-rearranged acute leukemias. *Blood* 2009;**113**:2375–85
87 Zeisig BB, So CW. Retroviral/Lentiviral transduction and transformation assay. *Methods Mol Biol* 2009;**538**:207–29
88 Papenhausen PR, Griffin S, Tepperberg J. Oncogene amplification in transforming myelodysplasia. *Exp Mol Pathol* 2005;**79**: 168–75
89 Herry A, Douet-Guilbert N, Gueganic N, Morel F, Le Bris MJ, Berthou C, De Braekeleer M. Del(5q) and MLL amplification in homogeneously staining region in acute myeloblastic leukemia: a recurrent cytogenetic association. *Ann Hematol* 2006;**85**:244–9
90 Zatkova A, Merk S, Wendehack M, Bilban M, Muzik EM, Muradyan A, Haferlach C, Haferlach T, Wimmer K, Fonatsch C, Ullmann R. AML/MDS with 11q/MLL amplification show characteristic gene expression signature and interplay of DNA copy number changes. *Genes Chromosomes Cancer* 2009;**48**:510–20
91 Ariyama Y, Fukuda Y, Okuno Y, Seto M, Date K, Abe T, Nakamura Y, Inazawa J. Amplification on double-minute chromosomes and partial-tandem duplication of the MLL gene in leukemic cells of a patient with acute myelogenous leukemia. *Genes Chromosomes Cancer* 1998;**23**:267–72
92 Pajuelo-Gamez JC, Cervera J, Garcia-Casado Z, Mena-Duran AV, Valencia A, Barragan E, Such E, Bolufer P, Sanz MA. MLL amplification in acute myeloid leukemia. *Cancer Genet Cytogenet* 2007; **174**:127–31
93 Lochner K, Siegler G, Fuhrer M, Greil J, Beck JD, Fey GH, Marschalek R. A specific deletion in the breakpoint cluster region of the ALL-1 gene is associated with acute lymphoblastic T-cell leukemias. *Cancer Res* 1996;**56**:2171–7
94 Chen J, Santillan DA, Koonce M, Wei W, Luo R, Thirman MJ, Zeleznik-Le NJ, Diaz MO. Loss of MLL PHD finger 3 is necessary for MLL-ENL-induced hematopoietic stem cell immortalization. *Cancer Res* 2008;**68**:6199–207
95 Muntean AG, Giannola D, Udager AM, Hess JL. The PHD fingers of MLL block MLL fusion protein-mediated transformation. *Blood* 2008;**112**:4690–3