



HHS Public Access

Author manuscript

Leukemia. Author manuscript; available in PMC 2017 August 14.

Published in final edited form as:

Leukemia. 2014 July ; 28(7): 1396–1406. doi:10.1038/leu.2014.94.

Chromatin modifiers and the promise of epigenetic therapy in acute leukemia

Sarah M. Greenblatt, Ph.D.¹ and Stephen D. Nimer, M.D.¹

¹University of Miami, Sylvester Comprehensive Cancer Center, Miami, FL 33136, USA

Abstract

Hematopoiesis is a tightly regulated process involving the control of gene expression that directs the transition from hematopoietic stem and progenitor cells to terminally differentiated blood cells. In leukemia, the processes directing self-renewal, differentiation, and progenitor cell expansion are disrupted, leading to the accumulation of immature, non-functioning malignant cells. Insights into these processes have come in stages, based upon technological advances in genetic analyses, bioinformatics, and biological sciences. The first cytogenetic studies of leukemic cells identified chromosomal translocations that generate oncogenic fusion proteins, and most commonly affect regulators of transcription. This was followed by the discovery of recurrent somatic mutations in genes encoding regulators of the signal transduction pathways that control cell proliferation and survival. Recently, studies of global changes in methylation and gene expression have led to the understanding that the output of transcriptional regulators and the proliferative signaling pathways, are ultimately influenced by chromatin structure. Candidate gene, whole genome, and whole exome sequencing studies have identified recurrent somatic mutations in genes encoding epigenetic modifiers in both acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). In contrast to the two hit model of leukemogenesis, emerging evidence suggests that these epigenetic modifiers represent a class of mutations that are critical to the development of leukemia and affect the regulation of various other oncogenic pathways. In this review, we discuss the range of recurrent, somatic mutations in epigenetic modifiers found in leukemia and how these modifiers relate to the classical leukemogenic pathways that lead to impaired cell differentiation and aberrant self-renewal and proliferation.

Keywords

chromatin; histone modification; acute leukemia

Introduction

Leukemia research has largely focused on regulators of signaling and cellular differentiation for the past 15 years. Although the classical model of leukemogenesis has suggested that a

Corresponding Author: Stephen D. Nimer, M.D., Sylvester Comprehensive Cancer Center, Clinical Research Building, 6th Floor, Suite 660, 1120 NW 14th Street, (C241), Miami, FL 33136-1000, SNimer@med.miami.edu, Office: (305) 243-1775, Fax: (305) 243-4435.

Conflict of interest statement: The authors have no conflicts of interest to disclose.

mutation in a gene encoding a regulator of signaling/proliferation is a requirement for transformation, recent characterization of de novo AML has suggested that over 40% of patients do not have an identifiable mutation in a signaling gene.¹ Similarly, mutations in myeloid transcription factors occur in around 20% of AML patients and gene fusion events occur in less than half of AML patients. Taken together these findings fail to completely explain the impaired differentiation that is a defining characteristic of leukemia. Similarly, genetic characterization of pediatric T-cell acute lymphoblastic leukemia (T-ALL) has indicated that somatic mutations in genes involved in development or signaling are not found in 20% of early T-cell precursor (ETP)-ALL and 69% of non-ETP-ALL.² Mutations in epigenetic modifiers are emerging as a large class of mutations that is critical in the development of both AML and subtypes of ALL. In contrast to the previous view that this class of mutations are rare, analysis of 200 cases of de novo AML by a combination of whole exome and whole genome sequencing showed that over 70% of patients had at least one non-synonymous mutation in a DNA methylation related gene, or another epigenetic modifier.¹ Adding to this importance, many mutations classically defined for their role in proliferation and differentiation are now understood to have important roles in regulating chromatin structure.

Somatic mutations and alterations in chromatin modifying enzymes

Chromatin modifiers are enzymes that catalyze the chemical conversion of cytosine residues in DNA, or lysine, arginine, tyrosine, and serine residues in histone proteins. The importance of epigenetic modifiers in leukemia was first suggested by the identification of recurrent translocations in histone acetyltransferase and methyltransferase genes (e.g. *CBP*, *P300*, *NSD1*, *MLL* and *MOZ*). In recent years, somatic mutations have also been identified in genes that encode the proteins controlling DNA cytosine modifications (e.g. *DNMT3A* and *TET2*). Figure 1 depicts the epigenetic regulation of methylation and acetylation, and their potential targeting in leukemia by “epigenetic modifying therapy”.

Cytosine Modifications

DNA methylation plays an important role in myeloid and lymphoid commitment, as well as HSC self-renewal.^{3–5} Methylation profiling of mouse multipotent progenitor cells has indicated that the promoters of several transcription factors become methylated during the cellular differentiation towards common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs).⁶ Furthermore, methylation profiling has been used to classify subtypes of leukemia, with prognostic significance.^{7–9} Genome wide cytosine methylation profiling, combined with copy number and gene expression analysis in childhood ALL, has suggested that there is an aberrant epigenetic signature that is common to all cases, regardless of disease subtype. This suggests that a common set of epigenetically deregulated genes may be required for the initiation or maintenance of hematopoietic transformation. However, DNA methylation patterns clearly associate with specific chromosomal rearrangements. Indeed, oncogenic translocations involving transcription factors such as *ETV6-RUNX1* have prognostic value (favorable in this case) and are associated with specific alterations in methylation.¹⁰ Overexpression of *EVII* has been associated with an aberrant hypermethylation signature and poor prognosis in AML.¹¹ Finally, DNA

methylation profiling in MDS/AML suggests that aberrant methylation may be the primary mechanism of tumor suppressor gene silencing and clonal evolution to acute leukemia.¹²

DNMT3a is an enzyme required for de novo methylation and a frequent target of somatic mutations, occurring in over 30% of cytogenetically normal AML (CN-AML) patients and 16% of T-ALL.¹³⁻¹⁷ Approximately 60% of the mutations in *DNMT3A* result in the heterozygous substitution of arginine 882 in the catalytic domain of the enzyme, leading to decreased methyltransferase activity in vitro.¹⁸ Interestingly, the wildtype *DNMT3A* allele is still expressed and recent data suggest that the DNMT3A mutant proteins exert a dominant negative effect through interactions with wildtype DNMT3A and DNMT3B.¹⁹ DNMT3A deficient mouse HSCs display altered patterns of cytosine methylation including both hypomethylated and hypermethylated regions.^{13, 18, 20} DNMT3A appears to be required for the normal self-renewal capacity of HSCs in adult mice and for maintaining the differentiation potential of serially transplanted HSCs in wildtype recipients.³ DNMT1 also appears to be critical for leukemia stem cell function, as haploinsufficiency of *Dnmt1* in an MLL-AF9-induced mouse model resulted in reduced DNA methylation and bivalent chromatin marks associated with tumor suppressor gene de-repression.²¹

The ten-eleven translocation (TET) family of proteins has recently been shown to contribute to the regulation of DNA methylation through the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethyl cytosine (5hmC).²² This modification is thought to block the binding of proteins that mediate transcriptional silencing by recognizing methylated DNA, thus it is found in regulatory regions of genes that are actively transcribed.^{23,24} 5hmC is also thought to be a critical step on the path to DNA demethylation.²⁵ *TET2* mutations occur in 7–23% of AML and 49% of CMML and are associated with poor prognosis in CN-AML.^{26–29} Deletion of *Tet2* in mice leads to increased self-renewal, expansion of the hematopoietic stem and progenitor cell (HSPC) compartment, and altered cell differentiation towards the monocytic/granulocytic lineages.^{30–32} *TET2* mutations in myeloid malignancies are generally associated with low 5hmC levels, and both DNA hypermethylation and hypomethylation at CpG sites in AML.³³ *TET2* mutations are mutually exclusive with gain of function mutations in the isocitrate dehydrogenase 1 and 2 enzymes (*IDH1/2*), that are found in 15–33% of AML.^{26, 34–38} In general, *IDH1/2* mutations are associated with poor prognosis, but outcome may vary somewhat based on the location of the *IDH1/2* mutation.³⁹ The reason for this mutual exclusivity was rapidly identified; *IDH1/2* regulate the conversion of isocitrate to α -ketoglutarate (α -KG), and mutations in the arginine residues of *IDH1/2* alter its enzymatic function, leading to the aberrant accumulation of a 2-hydroglutarate (2-HG), an “oncometabolite” that impairs the function of TET proteins and the activity of the jumonji (JmJ) family of histone demethylases, which also require α -KG.^{40,41} Thus, *IDH1* mutations impair histone demethylation, and biologically, appear to inhibit differentiation.⁴² In a bone marrow transplantation model, *IDH1* mutations cooperated with *HOXA9* to accelerate the development of an MPD-like disorder.⁴³ Knock-in mice that express the *IDH1* (R132H) mutation have increased (i.e. detectable) 2-HG serum levels, and expansion of the multipotent progenitor population.⁴⁴ The same increase in 2-HG is seen in patients with *IDH1/2* mutant AML.⁴⁵

Polycomb group proteins

Hematopoiesis requires the proper temporal and lineage specific regulation of gene expression, such as the homeotic genes, whose expression is reciprocally controlled by large protein complexes containing the polycomb group (PcG) proteins or trithorax group (Trx) proteins. The balance between these complexes is crucial for the normal regulation of embryonic development and cell differentiation, with alterations in *HoxA* and *HoxB* cluster gene expression being a characteristic of many hematologic malignancies.⁵² Figure 2 depicts the normal PcG complexes, the leukemia associated *MLL* fusion complexes, and their relevance to the epigenetic therapy of leukemia. The major PcG protein complexes, known as polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2), maintain transcriptional silencing. The PRC2 complex consists of four core members, EZH1/2, EED, SUZ12 and RBAP48. EZH2 contains the methyltransferase activity that catalyzes the di and tri methylation of H3K27, which is generally a repressive chromatin mark.⁴⁷ *EZH2* is the most frequently mutated PRC2 component in cancer and it is also upregulated in many solid tumors, often serving as an indicator of aggressive disease.⁴⁸ While heterozygous gain-of-function mutations of *EZH2* have been identified in diffuse large B-cell lymphomas, loss-of-function missense, nonsense, and frameshift mutations are typically observed in myeloid malignancies, especially MDS.^{49–51} *EZH2* mutations are rare in most acute leukemias (1–2% of de novo AML), but they are found they in 16–19% of T-ALL.⁵² Recurrent deletions or somatic mutations in *SUZ12* (21%), and *EED* (15%) were also identified in ETP-ALL.²

The site-specific recruitment of PRC complexes to chromatin is an important step in the regulation of histone methylation. Since PRC complexes do not contain DNA sequence specific binding activity, they are subject to interaction with other proteins, such as ASXL1 (addition of sex combs like 1). Deletions and point mutations in *ASXL1* occur in 6–30% of AML and 43% of CMML.^{28,50,53} These mutations promote transformation by decreasing PRC2 recruitment and thereby reducing H3K27 methylation, leading to loss of the transcriptional repression of genes whose expression can promote leukemogenesis, including *HOXA9*.⁵⁴ Loss-of-function *Asx1/1* mutations showed a mild phenotype in mice, with defects in myeloid and lymphoid progenitors, but no evidence of myelodysplasia or leukemic progression, while conditional knockout of *Asx1/1* in the hematopoietic compartment resulted in myelodysplasia.^{55,56} Another PRC2 interacting protein, JARID2, is also involved in recruiting the complex to target loci.⁵⁷ JARID2 inhibits the lysine methyltransferase activity of PRC2 and it is deleted during the progression of some chronic phase myeloid malignancies to acute leukemia.⁵⁸

The PRC1 complex recognizes H3K27me3 via its chromodomain-containing CBX proteins, and is involved in the maintenance of gene repression through histone H2A ubiquitination and the recruitment of DNA methyltransferases.⁵⁹ The diverse forms of the PRC1 complex consist of a core containing BMI1 and the ubiquitin ligases RING1A and RING1B, but also CBX proteins, Ph homologs (PHC 1–3), and other RING-finger domain containing proteins. PRC1 contains several proteins linked to cancer including BMI1, a protein associated with HSC self-renewal and the leukemic reprogramming of myeloid progenitors.^{60,61} RING1A and RING1B contain important histone ubiquitin ligase activity, and both BMI1 and RING1

components have been shown to be overexpressed in myeloid malignancies and play a critical role in hematopoiesis.⁶² *Bmi1* deficient granulocyte/macrophage progenitors (GMPs) transformed with MLL-AF9 showed impaired leukemia stem activity and increased differentiation potential. Conditional inactivation of Ring1B resulted in a reduction in total bone marrow cell numbers, while the proliferation of myeloid progenitors and percentage of lineage negative cells increased.⁶³ Other fusion proteins such as PLZF/RARA have been shown to interact with BMI1 and PRC1 complexes, recruiting the complex to retinoic acid response elements.⁶⁴ Taken together, these studies suggest that oncogenic fusion genes require PRC1 protein activity in order to establish the leukemic reprogramming of myeloid progenitors, including the block in their differentiation.

The recurrent loss-of-function mutations, copy number alternations, or overexpression of PRC components in lymphoid leukemia suggest that these complexes may act as epigenetic tumor suppressors. Supporting this notion is the finding that overexpression of PRC2 components can inhibit the function of the complex by altering the subunit composition, thereby leading to aberrant targeting of the complex.⁶⁵ The recruitment of DNA methyltransferases by PRC2 is another critical step in transcriptional repression and it too may be altered by somatic mutations in *DNMT3A*.⁶⁶ All these changes may lead to aberrant activation of PRC target genes or the inappropriate recruitment of the complex to other gene targets, leading to aberrant transcriptional repression.

MLL proteins

Mutations involving mammalian versions of trithorax genes are found in leukemia (*MLL1*) and in solid tumors as well (*MLL 2,3,4,5*). The MLL genes encode histone methyltransferases whose mutations can alter chromatin structure. MLL proteins are members of the SET domain containing protein lysine methyltransferase family; they methylate H3K4, generating a mark of transcriptional activation. Translocations involving *MLL* and its in-frame fusion partners have been observed in 5–10% of AML and greater than 70% of infant ALL; they are generally associated with poor prognosis.^{67–71} In-frame partial tandem duplications of MLL occur in 5–7% of de novo AML and they too are associated with an unfavorable prognosis.⁷² The direct binding targets of MLL fusion proteins include *HOXA* cluster genes and *MEIS1*.⁷³ Enforced expression of MLL-AF9, or a combination of HOXA9 and MEIS1, induces the leukemic transformation of HSPCs in mouse models.⁷⁴ *MLL*-rearrangement leads to acquisition of H3K79 methyltransferase activity at MLL target sites due to the recruitment of the methyltransferase DOT1L.^{75–77} MLL fusion proteins are also dependent on Menin, a component of the MLL-SET1-like histone methyltransferase complex, that serves as a link to the chromatin-associated protein LEDGF.^{78,79} Genetic loss of Menin induces differentiation and reverses aberrant *HOX* gene expression in leukemic blasts, while disrupting the Menin interaction domain of MLL downregulates MEIS1 and inhibits cell proliferation.⁸⁰ Peptides that directly disrupt the LEDGF-MLL interface have shown efficacy in MLL-AF9 induced AML, and it appears that numerous MLL-interacting proteins must remain fully functional for MLL-FP driven leukemias to persist.⁸¹

Other lysine methyltransferases and demethylases

NSD1 encodes another protein lysine methyltransferase involved in leukemia through its fusion with the nucleoporin 98 gene (*NUP98*), generating the *NUP98-NSD1* fusion protein that is associated with poor prognosis in patients.^{82–84} Histone lysine methylation can be reversed by the amine oxidase type lysine specific demethylases, including LSD1 and LSD2, which are generally referred to as “erasers” because they remove histone marks. LSD1 is of particular interest since it exhibits specificity for H3K4 and H3K9 methylation, is critical for erythroid differentiation, and is highly expressed in AML.⁸⁵ Global H3K4 methylation levels can also be altered by mutations or gene expression changes in the Jumanji C (JmjC) family of lysine demethylases. These genetic abnormalities include translocations involving the *JARID1* family of histone H3K4 demethylases and overexpression of KDM2B, an H3K36me2-specific demethylase that is required for initiation and maintenance of acute myeloid leukemia.⁸⁶ Fusion of the *NUP98* and *JARID1A* genes (also known as *KDM5A*) occur in 10% of acute megakaryoblastic leukemia.^{87,88} This translocation creates haploinsufficiency for Nup98 and JARID1A and it leads to alteration of JARID1 function, possibly due to recruitment of p300/CBP by the fusion protein.⁸⁹ *UTX*, another member of the JmjC family of lysine demethylases, is altered through inactivating mutations in AML and ALL.^{90–92}

Protein arginine methyltransferases

Protein arginine methyltransferases (PRMTs) catalyze the mono or di-methylation of arginine residues in histones, and other non-histone substrates, including transcription factors. Asymmetric di-methylation of histones is generally associated with gene activation whereas symmetric dimethyl-arginine is associated with gene repression. PRMTs appear to play some role in acute leukemia, as several members of the PRMT family are overexpressed in AML including PRMT4 and PRMT5.^{93–95} Our lab has recently reported that PRMT4 can block myeloid differentiation, at least in part by promoting the assembly of a repressive RUNX1 complex. Knockdown of PRMT4 in several human leukemia cell lines, and in human CD34⁺ cells, promotes myeloid differentiation.⁹⁶ PRMT1 appears to be a critical member of the MLL transcriptional complex, while PRMT6 has been shown to inhibit H3K4 methylation by MLL.^{97,98}

Histone acetyltransferases

Histone acetyltransferases (HATs) catalyze the transfer of an acetyl group to lysine residues, neutralizing the positive charge and promoting a less compact chromatin state that is associated with increased gene transcription. Mutations in the HAT *CBP* have been identified in 18% of relapsed acute lymphoblastic leukemia, resulting in impaired histone acetylation and aberrant transcriptional regulation of CBP targets.^{99–101} *MOZ*, a member of the MYST family of acetyltransferases, is a critical regulator of HSC maintenance and global Hox gene expression, through its effects on histone H3K9 acetylation at Hox loci.^{102–104} *MOZ* is involved in several chromosomal translocations in leukemia including fusion to *P300*, *CBP*, and *TIF2*.^{105–107} TIP60 is another HAT thought to play a tumor suppressive role in leukemia through the recognition of H3K9me3.¹⁰⁸ TIP60 protein levels are reduced in AML patients and TIP60 has been shown to interact with ETV6, a frequent

fusion partner in B-precursor ALL, and a frequent site of deletions or mutations in acute leukemia.¹⁰⁹

Crosstalk between chromatin regulatory complexes

Transcriptional activation requires both the addition of activating post-translational modifications and the removal of the repressive modifications, such as H3K27 methylation. Therefore, it is not surprising that leukemia cells often display changes in both PcG and Trx group proteins concomitantly. The first connection between PcG proteins and MLL leukemia associated factors emerged from the observation that MLL-AF9 expressing leukemic stem cells achieve transcriptional activation and overcome senescence through interactions between the PRC1 components BMI1 and CBX8.^{110,111} The crosstalk between normal or oncogenic epigenetic modifiers and other oncogenes may have a potential for therapeutic intervention. A prime example of this is the efficacy of inhibiting PRC2 activity in *MLL*-rearranged leukemias.^{112–114} Conditional deletion of *Ezh2* in GMPs expressing MLL-AF9, reduced their proliferation in culture and attenuated the progression to AML.¹¹⁵ While genetic loss of *Ezh2* resulted in a mild phenotype in the MLL-AF9 mouse model, loss of PRC2 function through deletion of *Eed* significantly inhibited leukemia cell growth.¹¹² Inhibition of PRC2 components has shown pre-clinical efficacy in acute promyelocytic leukemia as well, a disease driven by the PML-RAR α fusion, also known as the (15;17) translocation. PML-RAR α was shown to complex with the PRC2 components SUZ12, EZH2, and EED and recruit them to specific promoters. This study showed a link between PML-RAR α and PRC2 driven H3K27 methylation and DNA methylation. Inhibition of PRC2 induced demethylation of PML-RAR α target genes, reactivating the promoters and driving granulocytic differentiation.^{63,116}

The ability of oncogenic fusion proteins to interact with proteins that their wildtype constituents may not, can be used to develop new therapeutics. For example, MLL fusion interact with the disruptor of telomere silencing 1-like (DOT1L) protein, a histone methyltransferase that catalyzes the methylation of H3K79, while wildtype MLL does not.¹¹⁷ This association leads to aberrant recruitment of DOT1L and enhanced H3K79 methylation at MLL fusion protein directed loci.⁷⁵ Loss of DOT1L in MLL-rearranged leukemia cells promotes differentiation and apoptosis as well as the decreased expression of MLL fusion targets. Finally, the development and maintenance of *MLL*-rearranged leukemia appears to be dependent on DOT1L in vivo, so that DOT1L inhibitors have potential therapeutic promise in this disease.^{76,118–120}

One of the challenges in developing new epigenetic therapies is to understand how these pathways act in concert to regulate transcription. Integrating large amounts of genetic data may mean letting go of preconceived notions of mutation classification and protein function. One of the findings of The Cancer Genome Atlas (TCGA) recent characterization of de novo AML was the discovery of recurrent mutations in genes that encode components of cohesion and the spliceosome complexes¹. Mutations in the cohesion complex were found in 13% of de novo AML and were predicted to occur in the leukemic initiating clone.¹²¹ Recent work has shown the association of DNMT3B with this complex, possibly providing a link between chromatin condensation and cytosine methylation.¹²² Similarly, mutations in genes

encoding the spliceosome complex have been found in many myeloid and lymphoid malignancies including 14% of de novo AML, 10%–15% of CLL and 38% of myelodysplastic syndrome (MDS).^{123,124} Interestingly, in MDS patients, these mutations were more likely to co-occur with mutations in epigenetic modifiers, suggesting a possible crosstalk between these two pathways.¹²⁵

Significance of chromatin modifiers in leukemia

An important question is whether these mutations in epigenetic modifiers are truly leukemic drivers and therefore appropriate therapeutic targets. MLL fusion proteins may be sufficient to drive leukemogenesis, as MLL-rearranged leukemias have the fewest number of mutations of any of the known cancers for which TCGA data exists¹. The identification of *TET2* and *DNMT3A* mutations in a normal elderly population suggests that these mutations may be involved in clonal selection and clonal fitness over time, but they can exist without malignant transformation.^{126,127} This enhanced capacity for self-renewal, clonal expansion, and skewing towards the myeloid lineage may make individual cells more susceptible to malignant transformation by other genetic alterations. The rate of these mutations in pre-leukemic disorders supports a role for epigenetic modifiers in the early stages of leukemic development. *ASXL1* mutations have been identified in over 30% of patients with refractory anemia with excess blasts (RAEB) and in a similar percentage of AML that evolved from myelodysplastic syndrome (MDS).¹²⁸ Interestingly, chronic myelomonocytic leukemia (CMML), the disease with the highest rate of epigenetic modifier gene mutations, is a myelodysplastic/myeloproliferative neoplasms (MDS/MPN) rather than an acute leukemia.^{28,129,130}

Numerous studies show that introducing epigenetic modifier gene mutations into mouse hematopoietic stem/progenitor cells often confers increased self-renewal, and myeloproliferation with extramedullary hematopoiesis, but not transformation to acute leukemia. Genetic knock out of these genes in mouse models have shown that they are critical for driving HSC self-renewal and differentiation, thus they contribute to but are not themselves sufficient to cause acute leukemia. Given the remarkable demonstrations of clonal heterogeneity in cancer, the early occurrence of epigenetic mutations suggest that they may be the most relevant therapeutic targets, since they are present in a greater number of leukemic clones than those mutations that occur in the final stages of transformation.

Prognostic importance of chromatin modifiers and therapeutic intervention

The discovery of recurrent mutations in chromatin modifiers has led to efforts to correlate these genetic changes with clinical characteristics, and several such mutations do have prognostic significance (Table 1). *TET2* mutations have been associated with adverse overall survival in intermediate risk AML.²⁹ Similarly, multiple studies have shown that *DNMT3A* mutations confer adverse risk to intermediate risk AML patients, although this appears to be restricted to the subset of patients with *FLT3-ITD* mutations.^{13,14} *ASXL1* mutations are associated with adverse overall survival in CN-AML or intermediate risk AML, while specific *IDH1* mutations have been shown to be associated with favorable outcomes.^{29,39} When combined with current cytogenetic and mutations testing, these markers may be

useful in risk stratification or treatment selection. For example, genetic profiling of AML patients suggests that those with *DNMT3A* mutations have improved outcome when treated with high dose vs. lower dose daunorubicin.⁵³

Epigenetic Therapeutics

We now know that epigenetic (DNA and chromatin) modifications are in fact, generally reversible, allowing for lineage specific changes in gene expression during differentiation, cell division, and DNA repair. Indeed, it is the inherent plasticity of epigenetic modifications makes them susceptible to pharmacological intervention. Thus, the discovery of recurrent mutations in chromatin modifiers has provided additional insights into the pathogenesis of leukemia, as well as the development of new highly potent and directed epigenetic therapies. A summary of current epigenetic therapies and their relevance to leukemia can be found in Table 2.

DNA methyltransferase inhibitors and histone deacetylase inhibitors (HDACs) were the first epigenetically targeted inhibitors to be FDA-approved for the treatment of cancer in the United States. Azacitidine and decitabine are nucleoside analogs and inhibitors of the DNA methyltransferase enzymes DNMT1 and DNMT3. They are thought to act through the gene hypomethylation (e.g. of tumor suppressor genes), however, this has never been formally shown. Combinations of these inhibitors are currently being evaluated in clinical trials for AML and MDS. HDAC inhibitors demonstrated modest therapeutic potential in early clinical trials for B-cell lymphoma, myeloma, myelodysplastic syndrome, and AML. They are currently FDA approved only for the treatment of cutaneous T-cell lymphoma. Despite extensive research, we still do not fully understand the mechanism of action of these therapeutics.

The discovery of recurrent IDH1/2 mutations has led to the development of small molecule inhibitors that aim to target the mutant enzymes. These agents are being evaluated in preclinical studies for their use in patients with glioma or AML. In cell lines, a selective IDH1-R132H inhibitor decreased the production of R-2-hydroxyglutarate (R-2HG), induced demethylation of histone H3K9me3, and increased expression of genes associated with differentiation.¹³¹ A small molecule inhibitor specific for IDH2-R140Q induced the differentiation of an erythroleukemia cell line and also human acute leukemia cells.¹³² Similarly, inhibition of mutant IDH1 decreased 2-HG production, induced apoptosis in murine cells, and inhibited the proliferation of progenitor cells from obtained from AML patients with *IDH1* mutations.⁴³

The high frequency of mutations in components (or regulators) of the PRC2 complex in myeloid and lymphoid malignancies, and the resulting changes in the level of H3K27me3, have sparked interest in the lysine demethylases as therapeutic targets. Small molecule catalytic site inhibitors are being developed for this family of proteins and show selectivity for these enzymes. Inhibition of LSD1, by RNAi or small molecules, has recently been shown to induce the differentiation of *MLL*-rearranged leukemias. Additionally, there is evidence that LSD1 inhibitors may be efficacious in non-APL patients when combined with all trans retinoic acid (ATRA).¹³³ This could make a currently FDA-approved therapy (ATRA) more applicable to this larger number of patients.¹⁶⁴ Potent inhibitors of the JmjC

family of histone demethylases, which includes UTX, JMJD3, and JARID1 are being developed for use in cancer, and potent and selective inhibitors of EZH2 have been developed for use in lymphoma and possibly leukemia as well.^{135,136} Another strategy has been to develop peptides which disrupt EZH2-EED protein interactions, and these show efficacy in MLL-AF9 expressing leukemia cells.¹³⁷

Another level of transcriptional regulation is provided by the proteins that recognize specific histone residues based on posttranslational modifications, known as chromatin “readers”. The bromodomain containing proteins are responsible for recognizing acetylated lysine residues on histone tails. Quite unexpectedly, MLL fusion protein driven AML is sensitive to JQ1, a BRD4 inhibitor, and MYC driven malignancies are also quite sensitive to such bromodomain inhibitors in vitro and in animal models.^{138,139} Several bromodomain containing proteins are amenable to small molecule inhibition, and demonstrate therapeutic efficacy in preclinical models of AML.¹⁴⁰ Bromodomain inhibitor treatment of B-ALL cell lines decreased their viability and induced the loss of BRD4 at the *MYC* promoter, causing down-regulation of *MYC* transcription, and the reduced expression of c-Myc target genes.¹⁴¹ A Phase I study of a BRD2/3/4 inhibitor in patients with hematologic malignancy is ongoing (NCT01713582). Inhibitors of other “epigenetic readers” such as the chromodomain containing proteins that recognize methyl-lysine are also being explored.¹⁴²

Challenges for epigenetic-targeted therapy

A major challenge in the development of more effective epigenetic therapies is the lack of biomarkers to evaluate efficacy in a clinical setting. In contrast to the pharmacodynamics of receptor tyrosine inhibitors or chemotherapeutics, the effects of epigenetic therapies often take a long time to observe. Clinical trials using hypomethylating agents have suggested that maximal DNA hypomethylation may occur more than a week after their administration, while effects on proliferation, differentiation and cell survival may not occur for weeks.^{143–145} Therefore, established measures of clinical response may be unhelpful in evaluating the mechanism of action these compounds. There has been an effort to correlate response with the methylation status of specific genes, changes in gene expression, or levels of microRNA, however, the connection between these biomarkers and efficacy is generally weak.^{146–148} While methylation of tumor suppressor genes decrease in some patients, global methylation changes are not consistently associated with changes in gene expression or clinical responses. Also, there is a need to understand why certain subsets of patients fail to respond to treatment.¹⁴⁹

There is also a need to understand more about the biology of these large multiprotein chromatin modifying complexes and the mechanism of action of the inhibitors. The function of these complexes may be cell context dependent, hinging on the underlying genetic landscape of the cell and the available interacting proteins. Mutations in the methyltransferase EZH2 demonstrate how both gain-of-function and loss-of-function mutations can lead to aberrant transcription and proliferation, depending on the cellular context. Chromatin modifying enzymes may have much more diverse roles than previously thought, as many histone methyltransferases are now known to act on non-histone substrates. Despite these limitations in our understanding, chromatin-modifying

protein inhibitors will be an important tool to help define these functions and develop more effective therapeutic strategies.

Although leukemia cells have relatively few mutations compared to solid tumors, it is rare for cancer to be driven by a single genetic or epigenetic mutation. In solid tumors, targeting of an oncogenic driver often leads to activation of the same or distant oncogenic pathways that allow the tumor to escape treatment. To date, most preclinical studies of chromatin modifier inhibitors have focused on *MLL*-rearranged leukemia, which is highly dependent on fusion protein dependent effects for survival. To make “epigenetic modifying therapy” more applicable to patients, we will need to consider how chromatin modifying mutations fit into the larger genetic landscape, which include changes in signaling pathways. Epigenetic therapy might lead to resistance through epigenetic or non-epigenetic mechanisms. For example, clinical trials of DNMT inhibitors in MDS and CML have shown that cells present at the time of relapse have less methylation than those present prior to treatment, suggesting methylation independent clonal evolution. Hopefully, there will be successes in combining epigenetic modifying therapies with other classes of inhibitors. HDACs have shown promising preclinical activity in combination with chemotherapeutic drugs, inhibitors of heat shock proteins, proteasome proteins, and tyrosine kinases.^{150–151} It is also encouraging that the first studies of the inhibitors of epigenetic modifiers have demonstrated unexpected effects on previously identified leukemic drivers. For example, the ability of bromodomain inhibitors to target critical oncogenes such as c-Myc or Bcl-2, is promising and somewhat unexpected.^{152–153} Although the biology of epigenetic regulation is complex, novel epigenetic therapies show tremendous potential for improving our therapy of acute leukemia and other related diseases.

Summary

The recent completion of the Cancer Genome Atlas Research Network’s analysis of adult de novo AML has highlighted the importance of mutations in chromatin modifiers. While individual mutations in chromatin modifiers do not appear to be sufficient to induce leukemia in mouse models, the changes in self-renewal and differentiation they mediate are critical steps in the transformation of hematopoietic stem/progenitor cells. Moreover, it is becoming increasingly clear that the interaction of transcription factors with epigenetic modifiers is critical to their oncogenic activity. When these interactions are inhibited in mouse models, leukemic precursors lose the ability to differentiate, self-renew, and propagate in recipient mice. The challenge for the future will be to translate this knowledge into the development of new, combination therapies, targeting leukemic driver mutations and their dependence on chromatin modifying enzymes.

Acknowledgments

We thank the members of the Nimer lab, especially P.J. Hamard, Ezra Blumenthal and Madhavi Tadi for their helpful comments and thoughtful discussions on this manuscript. This work has been supported by the NCI under award number R01CA166835.

References

1. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *The New England journal of medicine*. 2013 May 30; 368(22):2059–2074. [PubMed: 23634996]
2. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature*. 2012 Jan 12; 481(7380):157–163. [PubMed: 22237106]
3. Tadokoro Y, Ema H, Okano M, Li E, Nakauchi H. De novo DNA methyltransferase is essential for self-renewal, but not for differentiation, in hematopoietic stem cells. *The Journal of experimental medicine*. 2007 Apr 16; 204(4):715–722. [PubMed: 17420264]
4. Broske AM, Vockentanz L, Kharazi S, Huska MR, Mancini E, Scheller M, et al. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nature genetics*. 2009 Nov; 41(11):1207–1215. [PubMed: 19801979]
5. Bock C, Beerman I, Lien WH, Smith ZD, Gu H, Boyle P, et al. DNA methylation dynamics during in vivo differentiation of blood and skin stem cells. *Molecular cell*. 2012 Aug 24; 47(4):633–647. [PubMed: 22841485]
6. Ji H, Ehrlich LI, Seita J, Murakami P, Doi A, Lindau P, et al. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature*. 2010 Sep 16; 467(7313):338–342. [PubMed: 20720541]
7. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. *Cancer cell*. 2010 Jan 19; 17(1):13–27. [PubMed: 20060365]
8. Deneberg S, Guardiola P, Lennartsson A, Qu Y, Gaidzik V, Blanchet O, et al. Prognostic DNA methylation patterns in cytogenetically normal acute myeloid leukemia are predefined by stem cell chromatin marks. *Blood*. 2011 Nov 17; 118(20):5573–5582. [PubMed: 21960591]
9. Figueroa ME, Chen SC, Andersson AK, Phillips LA, Li Y, Sotzen J, et al. Integrated genetic and epigenetic analysis of childhood acute lymphoblastic leukemia. *The Journal of clinical investigation*. 2013 Jul 1; 123(7):3099–3111. [PubMed: 23921123]
10. Lugthart S, van Drunen E, van Norden Y, van Hoven A, Erpelinck CA, Valk PJ, et al. High EVI1 levels predict adverse outcome in acute myeloid leukemia: prevalence of EVI1 overexpression and chromosome 3q26 abnormalities underestimated. *Blood*. 2008 Apr 15; 111(8):4329–4337. [PubMed: 18272813]
11. Lugthart S, Figueroa ME, Bindels E, Skrabanek L, Valk PJ, Li Y, et al. Aberrant DNA hypermethylation signature in acute myeloid leukemia directed by EVI1. *Blood*. 2011 Jan 6; 117(1):234–241. [PubMed: 20855866]
12. Jiang Y, Dunbar A, Gondek LP, Mohan S, Rataul M, O’Keefe C, et al. Aberrant DNA methylation is a dominant mechanism in MDS progression to AML. *Blood*. 2009 Feb 5; 113(6):1315–1325. [PubMed: 18832655]
13. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. *The New England journal of medicine*. 2010 Dec 16; 363(25):2424–2433. [PubMed: 21067377]
14. Thol F, Damm F, Ludeking A, Winschel C, Wagner K, Morgan M, et al. Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011 Jul 20; 29(21):2889–2896. [PubMed: 21670448]
15. Marcucci G, Metzeler KH, Schwind S, Becker H, Maharry K, Mrozek K, et al. Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012 Mar 1; 30(7):742–750. [PubMed: 22291079]
16. Grossmann V, Haferlach C, Weissmann S, Roller A, Schindela S, Poetzinger F, et al. The molecular profile of adult T-cell acute lymphoblastic leukemia: mutations in RUNX1 and DNMT3A are associated with poor prognosis in T-ALL. *Genes, chromosomes & cancer*. 2013 Apr; 52(4):410–422. [PubMed: 23341344]

17. Neumann M, Heesch S, Schlee C, Schwartz S, Gokbuget N, Hoelzer D, et al. Whole-exome sequencing in adult ETP-ALL reveals a high rate of DNMT3A mutations. *Blood*. 2013 Jun 6; 121(23):4749–4752. [PubMed: 23603912]
18. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. *Nature genetics*. 2011 Apr; 43(4):309–315. [PubMed: 21399634]
19. Kim SJ, Zhao H, Hardikar S, Singh AK, Goodell MA, Chen T. A DNMT3A mutation common in AML exhibits dominant-negative effects in murine ES cells. *Blood*. 2013 Dec 12; 122(25):4086–4089. [PubMed: 24167195]
20. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nature genetics*. 2012 Jan; 44(1):23–31.
21. Trowbridge JJ, Sinha AU, Zhu N, Li M, Armstrong SA, Orkin SH. Haploinsufficiency of Dnmt1 impairs leukemia stem cell function through derepression of bivalent chromatin domains. *Genes & development*. 2012 Feb 15; 26(4):344–349. [PubMed: 22345515]
22. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature*. 2010 Aug 26; 466(7310):1129–1133. [PubMed: 20639862]
23. Pastor WA, Pape UJ, Huang Y, Henderson HR, Lister R, Ko M, et al. Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. *Nature*. 2011 May 19; 473(7347):394–397. [PubMed: 21552279]
24. Wu H, D'Alessio AC, Ito S, Wang Z, Cui K, Zhao K, et al. Genome-wide analysis of 5-hydroxymethylcytosine distribution reveals its dual function in transcriptional regulation in mouse embryonic stem cells. *Genes & development*. 2011 Apr 1; 25(7):679–684. [PubMed: 21460036]
25. Hackett JA, Sengupta R, Zyllicz JJ, Murakami K, Lee C, Down TA, et al. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science*. 2013 Jan 25; 339(6118):448–452. [PubMed: 23223451]
26. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A, et al. Mutation in TET2 in myeloid cancers. *The New England journal of medicine*. 2009 May 28; 360(22):2289–2301. [PubMed: 19474426]
27. Chou WC, Chou SC, Liu CY, Chen CY, Hou HA, Kuo YY, et al. TET2 mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics. *Blood*. 2011 Oct 6; 118(14):3803–3810. [PubMed: 21828143]
28. Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F, et al. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. *Blood*. 2011 Oct 6; 118(14):3932–3941. [PubMed: 21828135]
29. Metzeler KH, Maharry K, Radmacher MD, Mrozek K, Margeson D, Becker H, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2011 Apr 1; 29(10):1373–1381. [PubMed: 21343549]
30. Li Z, Cai X, Cai CL, Wang J, Zhang W, Petersen BE, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood*. 2011 Oct 27; 118(17):4509–4518. [PubMed: 21803851]
31. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, et al. Tet loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer cell*. 2011 Jul 12; 20(1):11–24. [PubMed: 21723200]
32. Quivoron C, Couronne L, Della Valle V, Lopez CK, Plo I, Wagner-Ballon O, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer cell*. 2011 Jul 12; 20(1):25–38. [PubMed: 21723201]
33. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer cell*. 2010 Dec 14; 18(6):553–567. [PubMed: 21130701]
34. Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilemaker A, et al. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid

- leukemia: prevalence and prognostic value. *Blood*. 2010 Sep 23; 116(12):2122–2126. [PubMed: 20538800]
35. Boissel N, Nibourel O, Renneville A, Gardin C, Reman O, Contentin N, et al. Prognostic impact of isocitrate dehydrogenase enzyme isoforms 1 and 2 mutations in acute myeloid leukemia: a study by the Acute Leukemia French Association group. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2010 Aug 10; 28(23):3717–3723. [PubMed: 20625116]
 36. Marcucci G, Maharry K, Wu YZ, Radmacher MD, Mrozek K, Margeson D, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010 May 10; 28(14):2348–2355. [PubMed: 20368543]
 37. Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2010 Aug 1; 28(22):3636–3643. [PubMed: 20567020]
 38. Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. *Blood*. 2010 Dec 16; 116(25):5486–5496. [PubMed: 20805365]
 39. Green CL, Evans CM, Zhao L, Hills RK, Burnett AK, Linch DC, et al. The prognostic significance of IDH2 mutations in AML depends on the location of the mutation. *Blood*. 2011 Jul 14; 118(2): 409–412. [PubMed: 21596855]
 40. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009 Dec 10; 462(7274):739–744. [PubMed: 19935646]
 41. Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *The Journal of experimental medicine*. 2010 Feb 15; 207(2): 339–344. [PubMed: 20142433]
 42. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature*. 2012 Mar 22; 483(7390):474–478. [PubMed: 22343901]
 43. Chaturvedi A, Araujo Cruz MM, Jyotsana N, Sharma A, Yun H, Gorlich K, et al. Mutant IDH1 promotes leukemogenesis in vivo and can be specifically targeted in human AML. *Blood*. 2013 Oct 17; 122(16):2877–2887. [PubMed: 23954893]
 44. Sasaki M, Knobbe CB, Munger JC, Lind EF, Brenner D, Brustle A, et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. *Nature*. 2012 Aug 30; 488(7413):656–659. [PubMed: 22763442]
 45. Janin M, Mylonas E, Saada V, Micol JB, Renneville A, Quivoron C, et al. Serum 2-Hydroxyglutarate Production in IDH1- and IDH2-Mutated De Novo Acute Myeloid Leukemia: A Study by the Acute Leukemia French Association Group. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2013 Dec 16.
 46. Argiropoulos B, Humphries RK. Hox genes in hematopoiesis and leukemogenesis. *Oncogene*. 2007 Oct 15; 26(47):6766–6776. [PubMed: 17934484]
 47. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science*. 2002 Nov 1; 298(5595):1039–1043. [PubMed: 12351676]
 48. Kroeze LI, Nikoloski G, da Silva-Coelho P, van Hoogen P, Stevens-Linders E, Kuiper RP, et al. Genetic defects in PRC2 components other than EZH2 are not common in myeloid malignancies. *Blood*. 2012 Feb 2; 119(5):1318–1319. [PubMed: 22308284]
 49. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nature genetics*. 2010 Feb; 42(2):181–185. [PubMed: 20081860]

50. Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, et al. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. *Leukemia*. 2011 Jul; 25(7):1200–1202. [PubMed: 21455215]
51. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nature genetics*. 2011 Sep; 43(9):830–837. [PubMed: 21804550]
52. Ntziachristos P, Tsiganos A, Van Vlierberghe P, Nedjic J, Trimarchi T, Flaherty MS, et al. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nature medicine*. 2012 Feb; 18(2):298–301.
53. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *The New England journal of medicine*. 2012 Mar 22; 366(12):1079–1089. [PubMed: 22417203]
54. Fisher CL, Lee I, Bloyer S, Bozza S, Chevalier J, Dahl A, et al. Additional sex combs-like 1 belongs to the enhancer of trithorax and polycomb group and genetically interacts with Cbx2 in mice. *Developmental biology*. 2010 Jan 1; 337(1):9–15. [PubMed: 19833123]
55. Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, et al. JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature*. 2010 Mar 11; 464(7286):306–310. [PubMed: 20075857]
56. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer cell*. 2012 Aug 14; 22(2):180–193. [PubMed: 22897849]
57. Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, et al. Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell*. 2009 Dec 24; 139(7):1290–1302. [PubMed: 20064375]
58. Puda A, Milosevic JD, Berg T, Klampfl T, Harutyunyan AS, Gisslinger B, et al. Frequent deletions of JARID2 in leukemic transformation of chronic myeloid malignancies. *American journal of hematology*. 2012 Mar; 87(3):245–250. [PubMed: 22190018]
59. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nature reviews Cancer*. 2006 Nov; 6(11):846–856. [PubMed: 17060944]
60. Smith LL, Yeung J, Zeisig BB, Popov N, Huijbers I, Barnes J, et al. Functional crosstalk between Bmi1 and MLL/Hoxa9 axis in establishment of normal hematopoietic and leukemic stem cells. *Cell stem cell*. 2011 Jun 3; 8(6):649–662. [PubMed: 21624810]
61. Yuan J, Takeuchi M, Negishi M, Oguro H, Ichikawa H, Iwama A. Bmi1 is essential for leukemic reprogramming of myeloid progenitor cells. *Leukemia*. 2011 Aug; 25(8):1335–1343. [PubMed: 21527932]
62. Xu F, Li X, Wu L, Zhang Q, Yang R, Yang Y, et al. Overexpression of the EZH2, RING1 and BMI1 genes is common in myelodysplastic syndromes: relation to adverse epigenetic alteration and poor prognostic scoring. *Annals of hematology*. 2011 Jun; 90(6):643–653. [PubMed: 21125401]
63. Cales C, Roman-Trufero M, Pavon L, Serrano I, Melgar T, Endoh M, et al. Inactivation of the polycomb group protein Ring1B unveils an antiproliferative role in hematopoietic cell expansion and cooperation with tumorigenesis associated with Ink4a deletion. *Molecular and cellular biology*. 2008 Feb; 28(3):1018–1028. [PubMed: 18039844]
64. Boukarabila H, Saurin AJ, Batsche E, Mossadegh N, van Lohuizen M, Otte AP, et al. The PRC1 Polycomb group complex interacts with PLZF/RARA to mediate leukemic transformation. *Genes & development*. 2009 May 15; 23(10):1195–1206. [PubMed: 19451220]
65. Li G, Margueron R, Ku M, Chambon P, Bernstein BE, Reinberg D. Jarid2 and PRC2, partners in regulating gene expression. *Genes & development*. 2010 Feb 15; 24(4):368–380. [PubMed: 20123894]
66. Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*. 2006 Feb 16; 439(7078):871–874. [PubMed: 16357870]
67. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid

- leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood*. 2000 Dec 15; 96(13):4075–4083. [PubMed: 11110676]
68. Dohner K, Tobis K, Ulrich R, Frohling S, Benner A, Schlenk RF, et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2002 Aug 1; 20(15):3254–3261. [PubMed: 12149299]
69. Balgobind BV, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*. 2009 Sep 17; 114(12):2489–2496. [PubMed: 19528532]
70. Steudel C, Wermke M, Schaich M, Schakel U, Illmer T, Ehninger G, et al. Comparative analysis of MLL partial tandem duplication and FLT3 internal tandem duplication mutations in 956 adult patients with acute myeloid leukemia. *Genes, chromosomes & cancer*. 2003 Jul; 37(3):237–251. [PubMed: 12759922]
71. Whitman SP, Ruppert AS, Marcucci G, Mrozek K, Paschka P, Langer C, et al. Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and MLL partial tandem duplication: a Cancer and Leukemia Group B study. *Blood*. 2007 Jun 15; 109(12):5164–5167. [PubMed: 17341662]
72. Caligiuri MA, Schichman SA, Strout MP, Mrozek K, Baer MR, Frankel SR, et al. Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. *Cancer research*. 1994 Jan 15; 54(2):370–373. [PubMed: 8275471]
73. Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, et al. MLL targets SET domain methyltransferase activity to Hox gene promoters. *Molecular cell*. 2002 Nov; 10(5):1107–1117. [PubMed: 12453418]
74. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature*. 2006 Aug 17; 442(7104):818–822. [PubMed: 16862118]
75. Bernt KM, Zhu N, Sinha AU, Vempati S, Faber J, Krivtsov AV, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer cell*. 2011 Jul 12; 20(1):66–78. [PubMed: 21741597]
76. Jo SY, Granowicz EM, Maillard I, Thomas D, Hess JL. Requirement for Dot1l in murine postnatal hematopoiesis and leukemogenesis by MLL translocation. *Blood*. 2011 May 5; 117(18):4759–4768. [PubMed: 21398221]
77. Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. *Blood*. 2011 Jun 23; 117(25):6912–6922. [PubMed: 21521783]
78. Yokoyama A, Somervaille TC, Smith KS, Rozenblatt-Rosen O, Meyerson M, Cleary ML. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell*. 2005 Oct 21; 123(2):207–218. [PubMed: 16239140]
79. Yokoyama A, Cleary ML. Menin critically links MLL proteins with LEDGF on cancer-associated target genes. *Cancer cell*. 2008 Jul 8; 14(1):36–46. [PubMed: 18598942]
80. Caslini C, Yang Z, El-Osta M, Milne TA, Slany RK, Hess JL. Interaction of MLL amino terminal sequences with menin is required for transformation. *Cancer research*. 2007 Aug 1; 67(15):7275–7283. [PubMed: 17671196]
81. Mereau H, De Rijck J, Cermakova K, Kutz A, Juge S, Demeulemeester J, et al. Impairing MLL-fusion gene-mediated transformation by dissecting critical interactions with the lens epithelium-derived growth factor (LEDGF/p75). *Leukemia*. 2013 Jun; 27(6):1245–1253. [PubMed: 23318960]
82. Cerveira N, Correia C, Doria S, Bizarro S, Rocha P, Gomes P, et al. Frequency of NUP98-NSD1 fusion transcript in childhood acute myeloid leukaemia. *Leukemia*. 2003 Nov; 17(11):2244–2247. [PubMed: 12931227]

83. Wang GG, Cai L, Pasillas MP, Kamps MP. NUP98-NSD1 links H3K36 methylation to Hox-A gene activation and leukaemogenesis. *Nature cell biology*. 2007 Jul; 9(7):804–812. [PubMed: 17589499]
84. Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Pratcorona M, Abbas S, Kuipers JE, et al. NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. *Blood*. 2011 Sep 29; 118(13):3645–3656. [PubMed: 21813447]
85. Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*. 2004 Dec 29; 119(7):941–953. [PubMed: 15620353]
86. He J, Nguyen AT, Zhang Y. KDM2b/JHDM1b, an H3K36me2-specific demethylase, is required for initiation and maintenance of acute myeloid leukemia. *Blood*. 2011 Apr 7; 117(14):3869–3880. [PubMed: 21310926]
87. de Rooij JD, Hollink IH, Arentsen-Peters ST, van Galen JF, Berna Beverloo H, Baruchel A, et al. NUP98/JARID1A is a novel recurrent abnormality in pediatric acute megakaryoblastic leukemia with a distinct HOX gene expression pattern. *Leukemia*. 2013 Dec; 27(12):2280–2288. [PubMed: 23531517]
88. Gruber TA, Larson Gedman A, Zhang J, Koss CS, Marada S, Ta HQ, et al. An Inv(16)(p13.3q24.3)-encoded CBFA2T3-GLIS2 fusion protein defines an aggressive subtype of pediatric acute megakaryoblastic leukemia. *Cancer cell*. 2012 Nov 13; 22(5):683–697. [PubMed: 23153540]
89. Wang GG, Song J, Wang Z, Dormann HL, Casadio F, Li H, et al. Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. *Nature*. 2009 Jun 11; 459(7248):847–851. [PubMed: 19430464]
90. Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, et al. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature*. 2007 Oct 11; 449(7163):731–734. [PubMed: 17713478]
91. van Haaften G, Dalglish GL, Davies H, Chen L, Bignell G, Greenman C, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nature genetics*. 2009 May; 41(5):521–523. [PubMed: 19330029]
92. Mar BG, Bullinger L, Basu E, Schlis K, Silverman LB, Dohner K, et al. Sequencing histone-modifying enzymes identifies UTX mutations in acute lymphoblastic leukemia. *Leukemia*. 2012 Aug; 26(8):1881–1883. [PubMed: 22377896]
93. Jansson M, Durant ST, Cho EC, Sheahan S, Edelmann M, Kessler B, et al. Arginine methylation regulates the p53 response. *Nature cell biology*. 2008 Dec; 10(12):1431–1439. [PubMed: 19011621]
94. Zhao Q, Rank G, Tan YT, Li H, Moritz RL, Simpson RJ, et al. PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. *Nature structural & molecular biology*. 2009 Mar; 16(3):304–311.
95. Liu F, Zhao X, Perna F, Wang L, Koppikar P, Abdel-Wahab O, et al. JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation. *Cancer cell*. 2011 Feb 15; 19(2):283–294. [PubMed: 21316606]
96. Vu LP, Perna F, Wang L, Voza F, Figueroa ME, Tempst P, et al. PRMT4 Blocks Myeloid Differentiation by Assembling a Methyl-RUNX1-Dependent Repressor Complex. *Cell reports*. 2013 Dec 26; 5(6):1625–1638. [PubMed: 24332853]
97. Cheung N, Chan LC, Thompson A, Cleary ML, So CW. Protein arginine-methyltransferase-dependent oncogenesis. *Nature cell biology*. 2007 Oct; 9(10):1208–1215. [PubMed: 17891136]
98. Guccione E, Bassi C, Casadio F, Martinato F, Cesaroni M, Schuchlantz H, et al. Methylation of histone H3R2 by PRMT6 and H3K4 by an MLL complex are mutually exclusive. *Nature*. 2007 Oct 18; 449(7164):933–937. [PubMed: 17898714]
99. Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, et al. CREBBP mutations in relapsed acute lymphoblastic leukaemia. *Nature*. 2011 Mar 10; 471(7337):235–239. [PubMed: 21390130]

100. Inthal A, Zeitlhofer P, Zeginigg M, Morak M, Grausenburger R, Fronkova E, et al. CREBBP HAT domain mutations prevail in relapse cases of high hyperdiploid childhood acute lymphoblastic leukemia. *Leukemia*. 2012 Aug; 26(8):1797–1803. [PubMed: 22388726]
101. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nature genetics*. 2011 Sep; 43(9):830–837. [PubMed: 21804550]
102. Voss AK, Collin C, Dixon MP, Thomas T. Moz and retinoic acid coordinately regulate H3K9 acetylation, Hox gene expression, and segment identity. *Developmental cell*. 2009 Nov; 17(5): 674–686. [PubMed: 19922872]
103. Katsumoto T, Aikawa Y, Iwama A, Ueda S, Ichikawa H, Ochiya T, et al. MOZ is essential for maintenance of hematopoietic stem cells. *Genes & development*. 2006 May 15; 20(10):1321–1330. [PubMed: 16702405]
104. Perez-Campo FM, Borrow J, Kouskoff V, Lacaud G. The histone acetyl transferase activity of monocytic leukemia zinc finger is critical for the proliferation of hematopoietic precursors. *Blood*. 2009 May 14; 113(20):4866–4874. [PubMed: 19264921]
105. Chaffanet M, Gressin L, Preudhomme C, Soenen-Cornu V, Birnbaum D, Pebusque MJ. MOZ is fused to p300 in an acute monocytic leukemia with t(8;22). *Genes, chromosomes & cancer*. 2000 Jun; 28(2):138–144. [PubMed: 10824998]
106. Deguchi K, Ayton PM, Carapeti M, Kutok JL, Snyder CS, Williams IR, et al. MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer cell*. 2003 Mar; 3(3):259–271. [PubMed: 12676584]
107. Crowley JA, Wang Y, Rapoport AP, Ning Y. Detection of MOZ-CBP fusion in acute myeloid leukemia with 8;16 translocation. *Leukemia*. 2005 Dec; 19(12):2344–2345. [PubMed: 16193081]
108. Sun Y, Jiang X, Xu Y, Ayrapetov MK, Moreau LA, Whetstone JR, et al. Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nature cell biology*. 2009 Nov; 11(11):1376–1382. [PubMed: 19783983]
109. Nordentoft I, Jorgensen P. The acetyltransferase 60 kDa trans-acting regulatory protein of HIV type 1-interacting protein (Tip60) interacts with the translocation E26 transforming-specific leukaemia gene (TEL) and functions as a transcriptional co-repressor. *The Biochemical journal*. 2003 Aug 15; 374(Pt 1):165–173. [PubMed: 12737628]
110. Smith LL, Yeung J, Zeisig BB, Popov N, Huijbers I, Barnes J, et al. Functional crosstalk between Bmi1 and MLL/Hoxa9 axis in establishment of normal hematopoietic and leukemic stem cells. *Cell stem cell*. 2011 Jun 3; 8(6):649–662. [PubMed: 21624810]
111. Tan J, Jones M, Koseki H, Nakayama M, Muntean AG, Maillard I, et al. CBX8, a polycomb group protein, is essential for MLL-AF9-induced leukemogenesis. *Cancer cell*. 2011 Nov 15; 20(5):563–575. [PubMed: 22094252]
112. Neff T, Sinha AU, Kluk MJ, Zhu N, Khattab MH, Stein L, et al. Polycomb repressive complex 2 is required for MLL-AF9 leukemia. *Proceedings of the National Academy of Sciences of the United States of America*. 2012 Mar 27; 109(13):5028–5033. [PubMed: 22396593]
113. Shi A, Murai MJ, He S, Lund G, Hartley T, Purohit T, et al. Structural insights into inhibition of the bivalent menin-MLL interaction by small molecules in leukemia. *Blood*. 2012 Nov 29; 120(23):4461–4469. [PubMed: 22936661]
114. Thiel AT, Feng Z, Pant DK, Chodosh LA, Hua X. The trithorax protein partner menin acts in tandem with EZH2 to suppress C/EBPalpha and differentiation in MLL-AF9 leukemia. *Haematologica*. 2013 Jun; 98(6):918–927. [PubMed: 23349306]
115. Tanaka S, Miyagi S, Sashida G, Chiba T, Yuan J, Mochizuki-Kashio M, et al. Ezh2 augments leukemogenicity by reinforcing differentiation blockage in acute myeloid leukemia. *Blood*. 2012 Aug 2; 120(5):1107–1117. [PubMed: 22677129]
116. Villa R, Pasini D, Gutierrez A, Morey L, Occhionorelli M, Vire E, et al. Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. *Cancer cell*. 2007 Jun; 11(6):513–525. [PubMed: 17560333]
117. Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, et al. hDOT1L links histone methylation to leukemogenesis. *Cell*. 2005 Apr 22; 121(2):167–178. [PubMed: 15851025]

118. Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer cell*. 2011 Jul 12; 20(1):53–65. [PubMed: 21741596]
119. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood*. 2013 Aug 8; 122(6):1017–1025. [PubMed: 23801631]
120. Yu W, Chory EJ, Wernimont AK, Tempel W, Scopton A, Federation A, et al. Catalytic site remodelling of the DOT1L methyltransferase by selective inhibitors. *Nature communications*. 2012; 3:1288.
121. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012 Jul 20; 150(2):264–278. [PubMed: 22817890]
122. Geiman TM, Sankpal UT, Robertson AK, Chen Y, Mazumdar M, Heale JT, et al. Isolation and characterization of a novel DNA methyltransferase complex linking DNMT3B with components of the mitotic chromosome condensation machinery. *Nucleic acids research*. 2004; 32(9):2716–2729. [PubMed: 15148359]
123. Quesada V, Conde L, Villamor N, Ordonez GR, Jares P, Bassaganyas L, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nature genetics*. 2012 Jan; 44(1):47–52.
124. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *The New England journal of medicine*. 2011 Dec 29; 365(26):2497–2506. [PubMed: 22150006]
125. Mian SA, Smith AE, Kulasekararaj AG, Kizilers A, Mohamedali AM, Lea NC, et al. Spliceosome mutations exhibit specific associations with epigenetic modifiers and protooncogenes mutated in myelodysplastic syndrome. *Haematologica*. 2013 Jul; 98(7):1058–1066. [PubMed: 23300180]
126. Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nature genetics*. 2012 Nov; 44(11):1179–1181. [PubMed: 23001125]
127. Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, Quake SR, et al. Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Science translational medicine*. 2012 Aug 29.4(149):149ra118.
128. Boulwood J, Perry J, Pellagatti A, Fernandez-Mercado M, Fernandez-Santamaria C, Calasanz MJ, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. *Leukemia*. 2010 May; 24(5):1062–1065. [PubMed: 20182461]
129. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nature genetics*. 2010 Aug; 42(8):722–726. [PubMed: 20601953]
130. Grossmann V, Kohlmann A, Eder C, Haferlach C, Kern W, Cross NC, et al. Molecular profiling of chronic myelomonocytic leukemia reveals diverse mutations in >80% of patients with TET2 and EZH2 being of high prognostic relevance. *Leukemia*. 2011 May; 25(5):877–879. [PubMed: 21339759]
131. Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science*. 2013 May 3; 340(6132):626–630. [PubMed: 23558169]
132. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science*. 2013 May 3; 340(6132):622–626. [PubMed: 23558173]
133. Schenk T, Chen WC, Gollner S, Howell L, Jin L, Hebestreit K, et al. Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia. *Nature medicine*. 2012 Apr; 18(4):605–611.

134. Harris WJ, Huang X, Lynch JT, Spencer GJ, Hitchin JR, Li Y, et al. The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. *Cancer cell*. 2012 Apr 17; 21(4):473–487. [PubMed: 22464800]
135. Knutson SK, Wigle TJ, Warholc NM, Sneeringer CJ, Allain CJ, Klaus CR, et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nature chemical biology*. 2012 Nov; 8(11):890–896. [PubMed: 23023262]
136. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*. 2012 Dec 6; 492(7427):108–112. [PubMed: 23051747]
137. Kim W, Bird GH, Neff T, Guo G, Kerenyi MA, Walensky LD, et al. Targeted disruption of the EZH2-EED complex inhibits EZH2-dependent cancer. *Nature chemical biology*. 2013 Oct; 9(10):643–650. [PubMed: 23974116]
138. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature*. 2011 Oct 27; 478(7370):529–533. [PubMed: 21964340]
139. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature*. 2011 Oct 27; 478(7370):524–528. [PubMed: 21814200]
140. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, et al. Selective inhibition of BET bromodomains. *Nature*. 2010 Dec 23; 468(7327):1067–1073. [PubMed: 20871596]
141. Ott CJ, Kopp N, Bird L, Paranal RM, Qi J, Bowman T, et al. BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia. *Blood*. 2012 Oct 4; 120(14):2843–2852. [PubMed: 22904298]
142. Herold JM, Wigle TJ, Norris JL, Lam R, Korboukh VK, Gao C, et al. Small-molecule ligands of methyl-lysine binding proteins. *Journal of medicinal chemistry*. 2011 Apr 14; 54(7):2504–2511. [PubMed: 21417280]
143. Garcia-Manero G, Gore SD, Cogle C, Ward R, Shi T, Macbeth KJ, et al. Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011 Jun 20; 29(18):2521–2527. [PubMed: 21576646]
144. Negrotto S, Ng KP, Jankowska AM, Bodo J, Gopalan B, Guinta K, et al. CpG methylation patterns and decitabine treatment response in acute myeloid leukemia cells and normal hematopoietic precursors. *Leukemia*. 2012 Feb; 26(2):244–254. [PubMed: 21836612]
145. Tsai HC, Li H, Van Neste L, Cai Y, Robert C, Rassool FV, et al. Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. *Cancer cell*. 2012 Mar 20; 21(3):430–446. [PubMed: 22439938]
146. Shen L, Kantarjian H, Guo Y, Lin E, Shan J, Huang X, et al. DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010 Feb 1; 28(4):605–613. [PubMed: 20038729]
147. Fandy TE, Herman JG, Kerns P, Jiemjit A, Sugar EA, Choi SH, et al. Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. *Blood*. 2009 Sep 24; 114(13):2764–2773. [PubMed: 19546476]
148. Blum W, Garzon R, Klisovic RB, Schwind S, Walker A, Geyer S, et al. Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. *Proceedings of the National Academy of Sciences of the United States of America*. 2010 Apr 20; 107(16):7473–7478. [PubMed: 20368434]
149. Klcó JM, Spencer DH, Lamprecht TL, Sarkaria SM, Wylie T, Magrini V, et al. Genomic impact of transient low-dose decitabine treatment on primary AML cells. *Blood*. 2013 Feb 28; 121(9):1633–1643. [PubMed: 23297133]
150. Rao R, Fiskus W, Yang Y, Lee P, Joshi R, Fernandez P, et al. HDAC6 inhibition enhances 17-AAG-mediated abrogation of hsp90 chaperone function in human leukemia cells. *Blood*. 2008 Sep 1; 112(5):1886–1893. [PubMed: 18591380]

151. Yu C, Rahmani M, Conrad D, Subler M, Dent P, Grant S. The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce apoptosis in Bcr/Abl+ cells sensitive and resistant to STI571. *Blood*. 2003 Nov 15; 102(10):3765–3774. [PubMed: 12893773]
152. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell*. 2011 Sep 16; 146(6):904–917. [PubMed: 21889194]
153. Picaud S, Da Costa D, Thanasopoulou A, Filippakopoulos P, Fish PV, Philpott M, et al. PFI-1, a highly selective protein interaction inhibitor, targeting BET Bromodomains. *Cancer research*. 2013 Jun 1; 73(11):3336–3346. [PubMed: 23576556]
154. Rubnitz JE, Wichlan D, Devidas M, Shuster J, Linda SB, Kurtzberg J, et al. Prospective analysis of TEL gene rearrangements in childhood acute lymphoblastic leukemia: a Children's Oncology Group study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2008 May 1; 26(13):2186–2191. [PubMed: 18445843]
155. Bhojwani D, Pei D, Sandlund JT, Jeha S, Ribeiro RC, Rubnitz JE, et al. ETV6-RUNX1-positive childhood acute lymphoblastic leukemia: improved outcome with contemporary therapy. *Leukemia*. 2012 Feb; 26(2):265–270. [PubMed: 21869842]
156. Gang EJ, Hsieh YT, Pham J, Zhao Y, Nguyen C, Huantes S, et al. Small-molecule inhibition of CBP/catenin interactions eliminates drug-resistant clones in acute lymphoblastic leukemia. *Oncogene*. 2013 Jun 3.
157. Gao XN, Lin J, Ning QY, Gao L, Yao YS, Zhou JH, et al. A histone acetyltransferase p300 inhibitor C646 induces cell cycle arrest and apoptosis selectively in AML1-ETO-positive AML cells. *PloS one*. 2013; 8(2):e55481. [PubMed: 23390536]
158. Falk H, Connor T, Yang H, Loft KJ, Alcindor JL, Nikolakopoulos G, et al. An efficient high-throughput screening method for MYST family acetyltransferases, a new class of epigenetic drug targets. *Journal of biomolecular screening*. 2011 Dec; 16(10):1196–1205. [PubMed: 22086725]
159. He J, Nguyen AT, Zhang Y. KDM2b/JHDM1b, an H3K36me2-specific demethylase, is required for initiation and maintenance of acute myeloid leukemia. *Blood*. 2011 Apr 7; 117(14):3869–3880. [PubMed: 21310926]
160. Kruidenier L, Chung CW, Cheng Z, Liddle J, Che K, Joberty G, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature*. 2012 Aug 16; 488(7411):404–408. [PubMed: 22842901]
161. Sayegh J, Cao J, Zou MR, Morales A, Blair LP, Norcia M, et al. Identification of small molecule inhibitors of Jumonji AT-rich interactive domain 1B (JARID1B) histone demethylase by a sensitive high throughput screen. *The Journal of biological chemistry*. 2013 Mar 29; 288(13):9408–9417. [PubMed: 23408432]
162. Grembecka J, He S, Shi A, Purohit T, Muntean AG, Sorenson RJ, et al. Menin-MLL inhibitors reverse oncogenic activity of MLL fusion proteins in leukemia. *Nature chemical biology*. 2012 Mar; 8(3):277–284. [PubMed: 22286128]
163. Shi A, Murai MJ, He S, Lund G, Hartley T, Purohit T, et al. Structural insights into inhibition of the bivalent menin-MLL interaction by small molecules in leukemia. *Blood*. 2012 Nov 29; 120(23):4461–4469. [PubMed: 22936661]
164. Konze KD, Ma A, Li F, Barsyte-Lovejoy D, Parton T, Macnevin CJ, et al. An Orally Bioavailable Chemical Probe of the Lysine Methyltransferases EZH2 and EZH1. *ACS chemical biology*. 2013 Apr 24.
165. Vedadi M, Barsyte-Lovejoy D, Liu F, Rival-Gervier S, Allali-Hassani A, Labrie V, et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nature chemical biology*. 2011 Aug; 7(8):566–574. [PubMed: 21743462]
166. Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML, et al. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Molecular cell*. 2007 Feb 9; 25(3):473–481. [PubMed: 17289593]
167. Wan H, Huynh T, Pang S, Geng J, Vaccaro W, Poss MA, et al. Benzo[d]imidazole inhibitors of Coactivator Associated Arginine Methyltransferase 1 (CARM1)--Hit to Lead studies. *Bioorganic & medicinal chemistry letters*. 2009 Sep 1; 19(17):5063–5066. [PubMed: 19632837]

168. Sack JS, Thieffine S, Bandiera T, Fasolini M, Duke GJ, Jayaraman L, et al. Structural basis for CARM1 inhibition by indole and pyrazole inhibitors. *The Biochemical journal*. 2011 Jun 1; 436(2):331–339. [PubMed: 21410432]
169. Shia WJ, Okumura AJ, Yan M, Sarkeshik A, Lo MC, Matsuura S, et al. PRMT1 interacts with AML1-ETO to promote its transcriptional activation and progenitor cell proliferative potential. *Blood*. 2012 May 24; 119(21):4953–4962. [PubMed: 22498736]

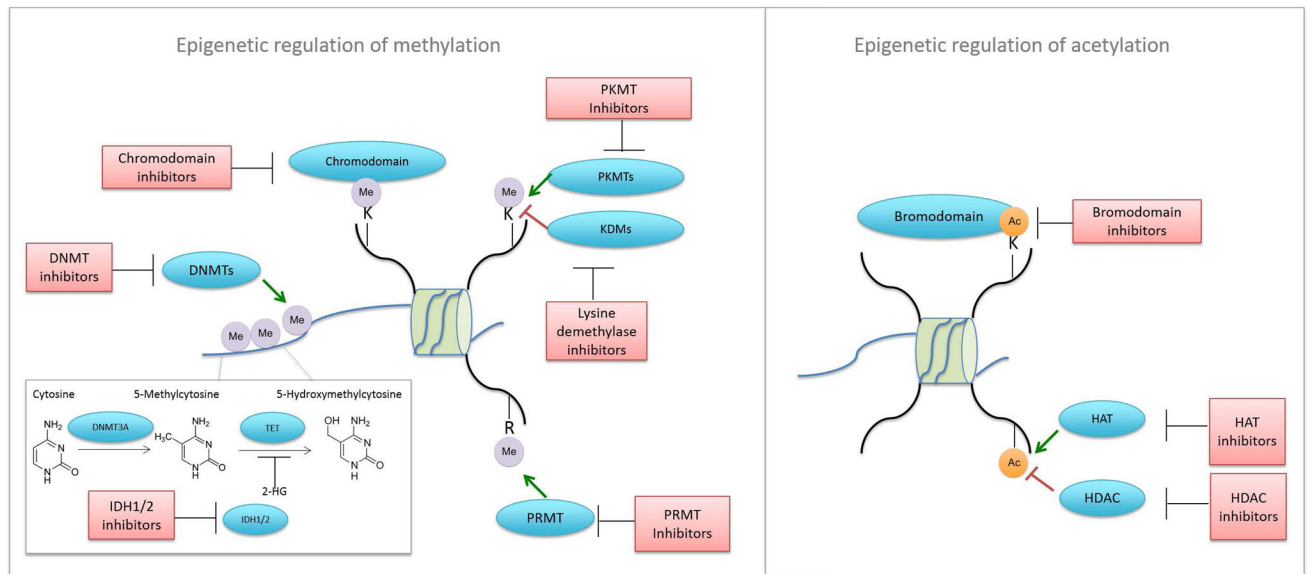


Figure 1. Regulation of methylation and acetylation in leukemia and their therapeutic potential

The figure shows a selection of proteins that add, remove, and recognize chromatin modifications, as well as the the proteins that regulate DNA methylation. The genes encoding these proteins can be altered through mutation, deletion or altered expression in leukemia. Me, methylation; Ac, acetylation. DNMT, DNA methyltransferase; PKMT, lysine methyltransferase; KDM, lysine demethylase; PRMT, arginine methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase

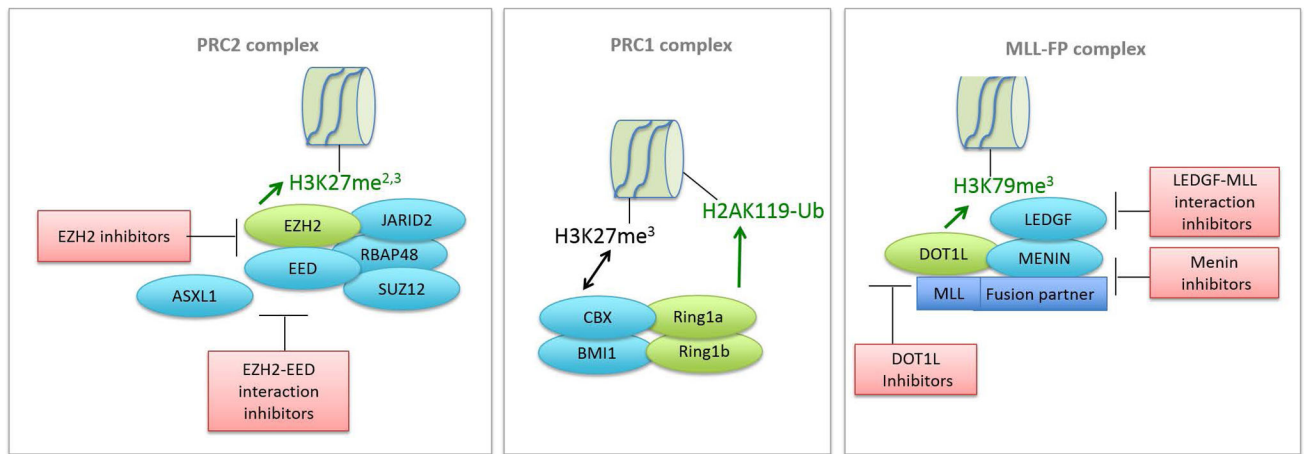


Figure 2. Polycomb repressive complexes and MLL-fusion complexes in leukemia and their therapeutic potential

The PcG protein complexes, known as PRC1 and PRC2, maintain transcriptional silencing. EZH2 contains the methyltransferase activity for PRC2 that catalyzes the di and tri methylation of H3K27. Recurrent deletions and sequence mutations in EZH2, SUZ12, and EED are found in T-ALL. ASXL1 mutations promote transformation by decreasing PRC2 recruitment, and contributing to loss of transcriptional repression. Another PRC2 interacting protein, JARID2, is involved in the recruitment of the complex to target loci and is deleted in the progression of chronic phase myeloid malignancies to acute leukemia. PRC1 complex recognizes H3K27me₃ via the chromodomain-containing CBX proteins and is involved in the maintenance of gene repression through histone H2A ubiquitination and the recruitment of DNA methyltransferases. PRC1 contains several proteins linked to cancer including Bmi-1, a protein associated with HSC self-renewal, and the ubiquitin ligases Ring1A and Ring1B. Several MLL fusion proteins can aberrantly recruit the DOT1L methyltransferase, leading to methylation of H3K79 and the activation of genes driving cellular transformation. MLL fusion proteins are also dependent on Menin, a component of the MLL-SET1-like histone methyltransferase complex and an adaptor to the chromatin associated protein LEDGF.

Table 1

Clinical significance of chromatin modifiers in leukemia

Mutation	Clinical association	Role in chromatin biology	References
TET2	Mutations found in 7–25% of AML. Mutations and copy number changes associated with worse prognosis in CN-AML.	Regulates demethylation through the conversion of 5-methylcytosine to 5-hydroxymethyl-cytosine. Mutations result in global DNA hypermethylation.	22, 26, 27, 29,33
IDH1/2	Missense mutations in the active site of the enzyme seen in 6–30% of adult AML. Adverse survival in CN-AML.	Altered enzyme activity leading to the accumulation of 2-HG; associated with aberrant locus-specific hypermethylation.	33–45
DNMT3A	Mutations occur in up to 36% of CN-AML and 16% of adult ETP-ALL. Associated with worse overall survival.	Enzyme involved in de novo DNA methylation. Mutations associated with gene expression changes in HoxB cluster.	13–20
ASXL1	Deletions and point mutations in 6–30% of AML patients.	Mutations result in decreased H3K27 methylation and decreased recruitment of PRC2 to target loci.	28, 53–55
EZH2	Mutations identified in 2% de novo AML.	Important H3K27 methyltransferase that is the enzymatic component of PRC2.	48–52
MLL-FP	MLL-rearrangement occurs in 5–10% of AML. MLL-rearrangements observed in 70% of infant ALL. Associated with poor prognosis.	Acquisition of H3K79 methyltransferase activity due to recruitment of Dot1L. Regulates HoxA cluster genes and Meis1 gene expression.	67–73
CBP	Mutations and deletions identified in 18% of relapsed ALL.	Transcriptional co-activator that has histone and non-histone protein acetyltransferase activity.	99–101
NUP98-NSD1	Detected in 5% of pediatric AML patients. Poor prognosis in children and adults.	Thought to upregulate HoxA cluster genes and Meis1 expression through recruitment of CBP/p300 and maintenance of H3K36 methylation.	82–84
ETV6-RUNX1	Translocations occur in 25% of B-precursor ALL. Associated with poor prognosis.	Associated with dominant negative RUNX1 and ETV6 functions, as well as aberrant DNA hypomethylation.	152,153

Table 2

Epigenetic targeted therapy in leukemia

Class of epigenetic target	Target of therapeutic	Mechanism and biological support	References
Histone acetyltransferases (HATs)	p300/CBP	Inhibits cellular proliferation, reduces colony formation, and induces apoptosis in AML1-ETO positive AML cell lines and primary blasts. Small-molecule inhibition of CBP/catenin interactions eliminate drug-resistant clones in ALL.	157,158
	MYST family (TIP60, MOZ)	Small molecule inhibition of histone acetyltransferase activity. Knockdown of Tip60 in a CML cell line results in loss of transcriptional repression at c-myc targets.	101–106
Readers of lysine acetylation	Bromodomain-containing proteins (BRD4)	Small molecule inhibitors targeting the acetyl-lysine binding pocket. Efficacy against MLL-fusion leukemia cell lines and mouse models through the induction of early cell cycle arrest and apoptosis. In Phase I clinical trials for patients with acute leukemia.	136–139
Histone lysine demethylases	LSD1 (KDM1A)	Disruption of histone demethylase activity. Inhibitors have been shown to induce differentiation in MLL-rearranged leukemias. Inhibitors may be efficacious when combined with ATRA in non-APL patients.	131,132
	JmjC-containing demethylases(UTX, JMJD3, JARID1, KDM2B)	Small molecular inhibitors are competitive for 2-oxoglutarate. A JMJD3/UTX inhibitor reduces proinflammatory cytokine production by macrophages. Depletion of Kdm2b in hematopoietic progenitors impairs Hoxa9/Meis1-induced leukemic transformation.	86, 158, 159
Metabolic modulators of methylation	IDH1/2	IDH1/2 inhibitors decrease the production of 2-HG, induce demethylation of histone H3K9me3, and increase expression of genes associated with differentiation.	40–45
Histone methyltransferases and associated proteins	Menin/LEDGF	Small molecule inhibitors that target the Menin-MLL interaction developed for MLL rearranged leukemias. Inhibitor induces growth arrest and inhibits transformation in MLL transduced bone marrow cells. Small peptides disrupting the LEDGF-MLL interaction show increase disease latency in an MLL-AF9 leukemia model.	77–81
	Dot1L	Selective for MLL rearranged acute leukemia cell lines. Inhibited H3K79 methylation and MLL-fusion target gene expression.	74–76, 115–118

Class of epigenetic target	Target of therapeutic	Mechanism and biological support	References
	EZH2	Small molecules which disrupt the methyltransferase activity of PRC2. Peptides have been developed which disrupt the EZH2-EED protein interactions. MLL-AF9 leukemia cells treated with inhibitor undergo growth arrest, and myeloid differentiation.	133–135
	G9a	Small molecular inhibitors targeting the histone peptide binding pocket G9a inhibition resulted in repression of JAK2 in a CML cell line.	160,161
	Arginine methyltransferases (PRMTs)	Knockdown of PRMT1 suppresses the self-renewal capability of AE9a cells. Down-regulation of PRMT4 promotes myeloid differentiation in leukemia cells and prolongs survival in a leukemia transplantation model.	162–164

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript