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The Role of the *MLL* Gene in Infant Leukemia

Mariko Eguchi, Minenori Eguchi-Ishimae, Mel Greaves

LRF Centre for Cell and Molecular Biology of Leukaemia, Institute of Cancer Research, London, UK

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Abstract

The *MLL* gene is a major player in leukemia, particularly in infant leukemia and in secondary, therapy-related acute leukemia. The normal *MLL* gene plays a key role in developmental regulation of gene expression (including *HOX* genes), and in leukemia this function is subverted by breakage, recombination, and chimeric fusion with one of 40 or more alternative partner genes. In infant leukemias, the chromosome translocations involving *MLL* arise during fetal hematopoiesis, possibly in a primitive lymphomyeloid stem cell. In general, these leukemias have a very poor prognosis. The malignancy of these leukemias is all the more dramatic considering their very short preclinical natural history or latency. These data raise fundamental issues of how such divergent *MLL* chimeric genes transform cells, why they so rapidly evolve to a malignant status, and what alternative or novel therapeutic strategies might be considered. We review here progress in tackling these questions. *Int J Hematol.* 2003;78:390-401.

Key words: MLL gene; Infant leukemia; In utero; Short latency; Oligomerization

1. Introduction

The diverse chromosomal and gene abnormalities in leukemia help define biological subsets of the disease and impact prognosis and choice of treatment. Translocations involving chromosome band 11q23 are present in various hematologic malignancies including acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), and myelodysplastic syndrome (MDS) but are particularly prevalent in two situations: infant leukemia (age <1 year) [1] and treatment-related secondary leukemias (mostly AML) associated with previous exposure to topoisomerase-inhibiting chemotherapeutic drugs [2]. The presence of an 11q23 translocation itself is an indicator of poor prognosis. The gene rearranged in 11q23 translocations, *MLL* (also called *ALL-1*, *HRX*, or *HTRX*) was first cloned from the breakpoint cluster region of 11q23 in 1991 [3-6]. *MLL* rearrangement can involve internal tandem duplication (see further below) but characteristically *MLL* joins up with a partner gene to form a chimeric fusion gene. Remarkably, approximately 40 genes are so far identified as alternative fusion partners for *MLL* in leukemias [7,8]. In this review we summarize recent progress in understanding the biology of leukemias with *MLL* gene rearrangement and new insights into the mechanisms of leukemogenesis.

2. Genomic Details of *MLL*: Clues for Understanding the Mechanism of Translocation

The *MLL* gene spans approximately 90 kb, consisting of 36 exons with nearly 12kb coding sequences. From the latest studies of exon-intron structure of the *MLL* gene [9-11], the total number of exons was revised and exon numbering is now different from that previously published [12,13]. However, as most of the reports still use the earlier exon numbering system, we do the same also.

In leukemic cells with *MLL* gene rearrangement most of the genomic breakpoints cluster within a 8.3-kb *Bam*HI fragment encompassing exon 5 to 11 of the *MLL* gene, the so-called breakpoint cluster region (bcr) [14] (Figure 1). *MLL* bcr has several DNA motifs that are implicated in recombination of DNA, such as topoisomerase-II-binding sites [15] and Alu sequences [14]. In addition, a high-affinity scaffold attachment region (SAR) was identified within the telomeric

Correspondence and reprint requests: Professor M. F. Greaves, FRS, LRF Centre, Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, UK; 44(0)20-7153-5160; fax: 44(0)20-7352-0266 (e-mail: mel.greaves@icr.ac.uk).

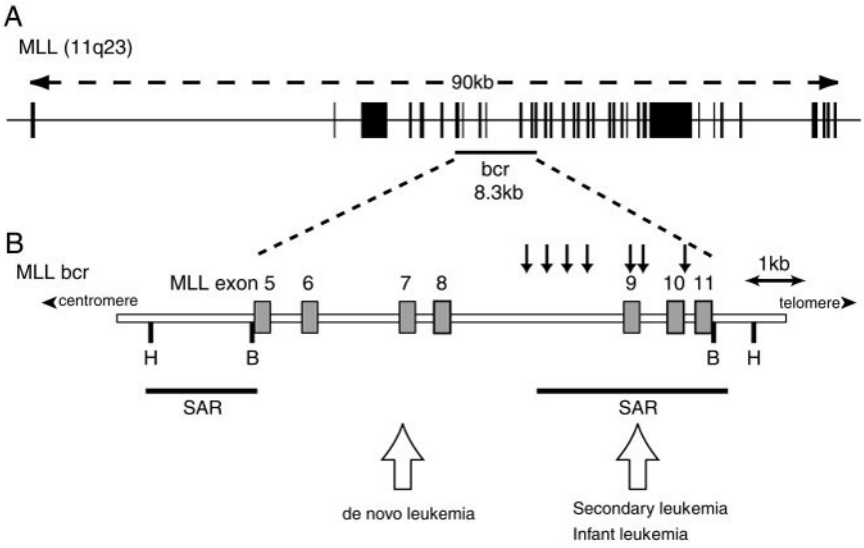

Figure 1. Exon/intron structure of the *MLL* gene. A, *MLL* spans 90 kb in chromosome 11 band q23, consisting of 36 exons and 12 kb coding sequences. B, Physical map surrounding the breakpoint cluster region (bcr) of the *MLL* gene. Narrow bars represent the introns, and black boxes represent the exons. The vertical arrows indicate the topoisomerase-II–consensus sites. High-affinity scaffold attachment region (SAR) exists within the telomeric region of bcr, weak affinity SAR at centromeric of bcr. Note that the breakpoint of infant/secondary leukemia and de novo leukemia (in non-infant children or adults) cluster in a different part of the bcr. B indicates *Bam*HI; H, *Hin*dIII.

(3′) region of the bcr, spanning from approximately the middle to the end of the bcr region [15]. SARs are sites for binding of DNA to nuclear scaffold/matrix proteins, functioning to maintain the structure of chromosomal loops and to provide venues for regulation of transcription, DNA replication, and recombination [16,17]. Notably, most of the topoisomerase-II–consensus sites are clustered in the telomeric part of the *MLL* bcr, overlapping the SAR [15].

The presence of these recombination-prone sequences in the small *MLL* bcr region indicates that rearrangement of the *MLL* gene might be the result of DNA breakage/recombination events. Alu repeats and topoisomerase-II–binding sites are thought to be involved in the recombination mechanism in *MLL* partial tandem duplications [18-20] and therapy-related *MLL* gene rearrangement after prior exposure to drugs that target topoisomerase-II (etoposide, teniposide, and anthracyclines) [21], respectively. This relationship between topoisomerase-II inhibitors and *MLL* gene breakage is supported by several studies showing in vitro induction of *MLL* gene cleavage (but not recombination) by topo-II inhibitors in human hematopoietic cells [22-24]. Error-prone repair leading to *MLL* translocations following gene breakage was also suggested from the scrutiny of sequences of *MLL* fusion genes [25-27]. Possible mechanisms leading to *MLL* translocations in secondary leukemia might include chromosomal breakage within the *MLL* gene induced by topoisomerase-II inhibitors or other therapeutic agents, followed by error-prone DNA repair mechanisms, eg, nonhomologous end joining (reviewed in [28]). Cleavage of the *MLL* gene was also shown to correlate to apoptotic stimuli [29,30]. A recent report showed that apoptotic stimuli not only caused double stand breaks in the *MLL* gene but also produced *MLL-AF9* genes in surviving cells [31].

There are significant differences between de novo and therapy-related acute leukemias in the distribution of genomic breakpoints within *MLL* bcr [15]. In therapy-related leukemias breakpoints tend to cluster in the telomeric part of the *MLL* bcr, where most of the topoisomerase-II consensus binding sites are present. In contrast, in de novo leukemias breakpoints cluster in the more centromeric region of *MLL* bcr. The same biased distribution of gene breakpoints within *MLL* bcr seen in treatment-related leukemias, ie, telomeric *MLL* bcr, has also been seen in infant leukemias [32]. This observation indicated that the mechanisms of cleavage of the *MLL* gene in infant leukemias might be similar to therapy-related, topoisomerase-II inhibitor–induced secondary leukemias. This finding in turn has led to the suggestion that infant exposure to some unknown agents with topoisomerase-II inhibitor–like activity or similar functions might be the initiating event of *MLL*-rearranged infant leukemia. Considering that *MLL* rearrangement takes place in utero in infant leukemia (see below) and that cells in the developing fetus have a high degree of cell turnover, in utero exposure to drugs, foods, or environmental factors that inhibit topoisomerase-II is considered a plausible initiating factor of *MLL* gene rearrangements for infant leukemia [33,34].

3. *MLL* Gene Rearrangements In Utero

There is compelling evidence that the *MLL* gene fusions characteristic of infant acute leukemia arise in utero. This is not surprising given the young age at diagnosis (average, 6 months for ALL), occasional congenital or neonatal presentations [35,36], and in one case, acute leukemia in a fetus that died at 36 weeks of gestation [37]. Definitive evidence

comes from two studies: an investigation of identical twins concordant for infant leukemia [38] and a retrospective scrutiny of archived neonatal blood spots (Guthrie cards) of infants with ALL [39]. Both studies exploited the fact that each patient with an *MLL* gene rearrangement and fusion has a unique or clonotypic breakpoint at the DNA level. Three pairs of identical infant twins with *MLL* fusion–positive ALL were found to share the same clonotypic but noninherited breakpoint, indicative of a single-cell origin. The only plausible interpretation of this is that *MLL* fusion occurred in one fetus and the resultant clone spread to the other co-twin via intraplacental anastomoses [38,40]. Other twin infants with ALL or AML and with shared *MLL* breakpoints have since been described [41,42].

Genomic fusion sequences of *MLL* and its common partner gene in infant ALL, *AF4*, were also used to screen the stored neonatal blood spots of 3 infants (aged 5, 6, and 24 months) with *MLL-AF4* positive ALL. In all 3, blood spots scored positively for the clonotypic fusion gene sequences. These data provide unequivocal evidence for an in utero origin of infant acute leukemia with *MLL* fusion. Similar twin and Guthrie card studies and results have been obtained for childhood leukemia with other gene fusions, including *TEL-AML1* and *AML-ETO* (reviewed in [28,40]). Clearly, these data endorse the likelihood that *MLL* gene fusions are a consequence of transplacental exposures during pregnancy.

The possible nature of the relevant exposures of pregnant women that may cause *MLL* gene breaks and chimeric fusions in the developing fetus is the subject of ongoing epidemiological investigations. The obvious parallel with *MLL* gene–associated secondary leukemia suggested that chemically similar exposures might be responsible [33,34,38]. This idea is supported by the observation that known topoisomerase-II inhibitors [22] and also dietary flavonoids [23] can cause DNA cleavage in the *MLL* gene in vitro. There might therefore be many other natural or medicinal substances that can biologically act as topoisomerase-II inhibitors. Epidemiological data so far is limited. An association has been reported between excess intake during pregnancy of fruit, possibly reflecting flavonoid excess, and resultant infant leukemia [33]. An international collaborative study involving patients in Japan, South America, Italy, and other countries found the selective link between pregnancy exposure to pesticides, in particular propoxur (Baygon), or consumption during pregnancy of the drug dipyrone, known as Mexican aspirin, and infant leukemias that had *MLL* gene rearrangements [43].

Inherited genetic background or allelic variation may alter susceptibility to *MLL* gene breakage in the developing fetus. Vulnerability to infant leukemia, particularly infant ALL with *MLL-AF4*, has been associated with low-function NAD(P)H quinone oxidoreductase (NQO1) alleles [44,45]. NQO1 detoxifies benzene metabolites and quinone ring–based chemicals (which include flavonoids). Loss of functional alleles in the enzyme methylenetetrahydrofolate reductase (MTHFR) are also associated significantly with decreased risk of leukemia with *MLL* translocations [46]. These genetic data are likely to be extended in the near future with the successful completion of the human genome project and the availability of new techniques for high-throughput single-nucleotide polymorphism (SNP) screening. The available data, however, strongly indicate that *MLL* gene fusions driving the pathogenesis of infant leukemia are probably a consequence of transplacental chemical exposures of the developing fetal hematopoietic system, with risk being modulated by genetic and possibly dietary factors.

Infant ALL with *MLL-AF4* fusions has a particularly poor clinical outcome, although the prognosis has been somewhat improved with more intensive induction chemotherapy including cytosine arabinoside (Ara-C) and bone marrow transplantation [1]. These leukemias respond poorly to lymphoid chemotherapy used to treat the common forms of childhood ALL, and one distinct possibility is that infant ALL is not in fact a lymphoid leukemia at all but rather originates in a lymphomyeloid or B/monocytic stem cell that is active in fetal hematopoiesis [1,28]. Evidence supporting this view comes from the lymphomyeloid phenotypes on single leukemic cells [47], myeloid gene expression profiles in microarray tests (see further below), patterns of drug sensitivity [1], and the observation of a rapid intraclonal switch from B lymphoid to monocytic cells in a congenital leukemia following administration of corticosteroid and Ara-C [36]. Modeling of leukemogenesis in mice via *MLL* fusions with *MLL-GAS7* also provides convincing evidence for lymphomyeloid stem cells as potential targets for transformation [48].

4. Functions of Normal MLL

The protein product encoded by *MLL* has 3969 amino acids and a molecular weight of nearly 430 kd. MLL is the mammalian equivalent of Drosophila trithorax [5,6,12], which is a key developmental regulator of stable gene expression from *HOX* and possibly other loci in humans, mice, and other vertebrate species [49,50].

Important insights into the normal function of MLL protein have been obtained from several gene-targeting or mutation studies. In a gene knock-out study, mice with a single targeted allele (ie, MLL heterozygous) showed axial-skeletal abnormalities with disordered identity of cervical, thoracic, and lumbar regions and a disturbed pattern of *HOXA7* and *HOXC9* gene expression [49]. Another consistent abnormality observed in these mice was anemia and thrombocytopenia indicating hematopoietic progenitor dysfunction. The homozygote with no functional MLL allele is an embryonic lethal with completely absent expression of *HOXA7* and *HOXC9* in the embryo [49]. Hematopoietic progenitors from homozygous embryos showed impaired proliferative activity [51,52]. These studies indicate that MLL is a positive regulator of *HOX* genes, probably via maintenance of gene expression rather than activation [50]. A critical role of MLL in hematopoietic development was further shown by experiments in vitro using hematopoietic progenitors from mice that were MLL null [51,53] or mice with MLL mutations [52]. Proliferation and differentiation of hematopoietic cells without MLL were greatly impaired. *HOX* gene expression has been identified in normal hematopoietic tissues including hematopoietic stem cells and confers a significant impact on proliferation and differentiation [54]. The hematopoietic dysfunction of MLL null cells is likely

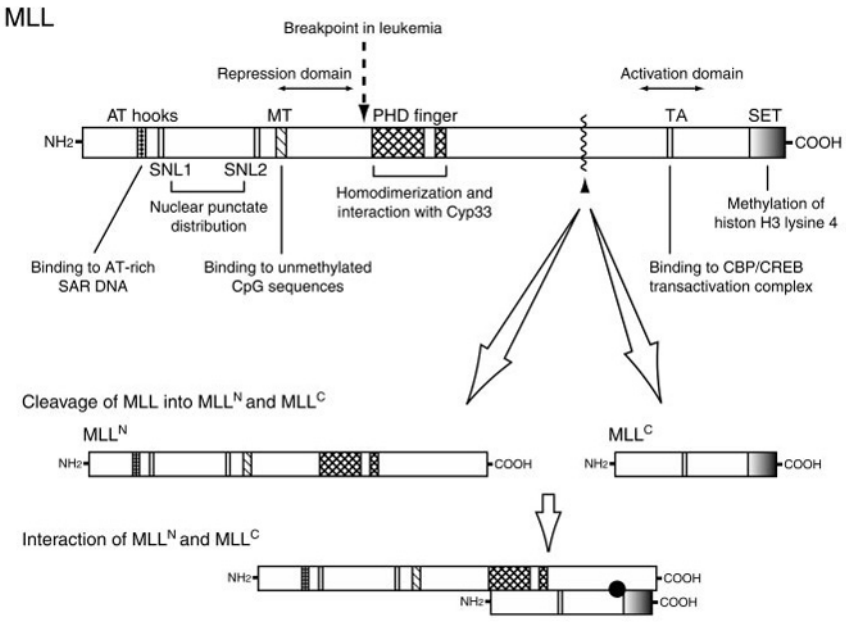

Figure 2. Protein structure of MLL. Wild-type MLL is proteolytically cleaved into N-terminal and C-terminal moiety (MLL^N and MLL^C). These 2 proteins interact with each other to confirm stability. MT indicates DNA methyltransferase homology region; PHD, PHD zinc fingers; TA, transactivation domain; SET, SET domain; SNL1 and 2, speckled nuclear localization signals 1 and 2. Dotted vertical arrow indicates the location of the breakpoint that occurs in leukemia with MLL gene translocation.

therefore to be attributed to deregulated patterns of *HOX* gene expression in hematopoietic stem cells or progenitors. The corollary is that this same transcriptional signaling pathway is probably corrupted as a consequence of *MLL* gene fusion in leukemogenesis.

MLL protein possesses at least 4 functional domains that could confer significant functional activity (Figure 2): AT hooks, DNA methyltransferase homology domain (MT), PHD zinc finger, and C-terminal SET domain. In addition to these domains, MLL also has nuclear localization signals in its N-terminal that determine a punctate localization pattern of the protein in the nucleus [55,56]. Of these functional domains, AT hooks and MT domain have DNA-binding activity that could be involved in the regulation of gene transcription through direct binding to DNA. The AT hook has homology to the high-mobility group I (HMG-I) protein, which binds to AT-rich regions of the minor groove of the DNA. Similar to HMG-I, MLL can bind to AT-rich sequences [57,58], but it recognizes DNA structure rather than specific consensus sequences. The MT domain is a cysteine-rich CXXC domain that is highly conserved among CpG methylation–related proteins such as DNMT1 and MBD1. Like these proteins, the MT domain of MLL has DNA-binding activity to CpG sequences, although only to unmethylated sequences, in contrast to MBD1, which binds methylated CpG sequences [59]. Although MLL can bind to CpG sequences, there is no evidence as yet for actual DNA methyltransferase activity conferred by MLL. The region of MLL including the MT domain is also known to have transcriptional repression activity [60] by recruiting repressor complex(es) such as HDAC or polycomb group proteins such as HPC2 or Bmi-1 [61].

Only a few reports concerning the PHD finger of MLL are available so far, and the precise functions of this evolutionarily conserved domain are still unknown. At least some part of transcriptional regulation/chromatin remodeling activity of MLL may be attributable to the PHD finger domain because most proteins with PHD fingers are components of chromatin remodeling/transcriptional regulation complexes [62]. One of the PHD fingers of MLL has homodimerization activity, although its significance is currently unclear [63]. In addition, nuclear cyclophilin Cyp33 can bind to the PHD finger of MLL, and overexpression of Cyp33 is known to have some effects on the expression pattern of *HOX* genes [63]. MLL also has a transcriptional activation domain between the PHD fingers and C-terminal SET domain. This activation domain of MLL can bind directly to CBP and facilitate the binding of CBP with CREB to promote transcriptional activation [64]. The SET domain is also an evolutionarily highly conserved region and has activity as a lysine-directed histone methyltransferase that impacts on chromatin structure and transcriptional regulation of *HOX* genes [65,66].

Full-length MLL protein is proteolytically cleaved into 2 parts (MLL N-terminal fragment MLL^N and C-terminal fragment MLL^C), and these 2 proteins need to interact with each other to avoid degradation and confer stability [67,68]. The cleavage sites within MLL and the interaction domain of MLL^N with the MLL^C fragment are located downstream of the MLL breakpoint cluster region and are thus deleted in MLL fusion proteins. The mechanism of degradation of MLL^N protein is still not clear, but loss of the interacting domain with the MLL^C fragment in the MLL fusion protein could result in instability of the fusion protein. A common

Table 1.
MLL Fusion Partners*

Gene	Chromosome Locus	Functions	Localization
Translocation partner genes of MLL (common)			
AF4	4q21	Transcriptional activator	N
AF6	6q27	Maintenance of cell–cell junctions and cell polarity	C
AF9	9p22	Transcriptional factor	N
AF10	10p12	Transcriptional factor	N
ELL/MEN	19p13.1	RNA polymerase II transcription elongation factor	N
ENL	19p13.3	Transcriptional activator	N
Translocation partner genes of MLL (rare)			
AFX	Xq13	Forkhead transcriptional factor	N
Septin6	Xq22	Septin family	C
AF1p	1p32	Regulation of endocytosis	C, N
AF1q	1q21	NK	NK
LAF4	2q11	Transcriptional activator	N
AF3p21	3p21	NK	N
GMPS	3q25	Guanosine monophosphate synthetase	C
LPP	3q28	Regulation of cell motility and focal adhesion	C, N
AF5q31	5q31	Transcriptional activator (?)	N (?)
GRAF	5q31	Negative regulator of RhoA	C
FKHRL1/AF6q21	6q21	Forkhead transcriptional factor	N
AF9q34/DAB2IP	9q34	Ras GTPase-activating protein	C
FBP17	9q34	NK	C
ABI1	10p11.2	Regulation of endocytosis (?), cell motility	C
LCX /TET1	10q22	NK	NK
CALM	11q14-q21	Regulation of endocytosis (assembly of clathrin coat to plasma membrane)	C, N
LARG	11q23.3	Activator of Rho GTPases	C
CBL	11q23.3	Negative regulator of receptor tyrosine kinases	C
GPHN	14q24	Gly and GABA receptors assembly, molybdenum cofactor biosynthesis	C
AF15q14	15q14	NK	NK
MPFYVE	15q14	NK	NK
CBP	16p13	Transcriptional coactivator, histone acetylase	N
GAS7	17p13	Actin assembly/crosslinking of actin filaments	C
AF17	17q21	Transcriptional factor, up-regulated by β-catenin	N
LASP1	17q21	NK	C
MSF/AF17q25	17q25	Septin family	C
EEN	19p13.3	Regulation of endocytosis (?)	C (?)
hCDCrel/AF22	22q11	Septin family	C
p300	22q13	Transcriptional coactivator, histone acetylase	N

*N indicates nuclear localization; C, cytoplasmic localization; NK, not known; (?), likely but not confirmed.

shared feature of MLL fusion partner proteins could be to stabilize this unstable MLL^N fragment.

5. Promiscuity of MLL Fusions

MLL has approximately 40 reported fusion partners that appear to contribute to a variety of functional domains [1,7,8,69] (Table 1). All fusions in leukemia are in frame and are believed to produce a full-length hybrid protein with novel properties. Despite this extensive genetic diversity of fusion the MLL fusion proteins always include, at the N-terminus, the DNA-binding AT hooks and MT homology domains of MLL but not the PHD zinc finger, and SET domain [60] (Figure 3A). The differences between various MLL fusions therefore reside in the domains contributed by fusion partners. Some of the fusion partner genes encode nuclear protein with presumed transcriptional modulation activity. However, a considerable number are cytoplasmic proteins with diverse functions. MLL fusion proteins form punctate patterns in the nucleus, regardless of whether the fusion partner itself is normally nuclear or cytoplasmic in origin [55,70] (M.E. et al, unpublished data). Several investigators have searched for structural and/or functional similarities among the different MLL partners. From the structural point of view, and with the exception of the homology shown by the group of AF4, LAF4, and AF5q31, the group of AF9 and ENL, the group of AF10 and AF17, the group of AFX and FKHRL1/AF6q21, and the group including MSF, septin6, and hCDCrel, sequence analysis does not reveal any universally shared or uniform features. These data highlight a conundrum: is there a common mechanism to explain the contribution of the many diverse partner genes in *MLL* gene chimeras? Any explanation would need to accommodate the fact that MLL internal duplications can also be leukemogenic [71], but MLL truncation alone is not.

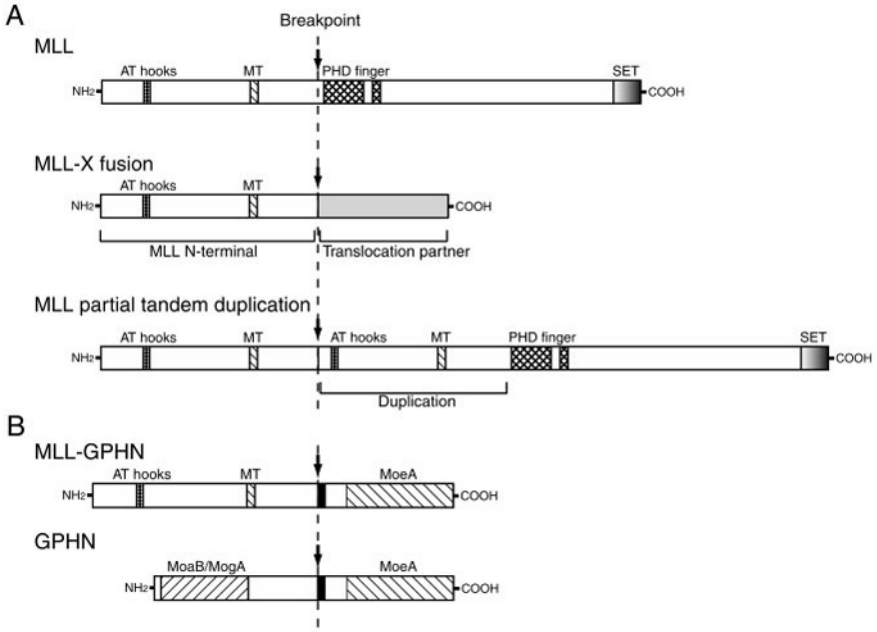

Figure 3. A, Schematic representation of wild-type and generic MLL mutant protein. N-terminal moiety of MLL is fused to the C-terminal portion of the fusion partner protein, or internally duplicated in leukemia. B, Schematic representation of MLL-GPHN fusion protein and wild-type GPHN. MT, SET, see legend of Figure 2; MoaB, MogA, and MoeA, regions in GPHN with sequence similarities to the respective *Escherichia coli* proteins involved in molybdenum cofactor biosynthesis. Arrows indicate the fusion site. Numbers refer to amino acid positions in wild-type MLL or GPHN. The black bar indicates the tubulin-binding site of GPHN (an oligomerization domain).

6. What Determines the Transforming Capacity of *MLL* Fusion Genes?

Obtaining in vivo and in vitro model systems to assess the transforming capacity of the fusion proteins has been important to elucidating the mechanism underlying leukemia with *MLL* plus its translocation partners. Lavau et al first described an in vitro myeloid clonogenic assay using retroviral-mediated transduction of murine bone marrow cells [72] (Figure 4). When an *MLL* fusion gene is retrovirally transduced into lineage-depleted murine bone marrow cells, colonies are formed in myeloid-conditioned methylcellulose. Immortalized myeloid progenitors maintain self-renewal and clonogenic capacity during serial replating and give rise to leukemia when introduced to irradiated or SCID mice. In contrast, normal progenitor cells display loss in clonogenic potential after one round of replating. This method not only allows assessment of the transforming capacity of *MLL* fusion genes in vivo and in vitro, but also facilitates mutational analyses for determining the minimal domains required for transformation.

The transforming capacity of MLL fusions with nuclear proteins as partners has been studied extensively using these retroviral gene transfer assays. MLL fusion between ENL, ELL, AF10, AFX, FKHRL1, and CBP all transform murine hematopoietic progenitors in the myeloid clonogenic assay [72-78]. When transferred into a mouse model, these different fusions result in leukemia development in vivo over variable time frames, with shorter latency noted for MLL-AF10 (mean 52 days) [75] compared with MLL-AFX (mean 185 days) [76]. One of the important conclusions from these studies is that neither N-terminal MLL alone or the C-terminal fusion partner alone was sufficient for transformation, indicating that dominant gain of function may be important [72].

Functional studies of the respective fusion proteins revealed that the minimal domains required for transformation display transactivation potentials [73-81]. This finding indicates that a common mechanism for transformation in some of MLL's *nuclear* protein fusion partners is to provide transcriptional effector domains to MLL, causing deregulation of its transcription-modulating functions. Further evidence comes from studies in which the transcriptional activation domain of herpes simplex virus VP16 conferred on MLL competence to transform murine primary bone marrow cells [82]. These data support the notion that a transcriptional domain may be an obligatory requirement for transformation by MLL fusion protein [76,77,79]. However, knock-in of an *MLL-β-galactosidase* (*LacZ*) gene also induced leukemia, albeit with a relatively long latency (approximately one third of mice leukemic by 18 months of age) [83]. LacZ itself has not been reported to have any transcriptional activity, but one possibly relevant feature of LacZ is its capacity to form tetramers [84]. The presence of this feature suggested the possibility that 3′ protein sequences contributed by some MLL fusion partners might provide regions that facilitate oligomerization of MLL which, in turn, alters 5′ MLL protein function, interaction with DNA for example, in such a way as to be transforming. The finding that *MLL* can contribute to AML via duplication of exons rather than by chromosome translocation and

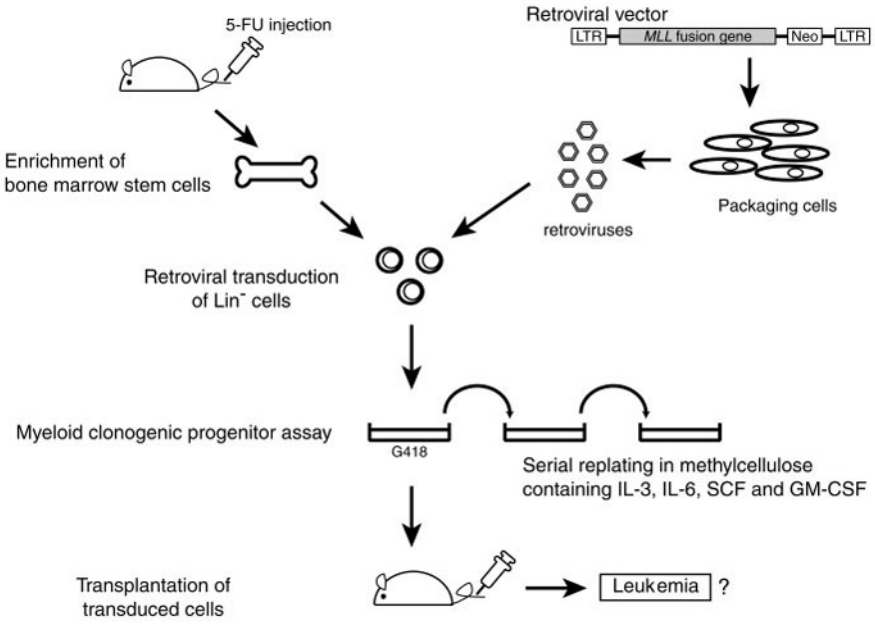

Figure 4. Experimental strategy for transduction of primitive myeloid progenitor cells. Murine bone marrow cells are harvested 5 days after 5-fluorouracil (5-FU) injection. Hematopoetic progenitors are enriched by lineage depletion of bone marrow cells, then transduced with a retroviral vector encoding an *MLL* fusion gene sequence. Transduced cells were serially replated in methylcellulose medium containing cytokines, and also transplanted into SCID or sublethally irradiated synergic mice.

fusion [71] also implies that partner genes may provide some generic function that alters *MLL* gene activity.

We have recently showed, using an *MLL-GPHN* fusion gene [85,86] (Figure 3B) as a model, that the small oligomerization domain of GPHN is necessary and sufficient, as a fusion partner with MLL, to transform hematopoietic stem cells (M.E. et al, unpublished data). So et al also recently reported that two cytoplasmic fusion partners of MLL, AF1p and GAS7 with coiled-coil domains, induce dimerization of MLL and activate its transcriptional and oncogenic properties [87]. These studies suggest that transcriptional activity is not an obligatory requirement for a partner gene to transform hematopoietic cells, and also suggest that oligomerization of truncated MLL protein may be important, possibly resulting in inappropriate recruitment of proteins and consequent deregulation of gene expression (Figure 5).

7. The Brief Latencies of Infant and Secondary Leukemia: Is *MLL* Fusion Sufficient to Cause Leukemia?

A striking feature of the natural history of infant ALL and secondary leukemias with *MLL* fusion genes is the remarkably brief preclinical natural history or latency. In the former, the average age at presentation is approximately 6 months [88]. For secondary leukemias with *MLL* fusions, the average latency from introduction of therapy is approximately 26 months [89], and *MLL* fusions are detectable very early in this period [90]. This brief latency is very unusual and perhaps unique for a malignant cancer. Coupled with the extremely high concordance of *MLL* fusion gene–positive leukemia in twins, these data suggest that *MLL* fusions are powerfully leukemogenic. The very high twin concordance rate for leukemia in infants [40] implies that in the presence of a functional *MLL* fusion, leukemia is inevitable and that all necessary genetic events occur prenatally. This finding has important etiological connotations. One biological explanation for this might be that the *MLL* fusion gene–encoded protein has a pleiotypic or global impact on chromatin structure and gene expression, simultaneously corrupting several signaling pathways, ie, an *MLL* chimeric gene is *sufficient* for leukemia. This was considered as a possible explanation of the infant twin data [38].

But there are other data that do not fit with this explanation. First, at diagnosis, leukemias with *MLL* fusions frequently have additional detectable chromosomal abnormalities (and gene mutations) [91-93]. Second, animal modeling tells a different story. Arguably the best in vivo model for *MLL* fusion–positive acute leukemia has until very recently been the *MLL-AF9* knock-in [94]. A striking feature of this and other in vivo murine models is, however, that latency is usually protracted (>9 months). The authors of these reports comment that latency reflects the time required for necessary secondary genetic events to arise. This theory accords with a chicken model for *MLL* fusion–positive leukemia in which MLL-ENL produced leukemia in vivo but only in combination with an activated kinase [95]. But these modeling observations ignore the paradox that the latency time frame is extraordinarily brief in the real clinical setting of *MLL* fusion–positive infant leukemia or secondary leukemia. A recent report describes an inducible cre-lox translocation system in which somatic *MLL-ENL* fusion can be generated in vivo [96]. A striking feature of this system is the high penetrance of leukemia (100%) and a shorter average latency of

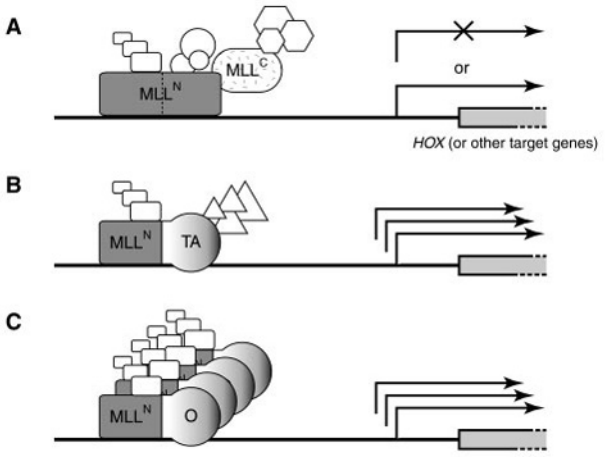

Figure 5. Different mechanisms for regulating target gene(s) by MLL proteins. A, Wild-type MLL is cleaved into MLL^N and MLL^C, which then interact with each other to form a multiprotein complex that can regulate *HOX* (or other) genes by remodeling chromatin through histone acetylation, deacetylation, and methylation. Wild-type MLL is necessary to maintain *HOX* gene expression in hematopoietic progenitor cells. B, MLL chimeric protein with nuclear fusion partner containing the activation domain is depicted. MLL fusion protein binds to DNA through MLL moiety. Fusion protein can recruit transcriptional modulators through MLL and partner protein included in the chimera, causing constitutive activation of the target genes. C, MLL chimeric protein with some cytoplasmic fusion partners can force oligomerization, which can also cause constitutive activation of the target genes. MLL^N, MLL^C, N-terminal and C-terminal moiety of MLL; TA, translocation partner with transactivation domain; O, translocation partner with oligomerization domain.

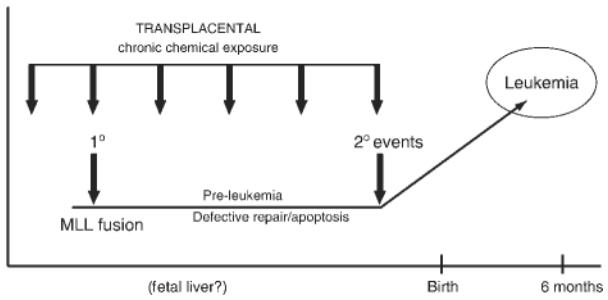

Figure 6. A hypothesis for brief latency in infant ALL. *MLL* gene fusion arises in utero as a consequence of transplacental exposure to chemical carcinogens. The presence of a *MLL* fusion gene positive clone of preleukemic cells with continued presence of the carcinogen would increase the risk of secondary genetic events arising in damaged cells surviving such damage. Note that the same hypothesis can be applied to therapy-related secondary leukemias with *MLL* fusions.

approximately 2 to 3 months. This latency period is still relatively long in mice compared with human infants, but it is sufficiently brief for the authors to suggest (with reference to the twin studies) that *MLL* gene fusion might, by itself, be sufficient to cause leukemia. The dilemma is then: are secondary mutations necessary to complement *MLL* fusions and, if so, how is it possible for them to be acquired so rapidly?

Previous laboratory and epidemiological studies suggest a possible resolution of this conundrum [40]. The twin data suggest that all necessary genetic events for infant ALL are accomplished prenatally, ie, in a few weeks or months, and that *MLL* gene fusion arises as a consequence of transplacental exposure to chemical carcinogens. Suppose therefore that:

(*a*) exposure to such chemicals during pregnancy (and in therapy preceding secondary leukemia) is chronic, and

(*b*) *MLL* fusion gene function includes both (1) a block to differentiation, probably via *HOX* gene dysregulation [97,98] and (2) abrogation of some critical aspect of cell cycle checkpoints, DNA damage recognition, apoptosis, and repair.

Then:

the presence of an *MLL* fusion gene–positive clone of preleukemic cells *plus* continued presence of the carcinogen would greatly increase the risk of secondary genetic events arising and/or of damaged cells surviving such damage. In the mouse model systems, the *MLL* fusion transgene is continually expressed but the DNA-damaging agent is not. An experimental parallel, or precedent, might be the very much abbreviated latency of leukemia in p53 mutant mice exposed to low-dose X-irradiation [99], coupled with our earlier finding that in a p53 null background, cells that have been X-irradiated and that would otherwise have died now survive as clonogenic mutants [100].

Data indirectly supporting this functional model for MLL-associated leukemia come from Adler et al [101]. The *GADD34* gene encodes a DNA damage–inducible factor that promotes apoptosis. MLL fusion protein appears to target and inhibit GADD34. This hypothesis (Figure 6) is currently being assessed in model systems using *MLL-GPHN*.

8. Clinical Implications

8.1. DNA Arrays as a Tool for Understanding Biology and Therapeutic Response

Although pediatric acute leukemia is a success story for cancer treatment [102], infant leukemias and de novo/secondary leukemias with *MLL* gene rearrangements, particularly infants with MLL-AF4, still have a relatively poor outcome [1,21,103-105]. A better understanding of the mechanism of leukemogenesis with *MLL* gene fusions might identify novel targets for improved therapy. Gene expression profiling studies are a step in this direction [98,106-110]. From these studies *MLL*-positive leukemias are seen to have unique gene expression signatures. Interestingly, genes that have a functional role in early B-cell development are underexpressed in infant pro-B ALL with MLL fusions, and some genes that are expressed in hematopoietic progenitors, such as *FLT3*, *LMO2*, are highly expressed [98,106,107,109]. Some *HOX* genes such as *HOXA9*, *HOXA5*, *HOXA4*, and *HOXC6* are expressed at high levels in leukemias with *MLL* fusions [98,106,107]. *MEIS1*, which encodes a cofactor for HOX proteins and is known to accelerate HOXA9-dependent leukemia, is also overexpressed [98,106,107]. Additionally Tsutsumi et al were able to recognize unique expression pro-

files that segregated infant ALL into good and very poor responders [109]. Genes whose expression provides this important distinction included the transcription factors *CBF2* and *CDP*. DNA microarrays provide therefore not only an incisive diagnostic tool to complement conventional karyotyping, fluorescence in situ hybridization, and reverse transcriptase polymerase chain reaction, but data that may suggest a biological rationale for treatment failure and identify molecular targets for novel therapies.

8.2. New Therapeutic Targets

There are some very encouraging precedents for targeted biological therapy in leukemia. In acute promyelocytic leukemia (APL) with the *PML-RARα* fusion gene, the aberrant protein product blocks differentiation of myeloid cells by recruiting repressor molecules such as histone deacetylase (HDAC) enzymes [111]. Successful targeted therapy for this type of leukemia exploits retinoic acid to reverse the block to normal myeloid cell development [112], or uses inhibitors for the HDAC repressor molecules [113]. The histone deacetylase inhibitors tricostatin and phenylbutyrate have been shown to reverse ETO-mediated transcription repression in *AML1-ETO*–positive AML [114]. These inhibitors have also been shown to restore retinoic acid-dependent transcriptional activation to cause terminal differentiation of the blasts [115]. *BCR-ABL* fusion in Philadelphia chromosome–positive CML and ALL results in active Abl kinase enzyme. Targeted therapy for this type of leukemia uses STI-571 (Gleevec/imatinib mesylate), a selective inhibitor of Abl and related kinases, and has been very effective in CML [116,117], despite the emergence of drug-resistant Abl mutations in some cases [118].

In *MLL* positive ALL 16%-18% have mutations in the activation loop of *Flt3*, indicating that this may be a common secondary hit in *MLL* leukemias [119,120]. In Flt3-mutated cases, constitutive activation of the kinase result in resistance to induction therapy. A recent report has shown that the Flt3-specific inhibitor PKC412 can inhibit aberrant cell proliferation in vitro and in vivo [119], suggesting that Flt3 may be a viable target for some leukemias with *MLL* fusions as well as for other leukemias (eg, many adult AMLs) in which Flt3 activation is implicated.

In diverse human cancers, hypermethylation of CpG islands in the promotors of tumor suppressor genes often exist [121,122], causing gene silencing. The demethylating agent 5-Aza-cytidine is an inhibitor of DNA methylation when incorporated into DNA [123]. This drug has worked successfully in MDS [124] and also was found to have a suppressive effect in cell lines bearing an *MLL* gene rearrangement [125]. Clearly its potential in leukemia with MLL fusions merits further exploration.

Finally, recent findings suggest that one of the common mechanisms in leukemia is abnormal recruitment of histone deacetylase (HDAC)-containing complexes to promoters of target genes. The MT domain of MLL, which is also retained in the chimeric protein, is known to bind to HDAC complexes [61]. The role of HDAC in *MLL* leukemia has not been fully clarified yet but HDAC inhibitors are candidate therapeutic molecules for these otherwise intransigent leukemias.

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References

1. Biondi A, Cimino G, Pieters R, Pui CH. Biological and therapeutic aspects of infant leukemia. *Blood*. 2000;96:24-33.
2. Felix CA. Secondary leukemias induced by topoisomerase-targeted drugs. *Biochim Biophys Acta*. 1998;1400:233-255.
3. Ziemin-van der Poel S, McCabe NR, Gill HJ, et al. Identification of a gene, MLL, that spans the breakpoint in 11q23 translocations associated with human leukemias. *Proc Natl Acad Sci U S A*. 1991; 88:10735-10739.
4. Cimino G, Moir DT, Canaani O, et al. Cloning of ALL-1, the locus involved in leukemias with the t(4;11)(q21;q23), t(9;11)(p22;q23), and t(11;19)(q23;p13) chromosome translocations. *Cancer Res*. 1991;51:6712-6714.
5. Tkachuk DC, Kohler S, Cleary ML. Involvement of a homolog of Drosophila trithorax by 11q23 chromosomal translocations in acute leukemias. *Cell*. 1992;71:691-700.
6. Djabali M, Selleri L, Parry P, Bower M, Young BD, Evans GA. A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. *Nat Genet*. 1992;2:113-118.
7. DiMartino JF, Cleary ML. Mll rearrangements in haematological malignancies: lessons from clinical and biological studies. *Br J Haematol*. 1999;106:614-626.
8. Rowley JD. The role of chromosome translocations in leukemogenesis. *Semin Hematol*. 1999;36:59-72.
9. Nilson I, Lochner K, Siegler G, et al. Exon/intron structure of the human ALL-1 (MLL) gene involved in translocations to chromosomal region 11q23 and acute leukaemias. *Br J Haematol*. 1996;93: 966-972.
10. Rasio D, Schichman SA, Negrini M, Canaani E, Croce CM. Complete exon structure of the ALL1 gene. *Cancer Res*. 1996;56: 1766-1769.
11. Marschalek R, Nilson I, Lochner K, et al. The structure of the human ALL-1/MLL/HRX gene. *Leuk Lymphoma*. 1997;27: 417-428.
12. Gu Y, Nakamura T, Alder H, et al. The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. *Cell*. 1992;71:701-708.
13. Bernard OA, Berger R. Molecular basis of 11q23 rearrangements in hematopoietic malignant proliferations. *Genes Chromosomes Cancer*. 1995;13:75-85.
14. Gu Y, Alder H, Nakamura T, et al. Sequence analysis of the breakpoint cluster region in the ALL-1 gene involved in acute leukemia. *Cancer Res*. 1994;54:2326-2330.
15. Broeker PL, Super HG, Thirman MJ, et al. Distribution of 11q23 breakpoints within the MLL breakpoint cluster region in de novo acute leukemia and in treatment-related acute myeloid leukemia: correlation with scaffold attachment regions and topoisomerase II consensus binding sites. *Blood*. 1996;87:1912-1922.
16. Mirkovitch J, Mirault ME, Laemmli UK. Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. *Cell*. 1984;39:223-232.
17. Laemmli UK, Kas E, Poljak L, Adachi Y. Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. *Curr Opin Genet Dev*. 1992;2:275-285.
18. Schichman SA, Caligiuri MA, Strout MP, et al. ALL-1 tandem duplication in acute myeloid leukemia with a normal karyotype involves homologous recombination between Alu elements. *Cancer Res*. 1994;54:4277-4280.
19. So CW, Ma ZG, Price CM, et al. MLL self fusion mediated by Alu repeat homologous recombination and prognosis of AML-M4/M5 subtypes. *Cancer Res*. 1997;57:117-122.
20. 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 U S A*. 1998;95:2390-2395.
21. Felix CA. Leukemias related to treatment with DNA topoisomerase II inhibitors. *Med Pediatr Oncol*. 2001;36:525-535.
22. Aplan PD, Chervinsky DS, Stanulla M, Burhans WC. Site-specific DNA cleavage within the MLL breakpoint cluster region induced by topoisomerase II inhibitors. *Blood*. 1996;87:2649-2658.
23. Strick R, Strissel PL, Borgers S, Smith SL, Rowley JD. Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. *Proc Natl Acad Sci U S A*. 2000;97: 4790-4795.
24. Ishii E, Eguchi M, Eguchi-Ishimae M, et al. In vitro cleavage of the MLL gene by topoisomerase II inhibitor (etoposide) in normal cord and peripheral blood mononuclear cells. *Int J Hematol*. 2002; 76:74-79.
25. Reichel M, Gillert E, Nilson I, et al. Fine structure of translocation breakpoints in leukemic blasts with chromosomal translocation t(4;11): the DNA damage-repair model of translocation. *Oncogene*. 1998;17:3035-3044.
26. Gillert E, Leis T, Repp R, et al. A DNA damage repair mechanism is involved in the origin of chromosomal translocations t(4;11) in primary leukemic cells. *Oncogene*. 1999;18:4663-4671.
27. Langer T, Metzler M, Reinhardt D, et al. Analysis of t(9;11) chromosomal breakpoint sequences in childhood acute leukemia: almost identical MLL breakpoints in therapy-related AML after treatment without etoposides. *Genes Chromosomes Cancer*. 2003;36:393-401.
28. Greaves MF, Wiemel J. Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer*. 2003;3:639-649.
29. Betti CJ, Villalobos MJ, Diaz MO, Vaughan AT. Apoptotic triggers initiate translocations within the MLL gene involving the nonhomologous end joining repair system. *Cancer Res*. 2001;61: 4550-4555.
30. Sim SP, Liu LF. Nucleolytic cleavage of the mixed lineage leukemia breakpoint cluster region during apoptosis. *J Biol Chem*. 2001;276: 31590-31595.
31. Betti CJ, Villalobos MJ, Diaz MO, Vaughan AT. Apoptotic stimuli initiate MLL-AF9 translocations that are transcribed in cells capable of division. *Cancer Res*. 2003;63:1377-1381.
32. Cimino G, Rapanotti MC, Biondi A, et al. Infant acute leukemias show the same biased distribution of ALL1 gene breaks as topoisomerase II related secondary acute leukemias. *Cancer Res*. 1997; 57:2879-2883.
33. Ross JA, Potter JD, Reaman GH, Pendergrass TW, Robison LL. Maternal exposure to potential inhibitors of DNA topoisomerase II and infant leukemia (United States): a report from the Children's Cancer Group. *Cancer Causes Control*. 1996;7:581-590.
34. Greaves MF. Aetiology of acute leukaemia. *Lancet*. 1997;349: 344-349.
35. Heim S, Bekassy AN, Garwicz S, et al. New structural chromosomal rearrangements in congenital leukemia. *Leukemia*. 1987;1:16-23.
36. Ridge SA, Cabrera ME, Ford AM, et al. Rapid intraclonal switch of lineage dominance in congenital leukaemia with a MLL gene rearrangement. *Leukemia*. 1995;9:2023-2026.
37. Hunger SP, McGavran L, Meltesen L, Parker NB, Kassenbrock CK, Bitter MA. Oncogenesis in utero: fetal death due to acute myelogenous leukaemia with an MLL translocation. *Br J Haematol*. 1998; 103:539-542.
38. Ford AM, Ridge SA, Cabrera ME, et al. In utero rearrangements in the trithorax-related oncogene in infant leukaemias. *Nature*. 1993;363:358-360.
39. Gale KB, Ford AM, Repp R, et al. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proc Natl Acad Sci U S A*. 1997;94:13950-13954.
40. Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. *Blood*. 2003;102:2321-2333.
41. Gill Super HJ, Rothberg PG, Kobayashi H, Freeman AI, Diaz MO, Rowley JD. Clonal, nonconstitutional rearrangements of the MLL gene in infant twins with acute lymphoblastic leukemia: in utero chromosome rearrangement of 11q23. *Blood*. 1994;83:641-644.
42. Megonigal MD, Rappaport EF, Jones DH, et al. t(11;22)(q23;q11.2) in acute myeloid leukemia of infant twins fuses MLL with hCDCrel, a cell division cycle gene in the genomic region of deletion in DiGeorge and velocardiofacial syndromes. *Proc Natl Acad Sci U S A*. 1998;95:6413-6418.
43. Alexander FE, Patheal SL, Biondi A, et al. Transplacental chemical exposure and risk of infant leukemia with MLL gene fusion. *Cancer Res*. 2001;61:2542-2546.
44. Wiemels JL, Pagnamenta A, Taylor GM, et al. A lack of a functional NAD(P)H:quinone oxidoreductase allele is selectively associated with pediatric leukemias that have MLL fusions. *Cancer Res*. 1999;59:4095-4099.
45. Smith MT, Wang Y, Skibola CF, et al. Low NAD(P)H:quinone oxidoreductase activity is associated with increased risk of leukemia with MLL translocations in infants and children. *Blood*. 2002;100: 4590-4593.
46. Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. *Proc Natl Acad Sci U S A*. 2001;98:4004-4009.
47. Matamoros N, Matutes E, Hernandez M, et al. Neonatal mixed lineage acute leukaemia. *Leukemia*. 1994;8:1236-1242.
48. 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;3:161-171.
49. Yu BD, Hess JL, Horning SE, Brown GA, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. *Nature*. 1995;378:505-508.
50. 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 U S A*. 1998;95:10632-10636.
51. Hess JL, Yu BD, Li B, Hanson R, Korsmeyer SJ. Defects in yolk sac hematopoiesis in Mll-null embryos. *Blood*. 1997;90:1799-1806.
52. Yagi H, Deguchi K, Aono A, Tani Y, Kishimoto T, Komori T. Growth disturbance in fetal liver hematopoiesis of Mll-mutant mice. *Blood*. 1998;92:108-117.
53. Fidanza V, Melotti P, Yano T, et al. Double knockout of the ALL-1 gene blocks hematopoietic differentiation in vitro. *Cancer Res*. 1996;56:1179-1183.
54. Owens BM, Hawley RG. HOX and non-HOX homeobox genes in leukemic hematopoiesis. *Stem Cells*. 2002;20:364-379.
55. Yano T, Nakamura T, Blechman J, et al. Nuclear punctate distribution of ALL-1 is conferred by distinct elements at the N terminus of the protein. *Proc Natl Acad Sci U S A*. 1997;94:7286-7291.
56. Caslini C, Alarcon AS, Hess JL, Tanaka R, Murti KG, Biondi A. The amino terminus targets the mixed lineage leukemia (MLL) protein to the nucleolus, nuclear matrix and mitotic chromosomal scaffolds. *Leukemia*. 2000;14:1898-1908.
57. Broeker PL, Harden A, Rowley JD, Zeleznik-Le N. The mixed lineage leukemia (MLL) protein involved in 11q23 translocations contains a domain that binds cruciform DNA and scaffold attachment region (SAR) DNA. *Curr Top Microbiol Immunol*. 1996;211: 259-268.
58. Aravind L, Landsman D. AT-hook motifs identified in a wide variety of DNA-binding proteins. *Nucleic Acids Res*. 1998;26: 4413-4421.
59. Birke M, Schreiner S, Garcia-Cuellar M-P, Mahr K, Titgemeyer F, Slany RK. The MT domain of the proto-oncoprotein MLL binds to CpG-containing DNA and discriminates against methylation. *Nucl Acids Res*. 2002;30:958-965.
60. Zeleznik-Le NJ, Harden AM, Rowley JD. 11q23 translocations split the "AT-hook" cruciform DNA-binding region and the transcriptional repression domain from the activation domain of the mixed-lineage leukemia (MLL) gene. *Proc Natl Acad Sci U S A*. 1994;91: 10610-10614.
61. Xia Z-B, Anderson M, Diaz MO, Zeleznik-Le NJ. MLL repression

domain interacts with histone deacetylases, the polycomb group proteins HPC2 and BMI-1, and the corepressor C-terminal-binding protein. *Proc Natl Acad Sci U S A*. 2003;100:8342-8347.
62. Aasland R, Gibson TJ, Stewart AF. The PHD finger: implications for chromatin-mediated transcriptional regulation. *Trends Biochem Sci*. 1995;20:56-59.
63. Fair K, Anderson M, Bulanova E, Mi H, Tropschug M, Diaz MO. Protein interactions of the MLL PHD fingers modulate MLL target gene regulation in human cells. *Mol Cell Biol*. 2001;21: 3589-3597.
64. Ernst P, Wang J, Huang M, Goodman RH, Korsmeyer SJ. MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Mol Cell Biol*. 2001;21:2249-2258.
65. Milne TA, Briggs SD, Brock HW, et al. MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol Cell*. 2002; 10:1107-1117.
66. Nakamura T, Mori T, Tada S, et al. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol Cell*. 2002;10:1119-1128.
67. 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;100:3710-3718.
68. 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;23:186-194.
69. Rowley JD. The critical role of chromosome translocations in human leukemias. *Annu Rev Genet*. 1998;32:495-519.
70. Joh T, Yamamoto K, Kagami Y, et al. Chimeric MLL products with a Ras binding cytoplasmic protein AF6 involved in t(6;11) (q27;q23) leukemia localize in the nucleus. *Oncogene*. 1997;15:1681-1687.
71. Schichman SA, Canaani E, Croce CM. Self-fusion of the ALL1 gene. A new genetic mechanism for acute leukemia. *JAMA*. 1995; 273:571-576.
72. Lavau C, Szilvassy SJ, Slany R, Cleary ML. Immortalization and leukemic transformation of a myelomonocytic precursor by retrovirally transduced HRX-ENL. *EMBO J*. 1997;16:4226-4237.
73. DiMartino JF, Miller T, Ayton PM, et al. A carboxy-terminal domain of ELL is required and sufficient for immortalization of myeloid progenitors by MLL-ELL. *Blood*. 2000;96:3887-3893.
74. Lavau C, Luo RT, Du C, Thirman MJ. Retrovirus-mediated gene transfer of MLL-ELL transforms primary myeloid progenitors and causes acute myeloid leukemias in mice. *Proc Natl Acad Sci U S A*. 2000;97:10984-10989.
75. DiMartino JF, Ayton PM, Chen EH, Naftzger CC, Young BD, Cleary ML. The AF10 leucine zipper is required for leukemic transformation of myeloid progenitors by MLL-AF10. *Blood*. 2002;99:3780-3785.
76. So CW, Cleary ML. MLL-AFX requires the transcriptional effector domains of AFX to transform myeloid progenitors and transdominantly interfere with forkhead protein function. *Mol Cell Biol*. 2002;22:6542-6552.
77. So CW, Cleary ML. Common mechanism for oncogenic activation of MLL by forkhead family proteins. *Blood*. 2003;101:633-639.
78. Lavau C, Du C, Thirman M, Zeleznik-Le N. Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia. *EMBO J*. 2000;19: 4655-4664.
79. Slany RK, Lavau C, Cleary ML. The oncogenic capacity of HRX-ENL requires the transcriptional transactivation activity of ENL and the DNA binding motifs of HRX. *Mol Cell Biol*. 1998;18: 122-129.
80. Ayton PM, Cleary ML. Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. *Oncogene*. 2001;20:5695-5707.
81. Luo RT, Lavau C, Du C, et al. The elongation domain of ELL is dispensable but its ELL-associated factor 1 interaction domain is essential for MLL-ELL-induced leukemogenesis. *Mol Cell Biol*. 2001;21:5678-5687.
82. Zeisig BB, Schreiner S, Garcia-Cuellar MP, Slany RK. Transcriptional activation is a key function encoded by MLL fusion partners. *Leukemia*. 2003;17:359-365.
83. Dobson CL, Warren AJ, Pannell R, Forster A, Rabbitts TH. Tumorigenesis in mice with a fusion of the leukaemia oncogene Mll and the bacterial lacZ gene. *EMBO J*. 2000;19:843-851.
84. Jacobson RH, Zhang XJ, DuBose RF, Matthews BW. Three-dimensional structure of beta-galactosidase from E. coli. *Nature*. 1994; 369:761-766.
85. Eguchi M, Eguchi-Ishimae M, Seto M, et al. GPHN, a novel partner gene fused to MLL in a leukemia with t(11;14)(q23;q24). *Genes Chromosomes Cancer*. 2001;32:212-221.
86. Kuwada N, Kimura F, Matsumura T, et al. t(11;14)(q23;q24) generates an MLL-human gephyrin fusion gene along with a de facto truncated MLL in acute monoblastic leukemia. *Cancer Res*. 2001;61:2665-2669.
87. 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.
88. Greaves MF. Infant leukaemia biology, aetiology and treatment. *Leukemia*. 1996;10:372-377.
89. Rowley JD, Olney HJ. International workshop on the relationship of prior therapy to balanced chromosome aberrations in therapy-related myelodysplastic syndromes and acute leukemia: overview report. *Genes Chromosomes Cancer*. 2002;33:331-345.
90. Megonigal MD, Cheung NK, Rappaport EF, et al. Detection of leukemia-associated MLL-GAS7 translocation early during chemotherapy with DNA topoisomerase II inhibitors. *Proc Natl Acad Sci U S A*. 2000;97:2814-2819.
91. Johansson B, Moorman AV, Haas OA, et al. Hematologic malignancies with t(4;11)(q21;q23)—a cytogenetic, morphologic, immunophenotypic and clinical study of 183 cases. European 11q23 Workshop participants. *Leukemia*. 1998;12:779-787.
92. Lanza C, Gaidano G, Cimino G, et al. Distribution of TP53 mutations among acute leukemias with MLL rearrangements. *Genes Chromosomes Cancer*. 1996;15:48-53.
93. Mahgoub N, Parker RI, Hosler MR, et al. RAS mutations in pediatric leukemias with MLL gene rearrangements. *Genes Chromosomes Cancer*. 1998;21:270-275.
94. Dobson CL, Warren AJ, Pannell R, et al. The mll-AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. *EMBO J*. 1999;18:3564-3574.
95. Schulte CE, Lindern Mv, Steinlein P, Beug H, Wiedemann LM. MLL-ENL cooperates with SCF to transform primary avian multipotent cells. *EMBO J*. 2002;21:4297-4306.
96. Forster A, Pannell R, Drynan LF, et al. Engineering de novo reciprocal chromosomal translocations associated with Mll to replicate primary events of human cancer. *Cancer Cell*. 2003;3:449-458.
97. Ernst P, Wang J, Korsmeyer SJ. The role of MLL in hematopoiesis and leukemia. *Curr Opin Hematol*. 2002;9:282-287.
98. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet*. 2002;30:41-47.
99. Kemp CJ, Wheldon T, Balmain A. p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. *Nat Genet*. 1994;8: 66-69.
100. Griffiths SD, Clarke AR, Healy LE, et al. Absence of p53 permits propagation of mutant cells following genotoxic damage. *Oncogene*. 1997;14:523-531.
101. Adler HT, Chinery R, Wu DY, et al. Leukemic HRX fusion proteins inhibit GADD34-induced apoptosis and associate with the GADD34 and hSNF5/INI1 proteins. *Mol Cell Biol*. 1999;19: 7050-7060.
102. Kersey JH. Fifty years of studies of the biology and therapy of childhood leukemia. *Blood*. 1998;92:1838.
103. Kawasaki H, Isoyama K, Eguchi M, et al. Superior outcome of infant acute myeloid leukemia with intensive chemotherapy: results of the Japan Infant Leukemia Study Group. *Blood*. 2001;98: 3589-3594.

104. Isoyama K, Eguchi M, Hibi S, et al. Risk-directed treatment of infant acute lymphoblastic leukaemia based on early assessment of MLL gene status: results of the Japan Infant Leukaemia Study (MLL96). *Br J Haematol*. 2002;118:999-1010.
105. Ishii E, Kawasaki H, Isoyama K, Eguchi-Ishimae M, Eguchi M. Recent advances in the treatment of infant acute myeloid leukemia. *Leuk Lymphoma*. 2003;44:741-748.
106. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell*. 2002;1:133-143.
107. Ferrando AA, Armstrong SA, Neuberg DS, et al. Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. *Blood*. 2003;102: 262-268.
108. Rozovskaia T, Ravid-Amir O, Tillib S, et al. Expression profiles of acute lymphoblastic and myeloblastic leukemias with ALL-1 rearrangements. *Proc Natl Acad Sci U S A*. 2003;100:7853-7858.
109. Tsutsumi S, Taketani T, Nishimura K, et al. Two distinct gene expression signatures in pediatric acute lymphoblastic leukemia with MLL rearrangements. *Cancer Res*. 2003;63:4882-4887.
110. Yagi T, Morimoto A, Eguchi M, et al. Identification of a gene expression signature associated with pediatric AML prognosis. *Blood*. 2003;102:1849-1856.
111. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature*. 1998;391:811-814.
112. Fenaux P, Chomienne C, Degos L. Treatment of acute promyelocytic leukaemia. *Best Pract Res Clin Haematol*. 2001;14:153-174.
113. Amin HM, Saeed S, Alkan S. Histone deacetylase inhibitors induce caspase-dependent apoptosis and downregulation of daxx in acute promyelocytic leukaemia with t(15;17). *Br J Haematol*. 2001;115: 287-297.
114. Wang J, Saunthararajah Y, Redner RL, Liu JM. Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells. *Cancer Res*. 1999;59: 2766-2769.
115. Ferrara FF, Fazi F, Bianchini A, et al. Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia. *Cancer Res*. 2001;61:2-7.
116. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344:1031-1037.
117. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med*. 2001;344: 1038-1042.
118. Shannon KM. Resistance in the land of molecular cancer therapeutics. *Cancer Cell*. 2002;2:99-102.
119. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell*. 2003;3:173-183.
120. Taketani T, Taki T, Sugita K, et al. FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant acute lymphoblastic leukemia (ALL) with MLL rearrangement and pediatric ALL with hyperdiploidy. *Blood*. 2003:[Epub ahead of print]. In press.
121. Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *J Pathol*. 2002;196:1-7.
122. Nephew KP, Huang TH. Epigenetic gene silencing in cancer initiation and progression. *Cancer Lett*. 2003;190:125-133.
123. Christman JK. 5-Azacytidine and 5-aza-2′-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene*. 2002;21:5483-5495.
124. List AF. New approaches to the treatment of myelodysplasia. *Oncologist*. 2002;7(suppl 1):39-49.
125. Niitsu N, Hayashi Y, Sugita K, Honma Y. Sensitization by 5-aza-2′-deoxycytidine of leukaemia cells with MLL abnormalities to induction of differentiation by all-trans retinoic acid and 1alpha,25-dihydroxyvitamin D3. *Br J Haematol*. 2001;112:315-326.