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EPIGENETIC THERAPY IN ACUTE MYELOID LEUKEMIA: CURRENT AND FUTURE DIRECTIONS

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Abstract

Epigenetic modifications affect gene expression without changes in the actual DNA sequence. Two most important mechanisms include DNA methylation and histone tail modifications (especially acetylation and methylation). Epigenetic modulation is a part of normal physiologic development; its dysregulation is an important mechanism of pathogenesis of some cancers including acute myeloid leukemia (AML). Despite significant progress in understanding the pathogenesis of AML, therapeutic options remain quite limited. Technological advances have facilitated understanding of aberrant DNA methylation and histone methylation/acetylation as key elements in the development of AML and uncovered several recurrent mutations in genes important for epigenetic regulation. However, much remains to be learned about how to exploit this knowledge for epigenetic therapeutic targeting. Currently, no epigenetic therapy is approved for the treatment of AML although two DNA methyltransferase inhibitors (azacitidine and decitabine) are commonly used in clinical practice. Among the other epigenetic modifiers undergoing research in AML, the histone deacetylase inhibitors are the most studied. Other promising drugs such as inhibitors of histone methylation (e.g. EZH2 and DOT1L inhibitors), inhibitors of histone demethylases (e.g. LSD1 inhibitors), inhibitors of bromodomain-containing epigenetic “reader” BET proteins, and inhibitors of mutant isocitrate dehydrogenases are at early stages of clinical evaluation.

Keywords

epigenetics; acute myeloid leukemia; DNA methylation; histones

INTRODUCTION

In AML, primitive malignant clonal hematopoietic cells proliferate and accumulate in bone marrow, peripheral blood and occasionally other tissues[1]. AML accounts for 90% of all acute leukemia in adults, and 15–20% in children[2]. Despite extensive clinical research of many agents and exponential progress in dissecting the underlying molecular mechanisms of pathogenesis, and in contrast to most other hematologic malignancies where we have seen

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the approval of multiple novel agents, the backbone of induction chemotherapy for AML remains the same anthracycline/cytarabine combination regimen (3+7) developed in the 1970s[3].

The etiologies for AML have not been fully elucidated. Risk factors include previous exposure to chemotherapy and radiation. Myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) can also progress to AML. Along with many genetic alterations resulting in activation of oncogenes or inactivation of tumor suppressor genes (TSG)[4], epigenetic changes have been shown to play an important role in carcinogenesis. Epigenetic modification refers to a stable, mitotically perpetuated regulatory mechanism of gene expression that is not associated with a change in the actual DNA sequence[5]. Main mechanisms of epigenetic modifications include DNA methylation, histone tail modifications such as acetylation and methylation[6, 7] and changes in the small regulatory non-coding anti-sense RNAs (i.e. microRNA) which can result in transcriptional or posttranscriptional gene silencing or activation[8]. However, the epigenetic dysregulation that contributes to leukemogenesis is not fully understood[9]. A better understanding of epigenetic dysregulation will shed light on the mechanism of leukemogenesis, provide prognostic information, and elucidate potential therapeutic targets. In this review, we overview the current understanding of the epigenetic dysregulation in AML, discuss the available epigenetic therapies, and forecast the future directions of research in this area. Due to space limitations, we will focus on DNA methylation and histone modifications and their therapeutic targeting while inhibitory RNAs and the use of antisense oligonucleotides will not be discussed.

DNA METHYLATION IN AML

DNA methylation involves the addition of a methyl group to cytosine or adenine residues in DNA resulting in impaired gene transcription and translation. As such, DNA methylation provides an epigenetic method for controlling gene expression. This process is an essential component of normal development and cell differentiation. Certain cancer cells have altered patterns of DNA methylation via the global change of DNA methylation, or hypermethylation in specific regions of the genome[10]. While global hypomethylation is associated with chromosomal instability *in vitro* and may play a role in carcinogenesis[11], it is believed that aberrant DNA hypermethylation contributes to carcinogenesis by silencing TSG.

In contrast to normal hematopoietic cells, hypermethylation of TSG promoters is found at a high frequency in AML[12]. However, only a few of the frequently methylated genes in AML have known tumor suppressor function[13]. In addition, promoter hypermethylation in AML *in vitro* does not always silence critical genes, especially TSG *in vivo*[13, 14]. Therefore, only some of the genes which are hypermethylated in promoters are clearly significant to the neoplastic process. Regardless of the role of hypermethylation of TSG in the pathogenesis of AML, frequent gene methylation is generally associated with poor prognosis in AML[13] and clinical outcomes could be predicted based on DNA methylation cluster, including the groups without specific mutation[14].

MUTATIONS IN GENES WHICH INFLUENCE DNA METHYLATION

DNMT mutations in AML

DNA methylation is induced by a group of epigenetic "writer" enzymes called DNA methyltransferases (DNMTs) that add a methyl group to the cytosine residues, mostly within CpG islands, leading to epigenetic silencing of target genes[15, 16]. Of the 3 described DNMTs, DNMT1 mostly maintains existing methylation during replication of the DNA while DNMT3A and DNMT3B on the other hand are generally responsible for *de novo* methylation of previously unmethylated DNA[17].

The frequent occurrence of recurrent mutations in enzymes associated with DNA methylation in AML cells suggests that aberrant epigenetic modulation of genome plays an important role in leukemogenesis. *DNMT3A* is one of the most commonly mutated genes in AML (4% to 22% of adult AML patients and up to 36% of cytogenetically normal [CN] AML)[17]. *DNMT3A* mutations are associated with monocytic subtype, older age, and concurrent mutations including *FLT3, NPM1*, and*isocitrate dehydrogenase (IDH)1* and*2*[18, 19]. Interestingly, AML patients with mutations in *DNMT3A* (along with *NPM1* or fusions in *MLL1*) appear to benefit from dose intensification of daunorubicin (90mg/m^2) during induction chemotherapy[20].

TET2

TET (*Ten-Eleven Translocation*) 1~3 genes encode for epigenetic "eraser" enzymes that induce hydroxymethylation by catalyzing the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, which is followed by passive demethylation[16, 21]. Mutations in *TET2* have been detected in 7–23% of AML[22, 23]. Recent study suggests that *TET2* mutations occur more frequently in CN-AML and are associated with older age, higher white blood cell counts, and lower platelet counts[16, 23]. In addition, *TET2* and *IDH* mutations are mutually exclusive, which supports the role of aberrant hydroxymethylation in leukemogenesis as *IDH* gain of function mutations produce the oncometabolite 2-hydroxyglutarate (2-HG) which inhibits TET2 catalytic activity (see next section)[16, 24]. Experimental models suggest that *TET2* mutations result in a dysregulation of hematopoietic cell-renewal control mechanisms and facilitate the acquisition of additional somatic mutations[24–26]. Nonetheless, the precise mechanism by which *TET2* mutations drive leukemogenesis are still to be defined.

MUTATIONS IN GENES THAT IMPACT BOTH DNA METHYLATION AND HISTONE POSTTRANSLATIONAL MODIFICATIONS

IDH1 and 2

IDH 1 and 2 are a group of $NADP^+$ dependent enzymes which catalyze the conversion of isocitrate to α-ketoglutarate (α-KG) in the Krebs cycle and are thought to be involved in the prevention of oxidative damage within the cell[16, 24, 27]. *IDH* mutations are found in 15~30% of *de novo* AML and secondary AML[24, 28]. These neomorphic mutations confer a novel activity to the enzymes catalyzing the conversion of α-KG to its D stereoisomer 2-HG[24, 27–29]. Indeed, high2-HGlevels are detectable in AML patients who have IDH

mutations, and serum levels of 2-HG have been shown to have a diagnostic utility, to function as a biomarker for monitoring of disease activity and therapeutic response, and to potentially predict clinical outcomes[27–29]. The mechanisms by which mutating *IDH 1* and *2* drive pathogenesis of AML are under active investigation but it appears that the functional loss of TET2 activity by the depleted level of the TET2 cofactor α-KG and the inhibitory effects of 2-HG on TET2 function play important roles[16, 24]. *IDH* mutations also contribute to leukemogenesis in TET2-independent manners[16]. The oncometabolite 2-HG can also inhibit several other key epigenetic modifiers involved in histone and DNA methylation and demethylation leading to aberrant DNA and histone methylation and epigenetic remodeling[30]. The activity of histone demethylases are also dependent on α-KG and the epigenetic functional impact of inhibition of histone demethylase by low levels of α-KG may contribute to leukemogenesis by blocking differentiation[30, 31]. In addition, it is thought that high levels of α-HG may increase the production of reactive oxygen species and subsequent DNA damage[31]. The prognostic significance of *IDH* mutations remains debated.

MUTATIONS IN GENES WHICH AFFECT HISTONE POSTTRANSLATIONAL MODIFICATIONS

ASXL-1

Somatic nonsense, missense, frameshift and point mutations of the *additional sex combs-like gene* (*ASXL-1*), one of the Polycomb group (PcG) of proteins are found in 3 to 30% AML[9, 16, 32]. *ASXL-1* mutations were initially detected in MDS and chronic myelomonocytic leukemia (CMML) but were subsequently identified in AML[32–37]. These mutations are more prevalent in older AML patients (vs. younger patients) and those with therapy-related AML (vs.*de novo* AML)[32–34]. *ASXL-1* is important for PRC2-mediated trimethylation of histone 3 lysine 27 (H3K27); an epigenetic modification with a transcriptionally repressive effect. Inactivating mutations of *ASXL-1* therefore lead to H3K27me3level reductions at critical sites and potentially promote leukemogensis by de-repressing important leukemogenic oncogenes[16, 34]. Mutations in*ASXL-1* are also reported to be associated with interruption of ubiquitin removal from specific histone lysine residues[35] and promoting *HOX* gene expression[34] which are important in leukemic transformation. However, the mechanisms underlying leukemogenesis in patients with *ASXL-1* mutations remain to be full identified. The prognostic implications of *ASXL-1* mutations in AML have been elusive due to several conflicting data. Still, it is generally accepted that *ASXL-1* mutation is an adverse prognostic indicator in intermediate-risk AML[32, 36, 37].

EPIGENETIC THERAPY

DNMT Inhibitors

5-Azacytidine (azacitidine) and its deoxy analogue 5-aza-2'-deoxycytidine (decitabine) are the two most extensively studied DNMT inhibitors and are approved for clinical use in hematologic malignancies (specifically MDS) in the USA. These medications were first invented in 1960s, but were clinically abandoned due to excessive toxicity and narrow therapeutic window[38]. Subsequently it was realized that dose-response curve of azacitidine

is U-shape suggesting that a much lower dose of the drug than initially used could be clinically effective and safe[39].

Despite being recognized for several decades and being commercially available for a decade, the mechanisms of action of both approved DNMT inhibitors remain to be fully elucidated. Azacitidine is metabolized to decitabine and the tri-phosphorylated product (i.e. decitabine triphosphate) is incorporated into DNA and subsequently binds to DNMT covalently at the C-6 position[40, 41]. The incorporation of active metabolites results in cytotoxicitic effect at high concentrations while at lower concentrations the predominant effect appears to be depletion of DNMTs with subsequent loss of DNA methylation following DNA replication and therapeutic epigenetic modulation[40–44]. In contrast to decitabine which has no direct effects on RNA, most of the phosphorylated azacitidine is actually incorporated into the RNA thereby interfering with protein synthesis[40, 43]. In addition, these drugs appear to have immune-mediated effects[45, 46]. The degree to which the beneficial clinical effects of DNMT inhibitors are dependent on any of these (or other) mechanisms and whether differential molecular mechanisms of azacitidine versus decitabine (e.g. direct impact on protein synthesis) affect the final clinical effects are yet to be established. Recently, the use of decitabine with 75 to 90% dose reduction in patients with MDS with a more frequent administration (1 to 3 days/week instead of 5 days every 28 days schedule) showed that DNMT depletion effect was predominant with minimial direct cytotoxic effect via conventional apoptotic pathways[44]. These data suggest that lower dose of decitabine (or possibly azacitidine) might be effective in MDS or AML with mutation of key apoptotic genes including *TP53* and that different mechanisms of DNMT inhibitors depending on doses, administration schedule and their clinical applicability should be further investigated[44].

Table 1 summarizes the results of some of the important clinical trials of DNMT inhibitors in AML patients. A subgroup analysis of the AZA-001, a trial that compared azacitidine to conventional care regimens (CCR) in patients with higher-risk MDS, in patients with 20–30% bone marrow blasts who are currently classified as having AML according to the World Health Organization (WHO) classification demonstrated a significantly better median overall survival (OS, 24.5 vs. 16.0 months, respectively, hazard ratio=0.47; 95% CI, 0.28 to 0.79; p=0.005) and probability of OS at 2-year OS rate (50% vs 16%, respectively, p=0.001)[47]. Another subgroup analysis of patients with 20–30% bone marrow blasts (reclassified by WHO criteria) in the Cancer and Leukemia Group B (CALGB) randomized trial that led to FDA approval of azacitidine in MDS demonstrated a significant survival advantage of azacitidine as well[48]. Subsequent two phase II trials of decitabine in AML demonstrated similar response rates (25% and 26%, respectively)[49, 50]. A phase III randomized study of first-line decitabine therapy in elderly patients with AML (>65 years old at time of diagnosis) with intermediate or poor cytogenetics showed that while decitabine significantly increased CR rate compared to best supportive care (BSC) or low-dose cytarabine (17.8% vs. 7.8%, odds ratio, 2.5; 95% CI, 1.4 to 4.8; p=0.001), the difference in median OS was not statistically significant between the two groups (7.7 months vs 5.0 months, p=0.108)[51].

HDAC Inhibitors

Histones play a role in forming nucleosomes and chromatins with DNA in eukaryotic cells[52]. Acetylation and deacetylation in histone tails by histone acetyltransferases (HAT) and histone deacetylases (HDAC) is one of the important epigenetic mechanisms of control of gene transcription[40]. Several clinical trials have been conducted with histone deacetylase (HDAC) inhibitors in patients with MDS and AML (Table 2). In general, single agent HDAC inhibitor therapy has been associated with low response rates, usually in the range of 10–20%[52]. Based on *in vitro* studies, revealing HDAC inhibitors and DNMT inhibitors act synergistically to induce re-expression of silenced genes in cancer cells[53], clinical trials have been conducted to use HDAC inhibitors in combination with other agents, such as DNMT inhibitors and conventional chemotherapeutic agents[52].

Panobinostat in an HDAC inhibitor under investigation especially for core-binding factor (CBF)-AML. CBF-AML and APL are characterized by chimeric proteins that aberrantly recruit HDAC to target gene promoters leading to differentiation block and blast proliferation[54]. In a murine model of t(8;21) AML, panobinostat resulted in a significant anti-leukemic effect and triggered terminal myeloid differentiation suggesting the need for further studies of HDAC inhibitors in this setting[55].

Vorinostat (Suberoylanilide hydroxamic acid, SAHA), another potent HDAC inhibitor containing a hydroxamic acid moiety, binds to the zinc-containing pocket in the catalytic site of HDAC 1, 2, 3 and 6, causing their reversible inhibition. In a phase I trial of oral vorinostat for hematologic malignancies, responses were observed in leukemia including AML (7 of 41 patients,17%), including 2 CR, 2 CRs with incomplete hematologic recovery, and 3 with hematologic improvement[56]. In another study, 37 patients with AML were treated with vorinostat in a phase II trial, but only one patient had a hematologic improvement[57].

Romidepsin (depsipeptide) is a cyclic tetrapeptide that is a potent HDAC inhibitor *in vitro*. In a multicenter phase II trial, romidepsin was administrated to 20 patients with refractory or relapsed AML[58]. Two patients had disappearance of bone marrow blasts with concomitant recovery of near-normal hematopoiesis following one or two cycles of therapy, and three additional achieved a >50% reduction in bone marrow blasts[58].

Valproic acid (VPA) is a short-chain fatty acid oral antiepileptic agent that has been shown to inhibit HDAC activity at low levels. In a phase II study of single agent VPA or in combination with all-*trans* retinoic acid (ATRA), 75 patients with MDS or AML (n=32) were treated with VPA, including nine patients who also received ATRA from the start of treatment[59]. Hematologic improvement was observed in 24% of patients (30% in MDS, 16% in AML). Other studies using VPA either as a single agent or in combination with ATRA as therapy of poor-risk AML and MDS have also reported minimal activity[60–62]. Therefore, it seems that the activity of single-agent VPA in AML and MDS is limited.

Mocetinostat, entinostat and panobinostat have been studied mostly in phase I studies[63–65]. In summary, single-agent HDAC inhibitor therapy results in modest responses in AML at the best. In order to improve outcomes, studies have been conducted using HDAC inhibitors in

combination with other agents such as DNMT inhibitors and conventional chemotherapeutic agents (See the next section).

Combinations of DNMT inhibitors and HDAC inhibitors

Elucidation of multiple interacting mechanisms of gene silencing has led to combining drugs that affect multiple epigenetic pathways (Table 3). A number of clinical trials have tested the combination of DNMT inhibitors with HDAC inhibitors (i.e. VPA, vorinostat, entinostat and mocetinostat etc.) in both MDS and AML. Since Gore *et al.* have developed a well-tolerated combination schedule of azacitidine and HDAC inhibitor which induces promoter methylation reversal and global histone acetylation[66], multiple studies demonstrated that the combination of DNMT inhibitors with HDAC inhibitors confers a synergistic effect but most of these studies are in phase I or II[63, 67–69]. DNMT inhibitors have been combined with non-epigenetic agents including gemtuzumab ozogamicin[70], and sorafenib[71]. Combination of DNMT inhibitors with other agents such as erythropoietin[72], romiplostim[73], arsenic[74], and lenalidomide[75] were investigated mostly in MDS. The combination of bortezomib with decitabine seems to be interesting as it showed clinical activity in a limited number of older patients with AML[76].

A phase II study suggested the combination of vorinostat with idarubicin and cytarabine is viable in AML, but phase III study will be necessary to confirm these results[77]. A recently published randomized phase II trial in 149 patients with high-risk MDS and AML showed that the addition of VPA to decitabine did not improve CR rates, ORR, or survival in comparison to decitabine alone[78]. The E1905 study in which 149 patients with MDS (97 patients) and AML with trilineage dysplasia (52 patients) were randomized to azacitidine or a combination of azacitidine with the HDAC inhibitor, entinostat did not only show lack of significant differences in responses and survival between the two groups, but also observed lower degree of demethylation with the combination versus azacitidine monotherapy suggesting pharmacodynamic antagonism[79]. These findings suggest that the choice of the individual agents, their target specificity, the administration schedules, and the particular doses are all likely to significantly impact the outcome of combination studies. Further studies will be needed to determine whether there is a synergistic or additive clinical effects to combining DNMT inhibitors with HDAC inhibitors.

Novel DNMT inhibitors

The high frequency of somatic alterations in epigenetic modifiers in AML patients, combined with the clinical importance of DNMT inhibitors in AML, has led to a great interest in the development of novel epigenetic therapies. Next generation DNMT inhibitors have been developed. For example, SGI-110 is a novel DNMT inhibitor that was designed to enhance the efficacy of decitabine by combining it with deoxyguanosine which induces resistance to degradation by cytidine deaminase therefore increasing the bioavailability of the drug[52, 80]. A phase II of SGI-110 in patients with relapsed and refractory AML or elderly treatment-naïve AML show that overall remission rate in treatment naïve elderly AML was 53%; a rate which is comparable to that using conventional DNMT inhibitors[81].

Novel epigenetic modifiers

Several classes of novel epigenetic modifiers are in early phase clinical trials (Figure 1). In addition to methylation of DNA, methylation of histones is another important form of epigenetic modulation that controls critical cellular functions and whose aberrant regulation has been shown to contribute to leukemogenesis especially in mixed lineage leukemia (MLL)-fusion leukemias (which account for approximately 10% of adult AML). The H3K27 methyltransferase Enhancer of Zeste Homolog 2 (EZH2), the main component in the polycomb repressive complex 2 (PRC2) which tri-methylates H3K27 to H3K27me3 and recruits PRC1 to promoters of target genes, and the H3K79 histone methyltransferase Disruptor of Telomere Silencing 1-like (DOT1L), which di-methylates H3K79 to H3K79me2, both contribute to important cellular functions including maintenance of stemness of cells[82]. Mutations of EZH2 have been observed in various hematologic malignancies including MLL-rearranged leukemias and Inhibition of EZH2 using selective inhibitors such as 3-deazaneplanocin A (DZNep) has shown promising efficacy in preclinical studies in MLL-fusion leukemias[82, 83]. Importantly, in murine models of MLL-rearranged leukemias not only did DZNep suppress leukemia proliferation, but it also reduced leukemia-initiating cells [LIC] frequency through up-regulation of p16[82]. Double inhibition of EZH2 and EZH1 with an oral selective inhibitor, UNC1999, prolonged survival in a MLL-rearranged murine model further suggesting that inhibition of EZH2 (and EZH1) is a promising therapeutic intervention in MLL-rearranged leukemias[84]. Furthermore, combined epigenetic therapy using a combination of an EZH2 inhibitor and a HDAC inhibitor (DZNep and panobinostat) and even a triple combination (DZNep, the HDAC inhibitor trichostatin-A and the DNMT inhibitor decitabine) was effective and synergistic in preclinical studies of AML suggesting that triple targeting of the 3 epigenetic mechanisms that silence TSG merits further evaluation in AML[85, 86].

Misguiding of DOT1L with subsequent aberrant H3K79 methylation and transcriptional up-regulation of *HoxA* and *Meis1* genes are important for the initiation and maintenance of MLL-fusion leukemias while the loss of DOT1L function selectively reduces expression of the MLL-fusion driven transcriptional programs; therefore identifying DOT1L as a rational therapeutic target in MLL-rearranged leukemias[87, 88]. Indeed, a potent and selective aminonucleoside inhibitor of DOT1L, EPZ-5676, caused complete and sustained regression of the tumor in a rat xenograft model of MLL-fusion leukemia[89] and is currently undergoing phase 1 clinical trials in relapsed/refractory adult and pediatric MLL-rearranged leukemias (Clinicaltrials.gov identifier: NCT02141828 and NCT01684150). Studies in MLL-rearranged leukemia cell lines showed synergistic effects when EPZ-5676 was combined with DNMT inhibitors, cytarabine or daunorubicin, providing a rationale for clinical evaluation of these combinations[90]. Furthermore, inhibition of DOT1L with another small molecular inhibitor, SYC-522, was shown to increase chemo-sensitivity of MLL-rearranged leukemia by preventing DNA damage response[91]. Recent data suggest activity of DOT1L inhibitors might extend to other subtypes of AML aside from MLL-rearranged leukemias. For example, EPZ004777, a DOT1L inhibitor revealed *in vitro* responses in primary AML cells with IDH1 or IDH2 mutations, therefore warranting further evaluation of these agents in other subtypes of AML[92].

The bromodomain and extra terminal (BET) family of proteins include a number of Bromodomain-containing “reader” proteins (BRD [BRD2, BRD3, BRD4, and BRDT]) which use their acetyl-lysine recognition motifs (bromodomains) to read the post-translational acetylated motifs of histones and influence transcription of target genes[93, 94]. Dysregulation of BET adaptors has been shown to contribute to leukemogenesis across a variety of AML subtypes driven by different mutations[95]. Dislocation of BRD3 or BRD4 in AML (for instance, dissociation of BRD4 from mutant NPM1) enhances the expression of oncogenes, such as *c-Myc, Bcl-2* and *CDK6*, by recruiting super elongation complexes[96]. Inhibition of BRD4 using small hairpin inhibitory RNAs (shRNAs) or small molecule inhibitors (e.g. JQ1 and I-BET151) induces robust *in vivo* and *in vitro* anti-leukemic effects via down-regulation of oncogenes, suggesting this approach could be a potentially effective therapeutic strategy in some subtypes of AML[94, 95, 97–99]. Preclinical studies of the selective small molecule BRD4 inhibitors JQ1 and I-BET151 showed terminal myeloid differentiation and elimination of leukemia stem cells in MLL-rearranged AML, synergistic activity with HDAC inhibitors and anthracyclines to enhance p53-mediated apoptosis in *DNMT3A*/*NPM1*-mutated leukemic cell lines, and activity against NPM1-mutated AML and JAK2V617F-driven neoplasms[94, 95, 98, 99]. BET inhibitors (e.g. CPI-0610 and OTX015) have entered phase 1 clinical trials for refractory acute leukemias and MDS (NCT02158858 and NCT01713582). Lysine-specific demethylase1 (LSD1) is an epigenetic eraser that removes methyl groups from H3K4me1/2 and H3K9me1/2 in a location- and context-specific fashion acting as a transcriptional repressor or activator[100]. Data suggest that LSD1 is involved in leukemogenesis and maintenance of leukemic stem cells especially in MLL-rearranged leukemias[101]. Preclinical data suggest that inhibitors of LSD1 have activity in AML[102] and some are already in phase 1 trials (e.g.NCT02177812 evaluating the LSD1 inhibitor GSK2879552).

Finally, inhibitors of mutant IDH1 have also demonstrated preclinical evidence of efficacy in IDH1-mutated AML[103]. There are ongoing phase 1 clinical trials of mutant IDH1inhibitors (e.g. AG-120 in NCT02074839) and mutant IDH2 inhibitors (e.g. AG-221 in NCT01915498) in advanced hematologic malignancies with these respective IDH mutations. In addition, given that IDH-mutated AML are addicted to glutamine as the main source of α-KG[104, 105], selective glutamine depletion is being explored clinically as a potential therapeutic approach in IDH-mutated AML using Erwinase (Asparaginase Erwinia Chrysanthemi; NCT02283190).

CONCLUSIONS

Recent DNA sequencing and other techniques have facilitated understanding of the role of epigenetics in leukemogenesis, especially in AML. Aberrant DNA methylation (hypermethylation in promoters of TSG and hypomethylation in promoters of oncogenes) and aberrant histone acetylation and methylation are widely seen in AML. Mutations in genes important in the regulation of the epigenome, such as *TET2, DNMT3A, ASXL-1, IDH1/2*, and *EZH2*, have also provided insights into the process of leukemogenesis in AML and suggested opportunities for therapeutic targeting. These mutations also contribute to prognostication and risk stratification in AML.

Currently, DNMT inhibitors which are approved for treatment of AMDS are widely used as an off-label treatment option for AML patients who are not candidates for conventional intensive chemotherapy. HDAC inhibitors are among the better-studied epigenetic modifiers but are yet to improve outcomes in AML. Several other promising epigenetic modifiers are undergoing early phase clinical research in AML including inhibitors of histone methylation (e.g. EZH2 and DOT1L inhibitors), inhibitors of histone demethylases (e.g. LSD1 inhibitors), inhibitors of bromodomain-containing epigenetic "reader" BET proteins, and inhibitors of mutant IDH. In addition, combination-based approaches of different epigenetic modifiers are under active investigation. While epigenetic therapy for AML is still in its infancy, it is fair to say the future is likely to see a significant increase in the use of these agents as single agents, in combinations with each other, or with conventional chemotherapy.

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REFERENCES

1. Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet. 2013; 381:484–495. [PubMed: 23399072]
2. O'Donnell MR, Abboud CN, Altman J, Appelbaum FR, Arber DA, Attar E, et al. Acute myeloid leukemia. Journal of the National Comprehensive Cancer Network : JNCCN. 2012; 10:984–1021. [PubMed: 22878824]
3. Yates JW, Wallace HJ Jr, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. Cancer chemotherapy reports Part 1. 1973; 57:485–488. [PubMed: 4586956]
4. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]
5. Bird A. DNA methylation patterns and epigenetic memory. Genes & development. 2002; 16:6–21. [PubMed: 11782440]
6. Jenuwein T, Allis CD. Translating the histone code. Science. 2001; 293:1074–1080. [PubMed: 11498575]
7. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000; 403:41–45. [PubMed: 10638745]
8. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–297. [PubMed: 14744438]
9. Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. Nature reviews Cancer. 2012; 12:599–612. [PubMed: 22898539]
10. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nature reviews Genetics. 2002; 3:415–428.
11. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature. 1998; 395:89–93. [PubMed: 9738504]
12. Melki JR, Vincent PC, Clark SJ. Cancer-specific region of hypermethylation identified within the HIC1 putative tumour suppressor gene in acute myeloid leukaemia. Leukemia. 1999; 13:877–883. [PubMed: 10360376]
13. Toyota M, Kopecky KJ, Toyota MO, Jair KW, Willman CL, Issa JP. Methylation profiling in acute myeloid leukemia. Blood. 2001; 97:2823–2829. [PubMed: 11313277]
14. 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; 17:13–27. [PubMed: 20060365]
15. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. The New England journal of medicine. 2003; 349:2042–2054. [PubMed: 14627790]

16. Conway O'Brien E, Prideaux S, Chevassut T. The epigenetic landscape of acute myeloid leukemia. Advances in hematology. 2014; 2014:103175. [PubMed: 24778653]
17. 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; 30:742–750. [PubMed: 22291079]
18. 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; 363:2424–2433. [PubMed: 21067377]
19. 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; 29:2889–2896. [PubMed: 21670448]
20. 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; 366:1079–1089. [PubMed: 22417203]
21. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, Wadleigh M, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood. 2009; 114:144–147. [PubMed: 19420352]
22. 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; 29:1373–1381. [PubMed: 21343549]
23. Weissmann S, Alpermann T, Grossmann V, Kowarsch A, Nadarajah N, Eder C, et al. Landscape of TET2 mutations in acute myeloid leukemia. Leukemia. 2012; 26:934–942. [PubMed: 22116554]
24. 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; 18:553–567. [PubMed: 21130701]
25. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer cell. 2011; 20:11–24. [PubMed: 21723200]
26. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature. 2010; 468:839–843. [PubMed: 21057493]
27. Fathi AT, Sadrzadeh H, Borger DR, Ballen KK, Amrein PC, Attar EC, et al. Prospective serial evaluation of 2-hydroxyglutarate, during treatment of newly diagnosed acute myeloid leukemia, to assess disease activity and therapeutic response. Blood. 2012; 120:4649–4652. [PubMed: 23074281]
28. 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. 2014; 32:297–305. [PubMed: 24344214]
29. DiNardo CD, Propert KJ, Loren AW, Paietta E, Sun Z, Levine RL, et al. Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia. Blood. 2013; 121:4917–4924. [PubMed: 23641016]
30. 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; 483:474–478. [PubMed: 22343901]
31. Rakheja D, Konoplev S, Medeiros LJ, Chen W. IDH mutations in acute myeloid leukemia. Human pathology. 2012; 43:1541–1551. [PubMed: 22917530]
32. Pratcorona M, Abbas S, Sanders MA, Koenders JE, Kavelaars FG, Erpelinck-Verschueren CA, et al. Acquired mutations in ASXL1 in acute myeloid leukemia: prevalence and prognostic value. Haematologica. 2012; 97:388–392. [PubMed: 22058207]

33. Schnittger S, Eder C, Jeromin S, Alpermann T, Fasan A, Grossmann V, et al. ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. Leukemia. 2013; 27:82–91. [PubMed: 23018865]
34. 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; 22:180–193. [PubMed: 22897849]
35. Gelsi-Boyer V, Brecqueville M, Devillier R, Murati A, Mozziconacci MJ, Birnbaum D. Mutations in ASXL1 are associated with poor prognosis across the spectrum of malignant myeloid diseases. Journal of hematology & oncology. 2012; 5:12. [PubMed: 22436456]
36. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrozek K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 2011; 118:6920–6929. [PubMed: 22031865]
37. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011; 118:5593–5603. [PubMed: 21881046]
38. Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. Cell. 1980; 20:85–93. [PubMed: 6156004]
39. Issa JP, Garcia-Manero G, Giles FJ, Mannari R, Thomas D, Faderl S, et al. Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies. Blood. 2004; 103:1635–1640. [PubMed: 14604977]
40. Oki Y, Issa JP. Epigenetic mechanisms in AML - a target for therapy. Cancer treatment and research. 2010; 145:19–40. [PubMed: 20306243]
41. Jackson-Grusby L, Laird PW, Magge SN, Moeller BJ, Jaenisch R. Mutagenicity of 5-aza-2'-deoxycytidine is mediated by the mammalian DNA methyltransferase. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94:4681–4685. [PubMed: 9114051]
42. Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nature biotechnology. 2010; 28:1069–1078.
43. Qin T, Jelinek J, Si J, Shu J, Issa JP. Mechanisms of resistance to 5-aza-2'-deoxycytidine in human cancer cell lines. Blood. 2009; 113:659–667. [PubMed: 18931345]
44. Sauntharajah Y, Sekeres M, Advani A, Mahfouz R, Durkin L, Radivoyevitch T, et al. Evaluation of noncytotoxic DNMT1-depleting therapy in patients with myelodysplastic syndromes. The Journal of clinical investigation. 2015
45. Hambach L, Ling KW, Pool J, Aghai Z, Blokland E, Tanke HJ, et al. Hypomethylating drugs convert HA-1-negative solid tumors into targets for stem cell-based immunotherapy. Blood. 2009; 113:2715–2722. [PubMed: 19096014]
46. Guo ZS, Hong JA, Irvine KR, Chen GA, Spiess PJ, Liu Y, et al. De novo induction of a cancer/testis antigen by 5-aza-2'-deoxycytidine augments adoptive immunotherapy in a murine tumor model. Cancer research. 2006; 66:1105–1113. [PubMed: 16424047]
47. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Gattermann N, Germing U, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010; 28:562–569. [PubMed: 20026804]
48. Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, Beach CL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006; 24:3895–3903. [PubMed: 16921040]
49. Cashen AF, Schiller GJ, O'Donnell MR, DiPersio JF. Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010; 28:556–561. [PubMed: 20026803]
50. Lubbert M, Ruter BH, Claus R, Schmoor C, Schmid M, Germing U, et al. A multicenter phase II trial of decitabine as first-line treatment for older patients with acute myeloid leukemia judged unfit for induction chemotherapy. Haematologica. 2012; 97:393–401. [PubMed: 22058219]

51. Kantarjian HM, Thomas XG, Dmoszynska A, Wierzbowska A, Mazur G, Mayer J, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012; 30:2670–2677. [PubMed: 22689805]
52. Navada SC, Steinmann J, Lubbert M, Silverman LR. Clinical development of demethylating agents in hematology. The Journal of clinical investigation. 2014; 124:40–46. [PubMed: 24382388]
53. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nature genetics. 1999; 21:103–107. [PubMed: 9916800]
54. Yang G, Khalaf W, van de Locht L, Jansen JH, van der Reijden BA, Muller-Tidow C, et al. Epigenetic regulation of tumor suppressors in t(8:21)-containing AML. Annals of hematology. 2004; 83:329–330. [PubMed: 15052372]
55. Bots M, Verbrugge I, Martin BP, Salmon JM, Ghisi M, Baker A, et al. Differentiation therapy for the treatment of t(8;21) acute myeloid leukemia using histone deacetylase inhibitors. Blood. 2014; 123:1341–1352. [PubMed: 24415537]
56. Garcia-Manero G, Yang H, Bueso-Ramos C, Ferrajoli A, Cortes J, Wierda WG, et al. Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. Blood. 2008; 111:1060–1066. [PubMed: 17962510]
57. Schaefer EW, Loaiza-Bonilla A, Juckett M, DiPersio JF, Roy V, Slack J, et al. A phase 2 study of vorinostat in acute myeloid leukemia. Haematologica. 2009; 94:1375–1382. [PubMed: 19794082]
58. Odenike OM, Alkan S, Sher D, Godwin JE, Huo D, Brandt SJ, et al. Histone deacetylase inhibitor romidepsin has differential activity in core binding factor acute myeloid leukemia. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008; 14:7095–7101. [PubMed: 18981008]
59. Kuendgen A, Knipp S, Fox F, Strupp C, Hildebrandt B, Steidl C, et al. Results of a phase 2 study of valproic acid alone or in combination with all-trans retinoic acid in 75 patients with myelodysplastic syndrome and relapsed or refractory acute myeloid leukemia. Annals of hematology. 2005; 84(Suppl 1):61–66.
60. Kuendgen A, Schmid M, Schlenk R, Knipp S, Hildebrandt B, Steidl C, et al. The histone deacetylase (HDAC) inhibitor valproic acid as monotherapy or in combination with all-trans retinoic acid in patients with acute myeloid leukemia. Cancer. 2006; 106:112–119. [PubMed: 16323176]
61. Bug G, Ritter M, Wassmann B, Schoch C, Heinzel T, Schwarz K, et al. Clinical trial of valproic acid and all-trans retinoic acid in patients with poor-risk acute myeloid leukemia. Cancer. 2005; 104:2717–2725. [PubMed: 16294345]
62. Raffoux E, Chaibi P, Dombret H, Degos L. Valproic acid and all-trans retinoic acid for the treatment of elderly patients with acute myeloid leukemia. Haematologica. 2005; 90:986–988. [PubMed: 15996941]
63. Garcia-Manero G, Assouline S, Cortes J, Estrov Z, Kantarjian H, Yang H, et al. Phase 1 study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukemia. Blood. 2008; 112:981–989. [PubMed: 18495956]
64. Gojo I, Jiemjit A, Trepel JB, Sparreboom A, Figg WD, Rollins S, et al. Phase 1 and pharmacologic study of MS-275, a histone deacetylase inhibitor, in adults with refractory and relapsed acute leukemias. Blood. 2007; 109:2781–2790. [PubMed: 17179232]
65. Giles F, Fischer T, Cortes J, Garcia-Manero G, Beck J, Ravandi F, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006; 12:4628–4635. [PubMed: 16899611]
66. Gore SD, Baylin S, Sugar E, Carraway H, Miller CB, Carducci M, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. Cancer research. 2006; 66:6361–6369. [PubMed: 16778214]

67. Soriano AO, Yang H, Faderl S, Estrov Z, Giles F, Ravandi F, et al. Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood. 2007; 110:2302–2308. [PubMed: 17596541]
68. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, Yang H, Rosner G, Verstovsek S, et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. Blood. 2006; 108:3271–3279. [PubMed: 16882711]
69. Blum W, Klisovic RB, Hackanson B, Liu Z, Liu S, Devine H, et al. Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007; 25:3884–3891. [PubMed: 17679729]
70. Walter RB, Medeiros BC, Gardner KM, Orlowski KF, Gallegos L, Scott BL, et al. Gemtuzumab ozogamicin in combination with vorinostat and azacitidine in older patients with relapsed or refractory acute myeloid leukemia: a phase I/II study. Haematologica. 2014; 99:54–59. [PubMed: 24142996]
71. Ravandi F, Alattar ML, Grunwald MR, Rudek MA, Rajkhowa T, Richie MA, et al. Phase 2 study of azacytidine plus sorafenib in patients with acute myeloid leukemia and FLT-3 internal tandem duplication mutation. Blood. 2013; 121:4655–4662. [PubMed: 23613521]
72. Fili C, Malagola M, Follo MY, Finelli C, Iacobucci I, Martinelli G, et al. Prospective phase II Study on 5-days azacitidine for treatment of symptomatic and/or erythropoietin unresponsive patients with low/INT-1-risk myelodysplastic syndromes. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013; 19:3297–3308. [PubMed: 23596104]
73. Kantarjian HM, Giles FJ, Greenberg PL, Paquette RL, Wang ES, Gabrilove JL, et al. Phase 2 study of romiplostim in patients with low- or intermediate-risk myelodysplastic syndrome receiving azacitidine therapy. Blood. 2010; 116:3163–3170. [PubMed: 20631375]
74. Welch JS, Klco JM, Gao F, Procknow E, Uy GL, Stockerl-Goldstein KE, et al. Combination decitabine, arsenic trioxide, and ascorbic acid for the treatment of myelodysplastic syndrome and acute myeloid leukemia: a phase I study. American journal of hematology. 2011; 86:796–800. [PubMed: 21815182]
75. Sekeres MA, Tiu RV, Komrokji R, Lancet J, Advani AS, Afable M, et al. Phase 2 study of the lenalidomide and azacitidine combination in patients with higher-risk myelodysplastic syndromes. Blood. 2012; 120:4945–4951. [PubMed: 22915641]
76. Blum W, Schwind S, Tarighat SS, Geyer S, Eisfeld AK, Whitman S, et al. Clinical and pharmacodynamic activity of bortezomib and decitabine in acute myeloid leukemia. Blood. 2012; 119:6025–6031. [PubMed: 22566605]
77. Garcia-Manero G, Tambaro FP, Bekele NB, Yang H, Ravandi F, Jabbour E, et al. Phase II trial of vorinostat with idarubicin and cytarabine for patients with newly diagnosed acute myelogenous leukemia or myelodysplastic syndrome. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012; 30:2204–2210. [PubMed: 22585696]
78. Issa JP, Garcia-Manero G, Huang X, Cortes J, Ravandi F, Jabbour E, et al. Results of phase 2 randomized study of low-dose decitabine with or without valproic acid in patients with myelodysplastic syndrome and acute myelogenous leukemia. Cancer. 2014
79. Prebet T, Sun Z, Figueroa ME, Ketterling R, Melnick A, Greenberg PL, et al. Prolonged administration of azacitidine with or without entinostat for myelodysplastic syndrome and acute myeloid leukemia with myelodysplasia-related changes: results of the US Leukemia Intergroup trial E1905. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014; 32:1242–1248. [PubMed: 24663049]
80. Griffiths EA, Choy G, Redkar S, Taverna P, Azab M, Karpf AR. SGI-110 DNA Methyltransferase Inhibitor Oncolytic. Drug Future. 2013; 38:535–543.
81. Kantarjian HM, Jabbour E, Yee K, Kropf P, O'Connell C, Stock W, et al. First Clinical Results Of a Randomized Phase 2 Study Of SGI-110, a Novel Subcutaneous (SQ) Hypomethylating Agent (HMA), In Adult Patients With Acute Myeloid Leukemia (AML). Blood. 2013; 122
82. Ueda K, Yoshimi A, Kagoya Y, Nishikawa S, Marquez VE, Nakagawa M, et al. Inhibition of histone methyltransferase EZH2 depletes leukemia stem cell of mixed lineage leukemia fusion leukemia through upregulation of p16. Cancer science. 2014; 105:512–519. [PubMed: 24612037]

83. Zhou J, Bi C, Cheong LL, Mahara S, Liu SC, Tay KG, et al. The histone methyltransferase inhibitor, DZNep, up-regulates TXNIP, increases ROS production, and targets leukemia cells in AML. Blood. 2011; 118:2830–2839. [PubMed: 21734239]
84. Xu B, On DM, Ma A, Parton T, Konze KD, Pattenden SG, et al. Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. Blood. 2014
85. Momparler RL, Cote S, Momparler LF, Idaghdour Y. Epigenetic therapy of acute myeloid leukemia using 5-aza-2'-deoxycytidine (decitabine) in combination with inhibitors of histone methylation and deacetylation. Clinical epigenetics. 2014; 6:19. [PubMed: 25313314]
86. Fiskus W, Wang Y, Sreekumar A, Buckley KM, Shi H, Jillella A, et al. Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. Blood. 2009; 114:2733–2743. [PubMed: 19638619]
87. Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. Blood. 2011; 117:6912–6922. [PubMed: 21521783]
88. 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; 20:66–78. [PubMed: 21741597]
89. 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; 122:1017–1025. [PubMed: 23801631]
90. Klaus CR, Iwanowicz D, Johnston D, Campbell CA, Smith JJ, Moyer MP, et al. DOT1L inhibitor EPZ-5676 displays synergistic antiproliferative activity in combination with standard of care drugs and hypomethylating agents in MLL-rearranged leukemia cells. The Journal of pharmacology and experimental therapeutics. 2014; 350:646–656. [PubMed: 24993360]
91. Liu W, Deng L, Song Y, Redell M. DOT1L inhibition sensitizes MLL-rearranged AML to chemotherapy. PloS one. 2014; 9:e98270. [PubMed: 24858818]
92. Sarkaria SM, Christopher MJ, Klco JM, Ley TJ. Primary acute myeloid leukemia cells with IDH1 or IDH2 mutations respond to a DOT1L inhibitor in vitro. Leukemia. 2014
93. Filippakopoulos P, Picaud S, Mangos M, Keates T, Lambert JP, Barsyte-Lovejoy D, et al. Histone recognition and large-scale structural analysis of the human bromodomain family. Cell. 2012; 149:214–231. [PubMed: 22464331]
94. Stewart HJ, Horne GA, Bastow S, Chevassut TJ. BRD4 associates with p53 in DNMT3A-mutated leukemia cells and is implicated in apoptosis by the bromodomain inhibitor JQ1. Cancer medicine. 2013; 2:826–835. [PubMed: 24403256]
95. Dawson MA, Gudgin EJ, Horton SJ, Giotopoulos G, Meduri E, Robson S, et al. Recurrent mutations, including NPM1c, activate a BRD4-dependent core transcriptional program in acute myeloid leukemia. Leukemia. 2014; 28:311–320. [PubMed: 24220271]
96. Luo Z, Lin C, Shilatifard A. The super elongation complex (SEC) family in transcriptional control. Nature reviews Molecular cell biology. 2012; 13:543–547. [PubMed: 22895430]
97. 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; 478:524–528. [PubMed: 21814200]
98. 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; 478:529–533. [PubMed: 21964340]
99. Wyspianska BS, Bannister AJ, Barbieri I, Nangalia J, Godfrey A, Calero-Nieto FJ, et al. BET protein inhibition shows efficacy against JAK2V617F-driven neoplasms. Leukemia. 2014; 28:88–97. [PubMed: 23929215]
100. Mould DP, McGonagle AE, Wiseman DH, Williams EL, Jordan AM. Reversible Inhibitors of LSD1 as Therapeutic Agents in Acute Myeloid Leukemia: Clinical Significance and Progress to Date. Medicinal research reviews. 2014
101. 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; 21:473–487. [PubMed: 22464800]

102. 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; 18:605–611.

103. 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; 122:2877–2887. [PubMed: 23954893]

104. Emadi A, Jun SA, Tsukamoto T, Fathi AT, Minden MD, Dang CV. Inhibition of glutaminase selectively suppresses the growth of primary acute myeloid leukemia cells with IDH mutations. Experimental hematology. 2014; 42:247–251. [PubMed: 24333121]

105. Elhammali A, Ippolito JE, Collins L, Crowley J, Marasa J, Piwnica-Worms D. A high-throughput fluorimetric assay for 2-hydroxyglutarate identifies Zaprinast as a glutaminase inhibitor. Cancer discovery. 2014; 4:828–839. [PubMed: 24740997]

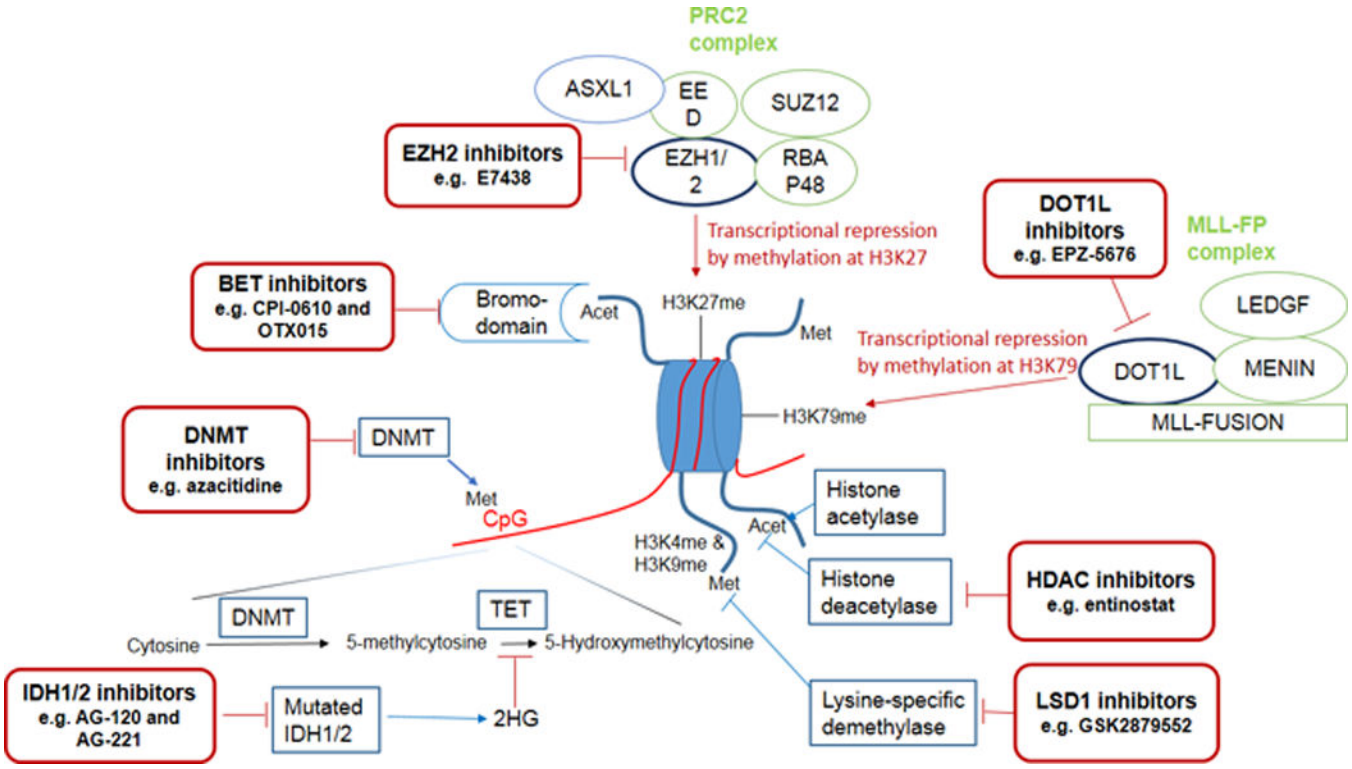

Figure 1.

Schematic view of the mechanism of epigenetic modifications and targeting sites for treatment

Table 1

Selected phase II and III clinical trials of DNMT inhibitors in AML

Study	Design	Participants	N	Responses	Median Overall survival (OS)	Ref
Azacitidine(AZA-001)	Randomized phase III	Reclassification of AML based on WHO criteria, Bone marrow (BM) blast 20~30%	55	18% CR	24.5ms (Aza) vs 16 ms (CCR) (p=0.005)	47
Azacitidine(CALBG 8421, 8921, 9221)	Randomized phase III	Reclassification of AML based on WHO criteria, BM blast 20~30%	103	35~48% (CR/PR/HI)	19.3 ms (Aza) vs 12.9 ms (observation) (only from CALGB 9221) (p value not indicated)	48
Decitabine(DACO-017)	Single arm phase II	AML with age>60, intermediate/poor risk (*de novo* or secondary)	55	25% (CR/PR)	7.7 ms	49
Decitabine (00332/AMLSG 14-09)	Single arm phase II	AML with age>60, intermediate/poor risk (*de novo* or secondary)	277	26% (CR/PR)	5.5 ms	50
Decitabine(DACO-016)	Randomized phase III	AML with age>65, intermediate/poor risk (*de novo* or secondary)	242	17.8% (CR+CRi) (vs. 7.8% treatment choice, p=0.001)	7.7 ms (decitabine) vs 5.0 ms (treatment choice) (p=0.0373)	51

Pts: patients

CR (complete remission): the disappearance of all signs and symptoms related to the disease, a bone marrow with 5% or fewer blasts and peripheral blood count with an absolute neutrophil count of 10^9/L or more and platelet count of 100 × 10^9/L or more.

CRp: CR except for a platelet count increase by 50% to more than 30 × 10^9/L but less than 100 × 10^9/L.

PR (Partial remission): A cellular marrow aspirate with 5% to 25% blasts, with a platelet count greater than 100×10^9/L and WBC less than 1.5×10^9/L

Bone marrow (BM) response: bone marrow blast of 5% or less but without meeting the peripheral blood count criteria for CR or CRp.

Hematologic improvement (HI): HI-E: a hemoglobin increase by at least 15g/L or transfusion independence, HI-P: an absolute increase of platelet counts from less than 20 to more than 20 × 10^9/L and by at least 100% or if more than 20 × 10^9/L, by an absolute increase of at least 30 × 10^9/L, HI-N: a granulocyte increase by at least 100% and by an absolute increase of at least 0.5 × 10^9/L.

CCR: conventional care regimen

N: number of patients

Aza: azacitidine

Table 2

Selected phase I and II clinical trials of HDAC inhibitors in AML

Study	Design	Participants	N	Responses	Median OS	Ref
Vorinostat	Single arm phase I	Relapsed or refractory leukemia, and MDS	41 (31 of AML)	17 % (2CR, 2CRi, 3 HI)	Not reported	56
Vorinostat (NCT00305773)	Two-stage, randomized phase II	Relapsed AML or untreated AML with age >65, antecedent MDS, poor cytogenetics	37 (15 pts in arm A, 22 pts in arm B, arm A and arm B have different administration schedule)	0% (arm A), 4.5% (CR) (arm B)	105 days (arm A), 153 days (arm B)	57
Romidepsin (Depsipeptide)	Two cohort, single arm phase II	Second relapsed AML <60 years, first relapsed AML >60 years, previously untreated AML>60 years who are not candidates for or who refuse conventional chemotherapy	20 (Cohort A: absence of chromosomal aberrations, Cohort B: presence of chromosomal aberrations (t(8;21), inv(16), t(15;17)	Cohort A: 0%, Cohort B: no objective responses by criteria, yet 75% >50% decrease in BM blasts, and HI in 3 out of 7 patients.	Not reported	58
VPA monotherapy or in combination with ATRA	Single arm phase II	AML/MDS, AML/MPN, *de novo* AML	75 (32 of AML)	16% (3% CR, 13% HI)	Not reported	59
MGCD0103 (Mocetinostat)	Single arm phase I	Relapsed or refractory AML, untreated AML with age >60	29 (22 of AML)	2 complete BM response (blasts<5%)	Not reported	63
MS-275 (Entinostat)	Single arm phase I	Relapsed AML or untreated AML with age >65, antecedent MDS, poor risk features (complex karyotype, antecedent hematologic disease)	38	No CR/PR. 12 pts in BM response, decreased transfusion requirement, ANC improvement etc.	Not reported	64
LBH589 (Panobinostat)	Single arm phase I	Relapsed or refractory AML	15 (13 of AML)	8 out of 11 pts with peripheral blasts showed transient reduction.	Not reported	65

Pts: patients

CR (complete remission): the disappearance of all signs and symptoms related to the disease, a bone marrow with 5% or fewer blasts and peripheral blood count with an absolute neutrophil count of 10^9/L or more and platelet count of 100 × 10^9/L or more.

CRp: CR except for a platelet count increase by 50% to more than 30 × 10^9/L but less than 100 × 10^9/L.

PR (Partial remission): A cellular marrow aspirate with 5% to 25% blasts, with a platelet count greater than 100×10^9/L and WBC less than 1.5×10^9/L

Bone marrow (BM) response: bone marrow blast of 5% or less but without meeting the peripheral blood count criteria for CR or CRp.

Hematologic improvement (HI): HI-E: a hemoglobin increase by at least 15g/L or transfusion independence, HI-P: an absolute increase of platelet counts from less than 20 to more than 20 × 10^9/L and by at least 100% or if more than 20 × 10^9/L, by an absolute increase of at least 30 × 10^9/L, HI-N: a granulocyte increase by at least 100% and by an absolute increase of at least 0.5 × 10^9/L.

CCR: conventional care regimen

N: number of patients

Aza: azacitidine

Table 3

Selected clinical trials of combination epigenetic therapy with DNMT inhibitors and HDAC inhibitors in AML

Study	Design	Participants	N	Responses	Median OS	Ref
Azacitidine/ phenylbutyate	Single arm II	MDS, AML/MDS, Relapsed AML or untreated AML with age >65, antecedent MDS, poor cytogenetics	29 (18 of AML)	38% (4 CR, 1PR, 6HI)	Not reported	66
Azacitidine/ VPA/ATRA	Single arm I/II	Relapsed or refractory AML, high-risk MDS, untreated AML with age >60, who refused or were not candidates for front-line chemotherapy	53 (49 of AML)	42% (12 CR, 3 CRp, 7 BM response)	With a median follow up of 21 wks, the median survival in patients achieving CR or CRp has not been reached	67
Decitabine/ VPA	Single arm I/II	Relapsed or refractory AML, high-risk MDS, untreated AML with age >60, who refused or were not candidates for front-line chemotherapy	54 (48 of AML)	22% (10 CR, 2 CRp)	15.3 ms in responders vs. 4.9 ms in non-responders (p value not reported)	68
Decitabine/ VPA	Single arm I	Relapsed AML or untreated AML with age >60, who were not candidates for intensive chemotherapy	25	44% (4 CR)	Not reported	69
Gemtuzumab ozogamicin/ vorinostat/ azacitidine	Single arm I/II	Primary refractory or relapsed AML (age>50)	43	41.9% (10 CR, 8 CRi)	7.4 ms in responders vs. 3.1 ms in non-responders (p=0.0023)	70
Azacitidine/ sorafenib	Single arm I/II	Refractory or relapsed AML	43	46% (6 CR, 10 CRi, 1 PR)	7.8 ms in responders vs. 6.0 ms in non-responders (p=0.01)	71
Bortezomib/ decitabine	Single arm I	Relapsed or refractory AML or previously untreated AML with age >65	19	36% (7 CR+CRi)	Not reported	76
Vorinostat/ idarubicin/ cytarabine	Single arm II	Previously untreated AML or higher-risk MDS with age 15 to 65 years	75 (52 of AML)	85% (57 CR, 7 CRi)	82 wks	77
Decitabine/ VPA	Randomized phaseII	Previously untreated AML or higher-risk MDS with age > 65 years	149 (62 of AML)	ORR: 51% in decitabine alone vs 35% in decitabine/VPA (p=0.208), CR: 33% in decitabine alone vs 9% in decitabine/VPA (p=0.729)	9.6 ms in decitabine alone vs. 7.9 ms in decitabine/VPA (p=0.729)	78
Azacitidine/ Entinostat (E1905)	Randomized phase II	MDS, CMML, AML with MDS related change (excluding therapy related MDS or AML)	149 (52 of AML)	44~46% (not analyzed in AML subgroup)	18 ms in aza alone vs 13 ms in aza/entinostat	79

Pts: patients

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