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REVIEW

The molecular mechanics of mixed lineage leukemia

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Mixed lineage leukemia caused by MLL fusion proteins is still a mostly incurable disease. Research on novel treatment strategies has gained momentum in the last years with the elucidation of the molecular mechanisms underlying the transforming potential of these powerful oncoproteins. This review summarizes the recent developments in this area including new attempts to treat MLL in a rational way by exploiting the biochemical vulnerabilities of the leukemogenic process.

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THE CLINICAL ASPECT OF MLL

Before the next-generation sequencing revolution, it was a daunting task to accurately pinpoint the genetic changes responsible for a disease like cancer. Therefore, it came as a breakthrough when it was realized that certain recurrent and cytogenetically observable genome alterations, like chromosomal translocations, were firmly associated with a specific subtype of neoplasia.[1,2] As correctly surmised, the visible chromosomal abnormalities served as a molecular beacon that enabled the localization, isolation and characterization of the genes causally involved in the transformation process. This was particularly true for leukemia where, in contrast to the often highly aberrant genomes of solid tumors, single chromosomal aberrations often stood out as sole genomic change. It was realized already in the early 1980s that a very aggressive subtype of infant leukemia presenting within the first year of life was characterized by translocations affecting a hotspot of genomic instability at '11q23'.[3,4] Microscopically, this aberration had been found in both acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML) cells. Closer inspection revealed that the transformed cells frequently carried surface markers of both major blood lineages and therefore this disease was called mixed lineage leukemia.[5–7] Today we know that up to 80% of infant ALL and about 35–50% of pediatric AML harbor 11q23 abnormalities.[8–12] A second peak of incidence for this chromosomal lesion has been recorded in patients who suffer from therapy-induced AML after treatment of an unrelated malignancy with certain genotoxic agents.[13,14] Particularly, the application of topoisomerase inhibitors like epipodophyllotoxins (etoposide) carries a high risk of secondary malignancy. Finally, 11q23 translocations do also occur in sporadic cases of adult ALL and AML adding up to a considerable 5% of adult ALL and 5–10% of AML with 11q23 involvement.

The common clinical denominator of all these cases is their dismal prognosis. Treatment of infant MLL depends on its initial classification as ALL or AML. Induction for 'ALL-type' disease is usually performed with prednisone followed either by a 'lymphoid' consolidation therapy (cyclophosphamide, cytarabine, 6-mercaptopurine) or, for some protocols adjusted to the more aggressive nature of MLL, by a more 'myeloid'-based therapy with cytarabine, daunorubicin/mitoxantrone and etoposide. The benefit of allogeneic hematopoietic stem cell transplants is still unclear for infant ALL. In contrast, infant AML with MLL involvement is treated similar to other pediatric AML with an intensive chemotherapy followed by hematopoietic stem cell transplant if donor marrow is available and if the health status of the recipient allows this procedure.[15] In adults, the mainstay of AML therapy is a cytarabine/anthracycline-based regimen followed by allo-hematopoietic stem cell transplant if circumstances permit.[16] The trend for high-risk ALL in older patients is to follow up induction therapy (usually including vincristine, prednisone, cyclophosphamide, doxorubicin and L-asparaginase) with an intensified regimen often containing cytarabine and methotrexate and subsequently with a maintenance therapy of methotrexate and 6-mercaptopurine. However, despite best supportive care, the 5-year survival rates after high-dose chemotherapy and bone marrow transplants for infants and adults with 11q23 abnormalities are still well below 50% with the possible exception of patients carrying t(11;9) translocations in AML.[17–19] Because of these extraordinary properties, 11q23 aberrations have been listed as a separate entity in the WHO (World Health Organization) classification of leukemia.[20] Underscoring the remarkable transforming power of 11q23 translocations, a recent sequencing study[21] uncovered that infant MLL is the only neoplasm besides low-grade glioblastomas that is almost devoid of secondary mutations besides the defining chromosomal aberration as founder event. On average, infant leukemia with MLL rearrangement contains 1.3 additional non-silent mutations (mostly in PI3K-RAS signaling pathways), yet in some instances, 11q23 aberrations are the only genetic lesion detectable.

MOLECULAR ANATOMY OF 11Q23 TRANSLOCATIONS: THE ADVENT OF EPIGENETICS IN ONCOGENESIS

MLL is a multi-functional chromatin modulator

Several groups contended to clarify the events underlying the 11q23 anomalies and to identify the genes involved. Four independent laboratories succeeded to clone and sequence the respective breakpoint genes.[22–25] On chromosome 11, they localized a gene coding for a very large protein of 3972 amino acids (432 kDa calculated MW) that showed clear homology to Drosophila *trithorax (trx)*. Trx is an embryonic regulator that is important for maintaining gene expression during development.

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It opposes the repressive activity of the so-called *polycomb* group proteins with the clustered *Hox*-homeobox genes as major physiological targets.[26] Because of this homology and the involvement in leukemogenesis, the human gene was initially called *MLL*, *ALL*-1 or *HRX* with *MLL* as the 'common name' prevailing until today. A knockout study in mice showed that the structural homology of *MLL* and *TRX* also extended to function as *Mll*$^{-/-}$ animals died *in utero* with homeotic malformations, aberrant *Hox* expression and hematopoietic problems.[27,28] A variety of functional domains have since been described in MLL and their function will be shortly summarized in their order of appearance (Figure 1a).

MLL-N: DNA-binding meets regulation

Under physiological conditions, full-length MLL is cleaved post-transcriptionally by an enzyme called Taspase1 into a larger MLL-N (also called N320, calculated MW 297kDa) and a MLL-C (C180, calculated MW 135 kDa) fragment (Figure 1a).[29–31] Both are kept together by intramolecular interactions mediated by two interaction motifs located in the N-terminal (FYRN) and C-terminal (FYRC) cleavage products, respectively.[30] The resulting dimer is incorporated into a larger protein assembly that associates with a variety of cofactors (WDR5, RBBP5, ASH2L, hDPY30) that are also present in other H3K4 methyltransferase complexes. As this topic has been summarized recently, readers interested in more detail are kindly referred to the respective review.[32] The physical separation into MLL-N and MLL-C subunits is also reflected by separable functions of the two cleavage products. MLL-N is involved in chromatin targeting and regulation of MLL activity, whereas MLL-C mediates transactivation. The first characterized domain at the very N-terminus of MLL-N is the LEDGF-menin interaction region (LMI). This moiety binds to menin (multiple endocrine neoplasia I) a protein that is lost in a familiar cancer syndrome.[33–36] The combined menin/MLL interface then allows interaction with LEDGF or lens epithelium-derived growth factor (encoded by the *PSIP1* gene, also known as p75).[37] LEDGF contains a PWWP domain that recognizes H3K36 methylation, a hallmark of actively transcribed chromatin. LEDGF seems to be necessary for recruiting MLL to active chromatin and, in an interesting side aspect, LEDGF is also used by HIV-integrase to enable insertion of the viral genome into the host chromosomes.[38] The LMI is followed by three AT-hooks that constitute an unusual DNA-binding domain with an affinity for 'distorted' DNA displaying a widened minor groove.[39] The AT-hooks of MLL have an additional function as they mediate interaction either with itself or with a domain further downstream[40] that is characterized by the occurrence of a duplicated Cysteine-n-n-Cysteine zinc-finger (CxxC). Similar structures have been found in proteins like DNA methyltransferase 1 that recognize CpG dinucleotides. In analogy, also the Cysteine-n-n-Cysteine zinc-finger motif of MLL binds CpG sequences with the additional capability to discriminate for unmethylated cytosine.[41] Together with a basic stretch of amino acids, this region also recruits PAF1, a member of the 'polymerase associated complex' (PAFc), which catalyzes histone H2B ubiquitination during transcription.[42,43] The LMI and the Cysteine-n-n-Cysteine zinc-finger domain are included in every known MLL fusion protein, and in structure–function experiments, they have been identified as the sole determinants that are necessary[44] and sufficient[45] to create a transforming protein if joined to a MLL fusion partner. Consequently, MLL fusion proteins never include any sequences downstream of the common and narrow 11q23 breakpoint region (Figure 1a, lower panel).

Thus, the central four PHD fingers and an intervening bromodomain of wt-MLL are invariably excluded from MLL fusions. Bromodomains read acetylated chromatin and PHD fingers recognize methylated proteins with PHD finger number 3 of MLL specifically interacting with methylated H3K4.[46–48] In this way, this region can establish a positive feedback loop as it will enable MLL to bind to chromatin that has been acetylated/methylated by itself (see below). Simultaneously, the PHD fingers are targets of ubiquitination and conformational regulation by interaction with the proline-isomerase CYP33.[49,50] This has been suggested as a regulatory mechanism that allows to turn off MLL activity. Binding of CYP33 to the third PHD finger in an artificial MLL-ENL fusion protein increased association with the histone

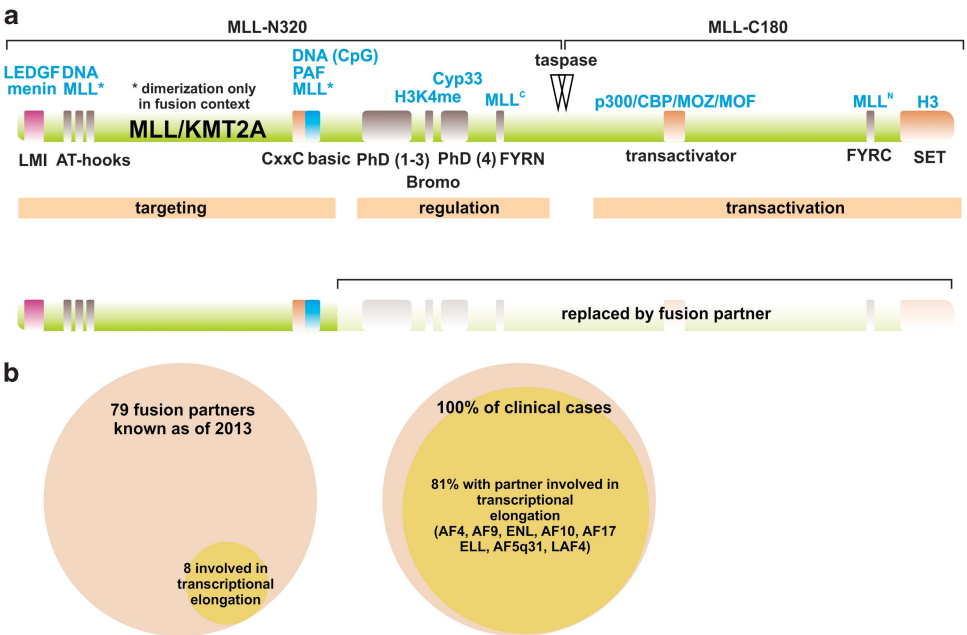

Figure 1. Wild-type MLL and the diversity of MLL fusion partners. (**a**) Schematic structure of wild-type MLL. Functional domains are labeled in black. Known interactions of these domains are annotated in blue. LMI, LEDGF, menin interaction domain; PHD, plant homeodomain; bromo, bromodomain; FYRN/FYRC, N-terminal and C-terminal interaction domains. For more details, see text. (**b**) Biochemical and clinical distribution of MLL fusion partners.

deacetylase HDAC1 leading to repression of *HOX* gene expression.[47] Thus, inclusion of this region into MLL fusion proteins incapacitates their transforming function[47,48] supposing that also the corresponding region in MLL serves as a 'regulatory module'.

MLL-C: a histone modification element

MLL-C contains two regions with chromatin modification capability. The first is a transactivator region that interacts either directly or genetically with the histone acetyltransferases p300/CBP, MOZ and MOF[51–53] transferring acetyl groups to H3K27, H3K9 and H4K16, respectively. The second histone modification activity is characterized by a C-terminal SET domain that works as histone methyltransferase.[54] SET is an acronym for Suppressor of variegation, Enhancer of zeste and Trithorax, the first proteins described with this functional unit. In general, SET domains catalyze the transfer of a methyl group from S-adenosylmethionine to a protein substrate. In the case of MLL, the acceptor is lysine 4 on histone H3, which can be monomethylated, dimethylated or trimethylated ($H3K4me_{1,2,3}$). H3K4me is a hallmark of actively transcribed chromatin with monomethylation marking enhancers while dimethylation and trimethylation are found around the transcription start sites of active genes.[55] The methyl-mark itself is recognized by 'reader' proteins that frequently recognize the methylated residue with the help of a PHD (plant homeodomain) domain. Although exact details remain to be worked out, one way how H3K4 methylation stimulates transcription is by attracting nucleosome remodeling complexes like NURF that can create nucleosome-free regions to facilitate transcriptional initiation.[56,57]

In an interesting new development, it has been demonstrated that actually H4K16 acetylation is the more important activity of MLL responsible for the loss-of-function phenotypes in *Mll* knockout animals.[58] After specific deletion of the *Mll* SET domain, H3K4me levels as well as expression of several genes critical for blood cell development remained unaffected, whereas a total knockout of *Mll* led to a rapid loss of transcription of the respective loci affecting primarily hematopoiesis. This corroborates previous reports demonstrating that a localized knockout specifically removing the SET domain of *Mll* produced viable offspring that survived into adulthood.[59] In line with these results, a simple pharmacologic inhibition of the MOF-counteracting histone deacetylase SIRT1 restored acetylation and *Mll* target gene expression in complete *Mll-/-* knockout cells.[58] Acetylated H4K16 provides a binding platform for BET-bromodomain proteins (bromodomain and extra terminal domain) like BRD4. BRD4 is a multifunctional activator that recruits and activates P-TEFb,[60] a kinase that is required for efficient transcriptional elongation. P-TEFb also has a major role in transformation by MLL fusions (see below) suggesting that wt-MLL and fusion derivatives use a similar mechanism to stimulate target transcription. In contrast, a loss of H3K4 methylation can be likely complemented by other H3K4 methyltransferases. In total, six enzymes with this catalytic activity have been identified in mammalian cells (MLL1-4, SET1A, SET1B) and the respective genes have been renamed *KMTxx* (from lysine = K methyltransferase) as KMT2A (MLL1), 2B (MLL4), 2C (MLL3), 2D (MLL2), 2 F (SET1A) and 2G (SET1B). It is not yet entirely clear how the task of global H3K4 methylation is distributed among the respective KMT proteins, but they seem to be generally highly exchangeable. For example, in ESC cells, *Mll* is localized virtually at every active promoter but only a few genes lose H3K4 methylation and expression upon *Mll* knockout, possibly because other KMT enzymes substitute.[61]

MLL FUSION PROTEINS: PERCEIVING ORDER IN CHAOS

The predominant MLL fusion partners function in transcriptional elongation

As anticipated by cytogenetic findings, the initial identification of MLL fusion partners yielded a very confusing picture. The 11q23 locus is translocated to partner loci distributed across the whole genome. Although a few translocations like t(4;11), t(11;19), t(9;11) and t(10;11) prevail, the respective partner genes (*AF4, ENL, AF9, AF10*) code for proteins with little resemblance except an occasional homology (for example, between *AF9* and *ENL*).[62] (The nomenclature of genes fused to MLL is still unsatisfactory as only some of the original genes have been included in a systematic scheme. For example, ENL was officially renamed MLLT1 (MLL translocated to number 1), whereas the translocation partner AF4 now is called AFF1 (AF4/FMR family member 1). For this reason, this review will use the older and better known denominations throughout.) Despite a lot of effort invested in cloning new MLL fusions, originally no unifying feature could be discovered. This changed with a series of reports where we and others purified protein complexes associated with MLL partners.[63–67] These studies revealed that 8 out of 79 known MLL partner proteins were involved in the process of transcriptional elongation (Figure 1b), a notion that had been first brought up for ELL, the only fusion partner for which a biological function could be assigned before.[68] Together these eight fusion partners cover more than 80% of all clinical cases of leukemia with MLL involvement. Thus, mixed lineage leukemia, in its majority, is caused by a loss of proper transcriptional regulation. The adjustment of transcriptional elongation rather than the classical control of initiation is the preferred mode of regulation for many genes that need to respond quickly like those involved in differentiation, proliferation and immediate early control. This step contributes to overall regulation to a varying degree also for other genes,[69] because RNA Polymerase II (RNA PolII) frequently stops after initiation and remains paused or 'stalled' until proper signals for further transcription are given. Elongation rates can be adjusted by the activity of various 'impeding' factors like NELF (negative elongation factor) and DSIF (DRB sensitivity inducing

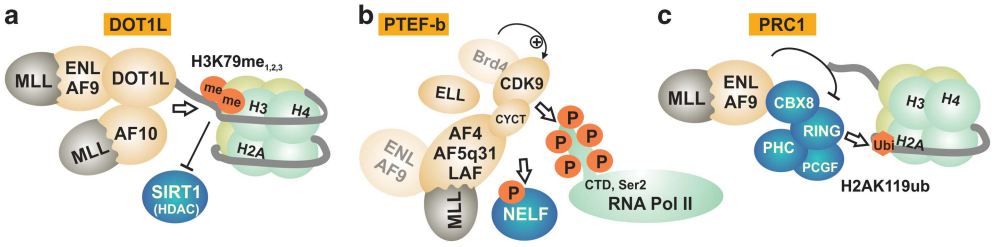

Figure 2. Schematic representation of the biochemical functions associated with common MLL fusion partners. (**a**) The DOT1L complex induces H3K79 methylation. (**b**) Positive transcription elongation factor b (P-TEFb) contains CDK9 that phosphorylates RNA PolII and various other proteins to promote transcriptional elongation. (**c**) Polycomb repressive complex 1 (PRC1) is inhibited by a direct interaction of ENL/AF9 with CBX8.

factor) and by adjusting the 'speed' of RNA PolII itself. In addition, the modification state of chromatin has an influence on transcription rates. Thus, it has been shown that the activity of the polycomb repressors can 'compact' chromatin to impede elongation.[70] MLL fusion partners are members of protein complexes that affect these set-points of elongation control with far reaching consequences. This was demonstrated first for ENL where affinity purification revealed that this protein (as well as its homolog AF9) is a member of a multi-subunit complex initially labeled EAP[65] (elongation-assisting proteins or ENL-associated proteins). Later it was shown that EAP actually is composed of separate subcomplexes (Figure 2) all of which impact on a different feature of transcriptional elongation[63,64,67] as detailed below.

DotCom: paving the road to transcription

One of the key activities within EAP is DOT1L (disruptor of telomeric silencing 1 like) representing the core component of the DOT1L sub-complex (DotCom) (Figure 2a). Initially discovered in yeast where it curbs the spread of repressive chromatin emanating from the telomere regions, DOT1L was later shown to be a histone methyltransferase.[71] It is the only known enzyme that either monomethylates, dimethylates or trimethylates lysine 79 in histone H3. It is also unique because it does not possess a SET domain, a fact that makes it vulnerable to specific pharmacological inhibition (see below). H3K79 methylation is associated with actively transcribed regions, and recently, it was shown that knocking-down the histone deacetylase SIRT1 can compensate for a loss of DOT1L activity by increasing histone acetylation.[72] This likely allows more BRD4/P-TEFb to bind as an alternative method to promote elongation (see next paragraph). Next to ENL and AF9 that bind DOT1L with a C-terminal region[66,73,74] also AF10 and AF17 interact directly with this enzyme[75] thereby stimulating DOT1L catalytic activity and thus allowing the accumulation of higher methylated states of H3K79 (di/trimethylation).[76] Interestingly, other studies have shown that ubiquitination of H2B, a modification catalyzed by the RNF20 subunit of the PAF complex, also facilitates DOT1L-mediated H3K79 methylation.[77] Thus, the transcriptional impact of the fusion partner is reinforced by PAF recruitment via the MLL portion.[77] As there is no known H3K79 demethylase, cells must remove this chromatin mark by dilution during cell division. H3K79me may therefore serve as a marker for past transcriptional states constituting the equivalent of a 'transcriptional memory'.

Positive transcription elongation factor b (P-TEFb) and the super elongation complex (SEC)

The second protein complex involving multiple MLL partner proteins is the super elongation complex or SEC[78] (Figure 2b). At the core of SEC, is positive transcription elongation factor b (P-TEFb), a dimer of cyclin-dependent kinase 9 and either a cyclin T (T1 or T2) or a cyclin K. Together, the active dimer recognizes multiple targets. Best known is its role in phosphorylating the C-terminal repeat domain of RNA PolII (CTD) that consists of a heptapeptide that is tandemly repeated 54 times for mammalian RNAPolII. The CTD contains several serines that can be phosphorylated, and depending on the location within the repeat, this creates a 'code' that determines the activity of RNAPolII in part by serving as docking site for accessory proteins. In this way, initiation, elongation, co-transcriptional RNA processing and termination can be coordinately regulated. P-TEFb specifically recognizes serine-2 in the heptarepeat, which needs to be modified for efficient elongation. In addition, P-TEFb inactivates impeding factors like NELF and DSIF. P-TEFb binds directly to AF4 and AF4-family members (AF5q31, LAF) through the cyclin moiety.[66] As ENL and AF9 interact with AF4 family proteins, P-TEFb can be indirectly recruited also by MLL-ENL/AF9. ELL has been identified as another component of SEC.[68] A further essential subunit of SEC is BRD4, a member of the BET (bromodomain and extra terminal domain) family that includes BRD2, BRD3 and BRDT. These proteins contain two bromodomains that recognize acetylated chromatin. BRD4 binds to CDK9, recruits it to chromatin and stimulates P-TEFb activity.[60] Thus, BRD4 couples elongation to acetyl-marks on chromatin in another intricate analogy to the transactivation mechanism of wild-type MLL.

Polycomb repressive complex 1 and MLL fusions

A further interaction of MLL fusion partners with transcriptionally active proteins has been uncovered for ENL and AF9 that bind to chromobox protein 8 (CBX8), a member of polycomb repressive complex 1[40,79] (PRC1) (Figure 2c). PRC1 is a repressor that impedes transcription by compacting chromatin and by imposing ubiquitination on histone H2A catalyzed by the RING1B subunit of the complex.[80] In fly, polycomb opposes trithorax activity and a perturbation of this balance manifests itself first as homeotic alteration caused by aberrant *Hox* expression. A similar situation exists in leukemia where interaction of the MLL-fused ENL moiety with CBX8 neutralizes PRC1-repressive activity, thus allowing an uncurbed expression of polycomb targets, foremost the clustered *HOX* homeobox genes.[40] Currently it is unknown how ENL inactivates PRC1, but as ENL generally acts as a scaffold bringing various proteins together, this activity may be mediated by some other ENL interaction partner. For the ENL homolog AF9, it has been shown that it tethers CBX8 to the histone acetyl-transferase TIP60, thus turning an inhibitor into an activator of transcription.[81]

The impact of MLL fusion proteins on gene expression patterns

One of the unsolved questions is whether MLL fusion proteins use all three of these mechanisms (induction of H3K79 methylation by DOT1L, stimulation of elongation through P-TEFb, suppression of polycomb function) simultaneously to activate their target genes. Biochemical assays have shown that most of the underlying protein–protein interactions do not occur at the same time. For example, ENL and its homolog AF9 can interact either with DOT1L, AF4 or CBX8, but all of these interactions are mutually exclusive.[40] This has been traced to a disordered interaction domain in ENL/AF9 that adopts a structured conformation dependent on the specific binding partner, thus excluding other interactions.[82] A potential solution for recruiting different effector molecules to the same molecular locus would be via dimerization or multimerization of individual MLL fusion molecules. Indeed, MLL fusions form large nuclear speckles that are visible by fluorescence microscopy and that must consist of more than a single molecule.[83] Consequently, MLL fusions contain two dimerization domains in the MLL portion (coinciding with the AT-hook and Cysteine-n-n-Cysteine zinc-finger motifs) that allow dimerization and likely also the formation of multimers.[40] Alternatively, various MLL fusion complexes of different composition may exist that are individually recruited to target genes depending on the respective necessities for transcriptional stimulation.

HOX genes as predominant targets of MLL fusion proteins

Regardless of the exact molecular mechanism of transcriptional activation, it has been confirmed in numerous studies that the clustered *HOX* homeobox genes and the genes for the HOX protein interaction partners MEIS1 and PBX3 are major targets of MLL fusion proteins.[84–86] Interestingly, a recent report quantifying *HOX* expression in normal and malignant cells showed that the presence of MLL fusion proteins does not really lead to the overexpression of *HOX* genes. Rather, they freeze *HOX* expression at a level that is present in normal early hematopoietic stem and precursor cells.[87] *HOX* genes, mainly of the so-called *HOX*-A cluster in combination with *MEIS1* and *PBX3*, are the foremost

determinants of self-renewing potential in the hematopoietic stem and precursor cell population. Their peculiar arrangement in genomic clusters parallels their special mode of regulation that is highly dependent on DOT1L-mediated H3K79 methylation even in wild-type cells and in the absence of MLL fusion proteins.[76] Conspicuously, there are many parallels between the transactivation mechanisms perused by MLL fusions and by wt-MLL suggesting that uncontrolled wt-MLL expression may be oncogenic by itself. Indeed, transcription of *MLL*, *MLL2* (a close homolog of MLL) and *MOZ* (coding for a HAT-enzyme) genes are activated by gain-of-function mutations of p53 in various solid cancers.[88] As a consequence, H3K4 methylation and H4K16 acetylation is increased at several gene loci supporting the transformed phenotype.

It is not clear whether MLL fusion targets besides *HOX, MEIS* and *PBX* genes are absolutely required to achieve leukemic transformation. In contrast to wt-MLL that can be found basically at every active promoter, MLL fusions have a relatively small set of confirmed binding sites with about 130 to approximately 400 loci identified in various ChIP studies.[89–91] A few of these 'non-*HOX*' MLL fusion targets have been tested in knockdown studies, and *CDK6*, *JMJD1C*, *MEF2C*, *EYA1* and the *MECOM* locus (from which *EVI*-1 and *MDS1* transcripts emanate) have been implied in the etiology of MLL fusion-induced leukemia by these studies.[92–98]

TARGETING THE BIOCHEMISTRY BEHIND MLL FUSION-INDUCED LEUKEMIA

Fusion proteins derived from recurrent chromosomal translocations have been the first examples of the potential success of a molecularly targeted therapy. The treatment of BCR-ABL-positive CML with Imatinib and related tyrosine kinase inhibitors as well as the medication of retinoic receptor alpha rearrangements in acute promyelocytic leukemia with all-trans retinoic acid and arsenic are substantial medical breakthroughs. It is therefore not surprising that numerous groups have investigated the possibilities to exploit the specific properties of MLL fusion proteins to treat this particularly aggressive leukemia (Figure 3).

The use of small molecule inhibitors aimed at the activity of DOT1L is most advanced in clinical development.[99] The unique structure of its catalytic domain enables the design of very specific pharmacons that block the methyltransferase activity of DOT1L with ID50s in the low nanomolar range.[100] No H3K79 demethylase exists in cells and tissues yet examined. Therefore, during incubation with DOT1L inhibitors, cell division leads to a slow dilution of this chromatin mark within several days. Despite the presence of H3K79 methylation on virtually all transcribed chromatin, blockage of DOT1L is surprisingly well tolerated in experimental animals with no obvious acute toxicity. This was unexpected because conditional knockout of DOT1L in adult mice is lethal within 8–12 weeks after deletion owing to hematopoietic failure.[101–103] Together, this indicates that DOT1L function, like MLL, does not completely rely on its methyltransferase activity. Nevertheless, the small molecule DOT1L inhibition is sufficient to block the proliferation of MLL fusion-induced leukemia cells of murine and human origin. Beyond that, DOT1L inhibitors seem to be also effective in every leukemia that depends on sustained *HOX* expression for transformation like NUP98 or CALM translocation cases.[76,104] This is mainly based on the exquisite and specific dependence of *HOX* transcription on H3K79 methylation.[76] A loss of DOT1L methyltransferase activity can be complemented by ectopic expression of *HOXA9* and *MEIS1* from non-DOT1L-dependent retroviral promoters. These promising properties have brought small molecule DOT1L inhibitors like EPZ5676 into phase I clinical studies. First data showed promising molecular efficacy in the majority of treated patients (reduction in H3K79 methylation) and some improvements in disease status.[105]

The second most clinically advanced strategy to target MLL fusion proteins involves the inhibition of BRD4 by small molecules (JQ1, i-Bet) that bind competitively to the bromodomain of BRD4. This blocks the binding of BRD4 to acetylated chromatin and affects P-TEFb activity. Originally thought to be specific for MLL fusion leukemia, later it was shown that these molecules have a broader effect on malignant cells.[106–111] Many genes with a role in cell proliferation with *MYC* as prime example seem to be regulated by special enhancers with a characteristic setup that have been controversially termed 'super-enhancers'.[112,113] Besides its function in the SEC complex, BRD4 is involved in the formation and function of these regulatory units.[114] Therefore, BRD4 inhibition affects *MYC* expression as a prerequisite for rapid cell proliferation in many cancer cells. At present, a clinical trial with the BRD4 inhibitor GSK525762 is recruiting for NUT midline carcinoma

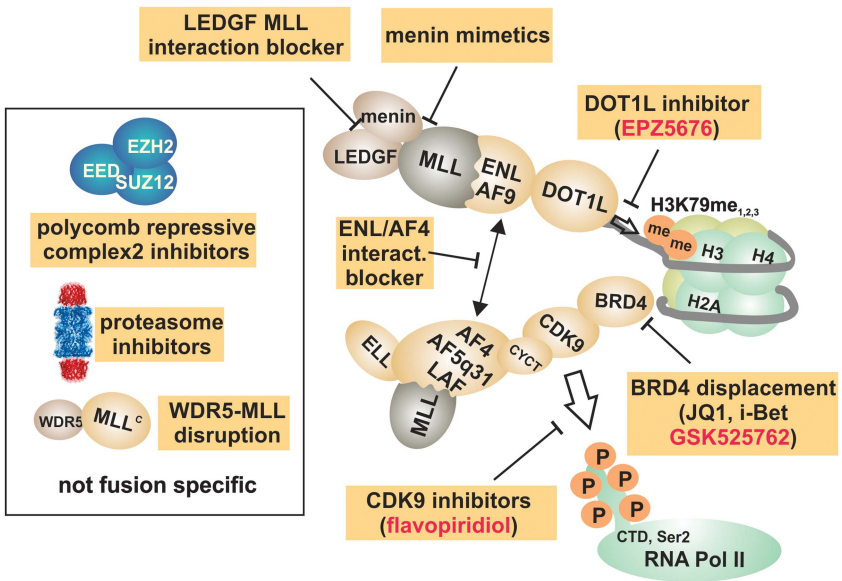

Figure 3. Different approaches to target MLL fusion-induced leukemia. A schematic representation of intervention points that have been used to block MLL fusion-mediated transformation. The boxed strategies target processes not directly related to MLL fusion-associated functions. Compounds already in clinical trials are highlighted in red font.

(clinicaltrials.gov #NCT01587703) where *BRD4* itself is involved in a chromosomal translocation with the *NUT* gene. However, it is to be expected that trials in leukemia patients will start soon.

In a similar direction CDK9, the enzymatically active subunit of P-TEFb, can be targeted by various inhibitors. Flavopiridol (alvocidib) is the most well-known representative in this inhibitor class. Animal experiments did report the efficacy of CDK9 inhibition in experimentally induced MLL.[115] Although flavopiridol is a widely researched substance, no trial has been set up targeting flavopiridol specifically at MLL. Several groups, however, report promising results for this substance if included in general treatment schedules for high risk leukemia including MLL-r cases. A small caveat is that many studies apply flavopiridol at doses where it acts as a general CDK inhibitor forsaking its moderate but existent specificity for CDK9.[116]

A variety of other approaches to disable specific functions of MLL fusion proteins have been explored either *in vitro* or in preclinical studies. Most of these target protein–protein interactions of the higher order MLL fusion complex. Most advanced are efforts to design small molecules that disrupt menin-MLL binding.[117–120] These reverse MLL fusion-mediated transformation *in vitro* and are one of the first examples that protein–protein interactions can be successfully targeted by small molecules. In analogy, it has been shown that expression of small LEDGF peptides that inhibit LEDGF interaction with the composite Menin/MLL-binding surface is antagonistic to MLL fusion-induced transformation.[121,122] Similarly, a small peptide interrupting the AF9/ENL-AF4 interaction showed anti-leukemic activity in MLL-rearranged cells.[123,124]

MLL-recombined leukemia has been also found to be sensitive towards the inhibition of processes that are not directly associated with the biochemistry of the fusion proteins. For example, a block of polycomb repressor complex 2 inhibited MLL fusion leukemia cell growth, likely because of a derepression of aberrantly silenced tumor suppressor genes.[125] In addition, proteasome inhibitors showed some efficacy against mixed lineage leukemia.[126] Reducing the rapid turnover of MLL-AF4 increased the cellular concentration of this oncoprotein beyond a tolerable level and induced apoptosis in ALL with t(4;11) translocations. Finally, an interesting new approach targets wt-MLL because an intact copy of MLL is essential to maintain the transformed state of MLL fusion cells.[127] The interaction of the MLL-C portion with WDR5, the common subunit of all H3K4 methyltransferase complexes, can be abolished by small molecule mimetics, an approach that may also benefit leukemia with C/EBPα mutations.[128,129]

OUTLOOK

On the basis of our increasingly complete knowledge of the MLL fusion-induced transformation mechanism, a variety of new compounds and principles have been developed that have at least the potential to stop MLL fusion-induced leukemogenesis. MLL fusions do use common pathways that are also utilized in normal transcription foreshadowing the potential for serious side effects. However, the surprising example of DOT1L inhibitors demonstrates that these are not predictable by biochemical knowledge alone. The hope now lies in the success of clinical trials testing these compounds in a real life setting. It is to expect that first results will be available soon to bring some light into the future of many patients suffering from MLL fusion induced leukemia.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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