Epigenetic therapies in acute myeloid leukemia: the role of hypomethylating agents, histone deacetylase inhibitors and the combination of hypomethylating agents with histone deacetylase inhibitors

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Abstract
Epigenetic regulation includes changes of DNA methylation and modifications of histone proteins and is essential for normal physiologic functions, especially for controlling gene expression. Epigenetic dysregulation plays a key role in disease pathogenesis and progression of some malignancies, including acute myeloid leukemia (AML). Epigenetic therapies, including hypomethylating agents (HMAs) and histone deacetylase (HDAC) inhibitors, were developed to reprogram the epigenetic abnormalities in AML. However, the molecular mechanisms and therapeutic effects of the two agents alone or their combination remain unknown. An overview of these epigenetic therapies is given here. A literature search was conducted through PubMed database, looking for important biological or clinical studies related to the epigenetic regimens in the treatment of AML until October 15th, 2019. Various types of articles, including original research and reviews, were assessed, identified, and eventually summarized as a collection of data pertaining the mechanisms and clinical effects of HMAs and HDAC inhibitors in AML patients. We provided here an overview of the current understanding of the mechanisms and clinical therapeutic effects involved in the treatment with HMAs and HDAC inhibitors alone, the combination of epigenetic therapies with intensive chemotherapy, and the combination of both types of epigenetic therapies. Relevant clinical trials were also discussed. Generally speaking, the large number of studies and their varied outcomes demonstrate that effects of epigenetic therapies are heterogeneous, and that HMAs combination regimens probably contribute to significant response rates. However, more research is needed to explore therapeutic effects of HDAC inhibitors and various combinations of HMAs and HDAC inhibitors.

Keywords: Acute myeloid leukemia; Decitabine; 5-azacytidine; Histone deacetylase inhibitors; Intensive chemotherapy

Introduction
Acute myeloid leukemia (AML) is a sub-type of acute leukemia, with pathogenetic heterogeneity. It is typically driven by different genetic abnormalities, influencing hematopoietic stem or progenitor cells, and eventually causing the emergence of abnormal clones.1 Although researchers have explored the mechanisms of leukemogenesis, and have conducted clinical trials for many potential therapies, intensive chemotherapy (IC) remains the mainstay of induction chemotherapy regimen for fit AML patients, which combines anthracyclines and cytarabine (known as the “7 + 3” regimen), followed by several courses of consolidation chemotherapy and/or hematopoietic stem cell transplantation (HSCT).2,3 Unfortunately, AML patients older than 65 years, with various complications, cannot tolerate IC, and have very limited survival rates of 10% or lower.4-6 Epigenetics encompass changes to chromatin structure occurring through histone modifications, DNA methylation, as well as abnormalities of the higher-order chromatin structures, affecting the level of gene expression.7,8 Epigenetic regulators can be categorized as “writers,” “erasers,” and “readers,” and they include DNA and histone methyltransferases, histone deacetylases (HDACs), and bromodomain-containing proteins, respectively.9

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Epigenetic dysregulation is essential for disease occurrence and progression of hematological malignancies. This is especially true for AML, in which several epigenetic mutations have been discovered and shown to be critical drivers in leukemogenesis and disease development. Such mutations, causing epigenetic alterations, include those related to regulation of histone modification (e.g., enhancer of zeste homolog 2) and DNA methylation (e.g., DNA (cytosine-5)-methyltransferase 3 alpha), as well as enzymes involved in metabolism (e.g., isocitrate dehydrogenase) causing consequences of epigenetics. A further exploration of epigenetic abnormality will illuminate the mechanisms of leukemogenesis, elucidate prognosis, and shed light on molecular biomarkers for response prediction.

Abnormal DNA methylation and DNA hydroxymethylation are critical steps towards silencing tumor suppressor genes and, eventually, fulfilling an essential role in the process of malignant transformation. DNA methyltransferase (DNMT) inhibitors (e.g., 5-azacitidine [5-AZA], and 5-aza-2′-deoxycytidine [decitabine, DAC]), are known as hypomethylating agents (HMAs). They have been used for more than 10 years to treat AML patients who are either ineligible for IC or are at high-risk for myelodysplastic syndromes (MDS). A prolonged survival has been observed from clinical trials under the treatment of HMAs compared to supporting treatment in MDS patients, with a tendency for better prognosis in AML cohorts. In real-world settings, however, treatment failure is often found.

Besides, gene transcription is modified during the dynamic process of histone acetylation, which is closely controlled by the competing effects of histone lysine acetyltransferases and HDACs with histone acetylation always causing a more accessible to be chromatin structure facilitating gene transcription. HDAC inhibitors include heterogenous structures that improve the level of histone acetylation, ultimately promoting transcription of different genes associated with cell differentiation, cell cycle modification, and cell apoptosis. Yet, low response rates have been shown in several clinical trials under the monotherapy of HDAC inhibitors in AML.

In the following sections, we present an overview of the mechanisms of action and relevant clinical trials, in which HMAs and HDAC inhibitors were used to treat AML patients. Due to the limited therapeutic effects of HDAC inhibitors and HMAs when used alone, combined strategies of HMAs and HDAC are explored currently in various phases of clinical trials, and will also be the emphasis of this review.

**HMAs**

**Mechanisms of DAC and 5-AZA treatment in AML**

The HMAs, 5-AZA and DAC, are most commonly used DNMT inhibitors, and have been approved for clinical treatment of hematologic malignancies (specifically for AML and high-risk MDS). It has also been found that lower doses of the drugs could be clinically effective and tolerable.

The mechanisms of the two approved HMAs remain to be fully elucidated. DAC is phosphorylated by deoxycytidine kinase, and ultimately transforms into decitabine triphosphate, which can be incorporated into DNA and has no direct influence on RNA. 5-AZA is initially phosphorylated and activated by uridine-cytidine kinase that can be integrated into the RNA structure and significantly inhibit the formation of proteins. Azacitidine diphosphate is then converted by ribonucleotide reductase into decitabine diphosphate, which is further phosphorylated by nucleoside diphosphate kinases to become decitabine triphosphate. It then competitively replaces cytosine in the CpG islands occurring in clusters of promoter regions. Subsequently, decitabine triphosphate suppresses methylation level of the promoter, based on a covalent bond with the DNMT enzymes, resulting in their eventual degradation. When compared with 5-AZA, DAC shows a more potent function in restraining the level of methylation at equimolar doses, probably because of more incorporation of 5-AZA into the structure of RNA than into DNA. High dose of active decitabine triphosphate can result in its incorporation into DNA, inhibiting DNA synthesis, and ultimately causing cytotoxic effect. At lower doses, it mainly depletes DNMT enzymes, followed by reduced DNA methylation level and methylation-induced gene silencing.

In vitro studies have shown a synergistic influence of HDAC inhibitors and HMAs [Figure 1], which will be further discussed in the following sections. Additionally, several studies have shown that the combination of HMAs with cytarabine or anthracycline also has a synergistic anti-leukemic influence, probably because the active phosphorylated form of cytobamine can be phosphorylated by deoxycytidine kinase induced by 5-AZA. HMAs can also work as chemosensitizers, restore expression level of tumor suppressor genes and eventually improve the susceptibility to chemotherapies. In more details, a recent study reported that DAC increased cytotoxicity in Kasumi-1 and HL60/ADR cells when combined with cytarabine, aclarubicin, and harringtonine. DAC-induced depletion of DNMT is S-phase-dependent and is more extensive in actively cycling cells. Leukemic cells at the G0/G1 phase could be induced into the S-phase by granulocyte colony-stimulating factor (G-CSF), making them more responsive to DAC. Based on these synergistic effects, several completed clinical trials combining HMAs and chemotherapy have been reported.

**Selective clinical trials of HMAs in AML**

In the early clinical trials of HMAs, DAC was used at a tolerated dose of 1500 to 2500 mg/m² to induce DNA synthesis arrest and cytotoxicity. More recently, DAC at high-dose was stopped due to severe hematological toxicity and prolonged myelosuppression.

A phase I/II clinical trial was performed to explore the therapeutic effects of low-dose DAC. Elderly AML patients were given doses of 30 to 90 mg/m² of DAC, three times per day for 3 days. These low doses resulted in an overall response rate (ORR) of 45% after about two intensive
courses, including 15% complete remission (CR) rate and 30% partial remission rate. For these responders, the median survival duration was 19 weeks.\textsuperscript{[38]} A phase II multicenter study in older AML cohorts undergoing DAC treatment at a dose of 135 mg/m\textsuperscript{2} every 6 weeks demonstrated that DAC is tolerable and patients had a relatively interesting ORR of 26% and median overall survival (OS) of 5.5 months, free of adverse-risk karyotypes [Table 1].\textsuperscript{[39]} A similar trial with DAC at a dose of 20 mg/m\textsuperscript{2} for five days within a 4-week cycle, obtained a CR of 24%.\textsuperscript{[18]} These results contributed to a randomized phase III multicenter trial for older AML cohorts (the DACO-016 trial), in which patients received DAC at a dose of 20 mg/m\textsuperscript{2} given daily for 5 days in a 4-week cycle \textit{vs.} other therapies composed of the best supportive care (BSC) and low-dose cytarabine (LDAC).
| References | Phase | n   | Inclusion criteria                                                                 | Treatment          | Response outcomes                                                                 | Survival outcomes                                                                                                                                                                                                                                                                                                                                 |
|------------|-------|-----|-----------------------------------------------------------------------------------|--------------------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 39         | II    | 227 | Patients >60 years old with untreated AML ineligible for induction chemotherapy     | Decitabine + ATRA  | ORR: 26% (95% CI: 20%–32%), including CR: 15.2%, and PR: 12.8%                   | The median survival time was 5.5 months, with a 1-year OS rate of 28%, 95% CI: 22%–34% and a 2-year OS rate of 13%, 95% CI: 8%–17%.                                                                                               |
| 18         | II    | 55  | Patients ≥60 years old with AML and intermediate- or poor-risk cytogenetics, including *de novo* AML, AML secondary to prior therapy, or transformed from MDS | Decitabine         | ORR: 25%; CR rate: 24%                                                           | The median EFS was 5.8 months (3 days–23.6 months). The median RFS for patients with a CR was 16.3 months (0.9–18.4 months). The median survival for patients who achieved a CR or CRi was 14 months.                                                                                                         |
| 40         | III   | 485 | Patients ≥65 years old with previously untreated *de novo* or secondary AML (≥20% blasts) and poor- or intermediate-risk cytogenetics | Decitabine vs. TC, either SC (to maximize the quality of life) or LDAC | CR: Decitabine vs. TC, 17.8% vs. 7.8%, *P* = 0.001                              | The median OS with decitabine vs. TC: 7.7 months (95% CI: 6.2–9.2 months) vs. 5.0 months (95% CI: 4.3–6.3 months), *P* = 0.108; HR, 0.82, 0.68–0.99, *P* = 0.373.                                                                                      |
| 42         | II    | 84  | Patients with AML/MDS                                                              | Decitabine         | Patients with *TP53* mutation vs. patients with wild-type *TP53*: CR 100% vs. 21%, *P* < 0.001; ORR 100% vs. 52.6%, *P* < 0.05 | OS was not negatively affected by cytogenetic abnormalities related to unfavorable risk (median survival, 11.6 months among patients with unfavorable risk and 10 months among patients with favorable or intermediate risk, *P* = 0.29) or the presence of *TP53* mutations (median survival, 12.7 months among patients with *TP53* mutations and 15.4 months among patients with wild-type *TP53*, *P* = 0.79). |
| 43         | II    | 53  | Patients ≥60 years with previously untreated AML who were not candidates for or who refused intensive chemotherapy | LDAC               | CR: 47% (95% CI: 33%–61%); ORR: 64% (95% CI: 50%–77%)                              | Median OS for all subjects was 55 weeks (95% CI: 36–72 weeks). Median DFS for CR subjects was 46 weeks (95% CI: 30 weeks – not yet reached).                                                                                                                                   |
| 45         | I/II  | 309 | Patients with AML/MDS                                                              | Azacitidine vs. SC  | Response rate: 35%–48%                                                           | Among the 33 AML responders, the median duration of response was 7.3 months (2.2–25.9 months); Median survival time for the 27 AML patients in the azacitidine group was 19.3 months compared with 12.9 months for the 25 AML patients randomly assigned to observation.                          |
| 46         | III   | 113 | Older patients with low marrow blast count (20% to 30%) WHO-defined AML            | Azacitidine vs. BSC vs. LDAC vs. IC (cytarabine + daunorubicin/ idarubicin/mitoxantrone) | The morphologic CR rate: Azacitidine vs. BSC + LDAC + IC, 18% vs. 16%, *P* = 0.80, LDAC, 15%; IC, 55%; BSC, 0% | Median OS: Azacitidine vs. BSC, 19.1 vs. 13.4 months, respectively (HR, 0.48, 95% CI: 0.24–0.94; *P* = 0.03). Azacitidine vs. LDAC, 24.5 vs. 17.0 months, respectively (HR, 0.37; 95% CI: 0.12–1.13; *P* = 0.08). |
(Continued)

Table 1: Selected clinical trials for HMAs with or without IC in AML treatment.

| References | Phase | n | Inclusion criteria | Treatment | Response outcomes | Survival outcomes |
|------------|-------|---|--------------------|-----------|-------------------|------------------|
| 47         | III   | 488| Patients ≥65 years old with newly diagnosed de novo or secondary AML with >30% BM blasts who were not eligible for hematopoietic stem cell transplantation, with intermediate- or poor-risk cytogenetics | Azacitidine vs. CCRs, including BSC, LDAC or IC (cytarabine + daunorubicin/idarubicin) | CR + CRi rates: Azacitidine vs. CCR, 27.8% vs. 25.1%, P = 0.5384; Within the CCR arm, ORR were 0% (BSC), 25.9% (LDAC), and 47.7% (IC) | Median OS for patients receiving azacitidine and CCR was 10.4 and 6.5 months (HR, 0.85; 95% CI: 0.69–1.03; P = 0.1009); After censoring AML patients treated with azacitidine at the beginning of second-line treatment, the median OS in the azacitidine arm was 12.1 vs. 6.9 months in the CCR arm (HR, 0.76; 95% CI: 0.60–0.96; P = 0.0190). In Cox regression analyses, azacitidine improved OS compared with CCRs (HR, 0.75; 95% CI: 0.59–0.94; P = 0.0130). The difference in DFS between the two arms was statistically significant (Cox regression; P = 0.005). The 12 months DFS was estimated at 39% for the control group and at 63% for the aza group. The difference in OS between the two groups is not statistically significant (Cox regression; P = 0.35). With a median follow-up of 32 months, 16 of 30 subjects (53%) are alive in CR; The median survival has not been reached, but the lower bound of the 95% CI is 15 months. After a median follow-up of 616 days from start of therapy, median OS and EFS were 266 days and 215 days, respectively. |
| 48         | III   | 117| Patients ≥60 years old with AML or MDS-RAEB in CR/CRi after at least two cycles of intensive chemotherapy | Maintenance therapy: Azacitidine vs. observation | – | – |
| 35         | I     | 30 | Patients ≤60 years old with untreated AML and the absence of a favorable karyotype | Decitabine followed by standard induction chemotherapy with infusional cytarabine and daunorubicin | ORR: 90%, CR: 57%, and PR 33%; No significant difference in response rate was identified among the treatment arms, cohorts, or dose levels | – |
| 50         | II    | 12 | Patients >60 years old with untreated AML | Azacitidine followed by “7 + 3” induction chemotherapy consisting of cytarabine and daunorubicin | Response rate: 58.3% | – |
| 51         | II    | 30 | Patients with a diagnosis of high-risk MDS, AML evolving from MDS, or relapsed/refractory AML | Decitabine followed by idarubicine combined with cytarabine | The overall CR rate (CR + CRi + CR2): 66.67%, 2 achieved PR | – |
| 52         | II    | 214| Patients >60 years old with newly diagnosed AML and without previous treatment except hydroxyurea to control leukocyte counts. | Azacitidine/chemotherapy (arm-A) or chemotherapy alone (arm-B); Chemotherapy included two courses of “7 + 3” induction therapy (cytarabine and daunorubicin) | CR rate: arm-A vs. Arm-B, 48% vs. 52%, P = 0.58 | The median EFS was 6 months in both arms (P = 0.96). Median OS was 15 months in arm-A and 21 months in arm-B (P = 0.35). Morphologic leukemia-free status was observed in 10% of patients in each arm, adding to a total response rate of 38% in arm-A and 62% in arm-B. |

AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; WHO: World Health Organization; ATRA: All-trans retinoic acid; CR: Complete remission; CRi: Complete remission with incomplete blood count recovery; TC: Treatment choice; SC: Supportive care; LDAC: Low-dose cytarabine; BSC: Best supportive care; IC: Intensive chemotherapy; CCR: Conventional care regimen; ORR: Overall response rate; PR: Partial response; OS: Overall survival; EFS: Event-free survival; HR: Hazard ratio; CI: Confidence interval; DFS: Disease-free survival; --: No data.
The findings demonstrated a remarkably improved response rate for DAC (18% vs. 8%; \( P = 0.001 \)) and a tendency for improved OS (8 vs. 5 months, \( P = 0.108 \)). And for 271 patients having white blood cells \( < 15 \times 10^{9} / \text{L} \) and bone marrow (BM) blasts \( > 30\% \) in this trial, a better response rate of 27\% for DAC could be observed when compared to the other treatment regimens (11\%), contributing to a remarkably improved OS for the DAC arm (8.6 vs. 4.7 months, \( P = 0.0033 \)).

In a single-center study including 84 patients with AML or MDS and treated with 10-day DAC 20 mg/m², the results illustrated patients with adverse-risk karyotypes had better DAC response rates than those with intermediate or low-risk cytogenetics (67\% vs. 34\%, \( P < 0.001 \)). Furthermore, response rate in patients harboring \( TP53 \) mutation was significantly higher compared to all other patients (100\% vs. 41\%, \( P < 0.001 \)). However, responses in patients with adverse-risk karyotypes remained to be limited and caused OS similar to those with intermediate-risk karyotypes.

Based on these findings, it remains controversial whether the 5 or 10-day DAC regimen should be recommended. In a phase II trial containing a smaller cohort, 53 AML patients presented a better CR rate (47\%) and ORR (64\%) after a median of three 10-day therapy courses. Cases with patients unclassified and bone marrow (BM) blasts \( \geq 30\% \) the second-line treatment (12.1 vs. 6.9 months, \( P = 0.019 \)).

For consolidation regimen after two or more courses of induction chemotherapies, 5-AZA might also be considered as a potential treatment option as was shown in a phase III clinical trial, demonstrating prolonged disease-free survival of 63\% vs. 39\% for 5-AZA vs. control, respectively (\( P = 0.005 \)).

In a retrospective study on relapsed or refractory AML (r/r AML), 5-AZA given for a median of four courses obtained an ORR of 17\% and a median OS of 8.4 months (Table 1).

Due to the synergistic anti-leukemic effects in the combination of HMAs and anthracycline or cytarabine mentioned above, several clinical trials have been performed to explore the therapeutic effect of this combination. A regimen of HMAs plus cytarabine was proposed to be an interesting option for older AML patients unsuitable for IC, due to the moderate and transient response rates from both agents when used alone.

Initial phase I or II studies were performed in AML patients who received sequential treatment of HMAs followed by “7 + 3” regimen, showing CR rate of 83\%, without increased toxicity.

However, a larger phase II clinical trial in older AML cohorts presented opposite results, showing that a combination of 5-AZA + “7 + 3” induction regimen not only failed to show any prolonged survival, but also caused more adverse events (AEs) when compared with intensive induction chemotherapy alone (Table 1). Identifying molecular biomarkers as predictors for responses to HMAs, fine-tuning of the therapeutic schedules, and checking this combination in patients with intermediate- or favorable-risk cytogenetics might be a viable option to explore in the future.

**HDAC Inhibitors**

**Mechanisms of HDAC inhibitors in AML**

Histones are critical for packaging the DNA into formulations such as nucleosomes and chromatin. Histone acetyltransferases and HDAC fulfill the functions of histone tails’ acetylation and deacetylation, respectively, playing a key role in epigenetic mechanisms that regulate genes transcription.

Generally speaking, HDACs deacetylate lysine residue on proteins regulating cell apoptosis and proliferation, and are divided into four types according to their similarity to yeast HDACs.

From structural aspect, HDAC inhibitors most commonly contain a hydroxamic acid or a benzamide zinc-binding cluster. However, several HDAC inhibitors can also attach to zinc in the absence of hydroxamic acid or benzamide.

HDAC inhibitors are employed to treat malignancies because they inhibit excessive histone deacetylation and modulate transcription factors, particularly those controlling expression level of tumor suppressor genes. A study on HDAC inhibitors showed that they could function in a number of ways: (i) inhibit the G1 phase of the cell cycle, (ii) promote cell apoptosis by inducing the mitochondria-driven apoptotic pathway or up-regulating apoptosis ligands and receptors, (iii) lead to DNA defects that cause cell death during mitosis, and (iv) contribute to autophagy through a number of pathways. Through these functions, HDAC inhibitors mainly mediate
the death of cancerous cells and revealed less cellular cytotoxicity in healthy cells,[54] thereby making these agents attractive for clinical treatment.

The drug panobinostat inhibits Class I of HDACs and degrades the oncoprotein AML1/ETO9a, a trigger of AML.[55] Panobinostat contributed to a remarkable anti-leukemic effect without the requirement of functional p53 gene nor activation of conventional apoptotic pathways[57] and could also affect cell cycle regulation.[58] In a mouse model with t(8;21) AML, panobinostat achieved an obvious anti-leukemic effect and induced cell differentiation.[57]

Vorinostat is another HDAC inhibitor. It contains hydroxamic acid, and it binds to the zinc-containing pocket in the catalytic site of HDAC 1, 2, 3, and 6, leading to reversible inhibition of HDACs, promoting protein acetylation, regulating gene expression, and inducing differentiation, growth arrest, and apoptosis of cancer cells.[59] Anti-tumor activity of vorinostat might arise from the oxidative stress it promotes, its modulation of gene expression, and its induction of DNA damage and genomic instability.[60,61]

Valproic acid (VPA) is a short-chain fatty acid that also inhibits HDAC activity. It can be induced differentiation by targeting the AML1/ETO-complex, and functions as AML cell proliferation inhibitor and apoptosis inducer, especially in t(8;21) AML.[62] The susceptibility to natural killer (NK) cell-mediated lysis can also be improved by this drug. This is done through up-regulation of NK cell ligands on the leukemic cells[63,64] and the result is that NK cells attack leukemic stem cells.[65] The orally bioavailable benzamide HDAC inhibitor, entinostat, inhibits class I of HDAC enzymes,[66] resulting in inhibition of cell growth and increased cytotoxicity to human cancer cells.[67] Entinostat is also shown to promote loss of leukemia maintenance and prolonged survival in murine model.[67,72] These growth inhibition and cytotoxicity might be due to transcription activation of anti-proliferation genes (eg, p21), transformation of growth factor-β type II receptor, and the induction of the maturation marker gelsolin.[67,71,73] Chidamide is a structural analog of entinostat that selectively inhibits HDAC1, 2, 3, 10. Through the function of reactive oxygen species, chidamide exhibited efficient anti-proliferative activity in AML cells, including stem cells, accompanied by the arrest at G0/G1 phase of the cell cycle and cell apoptosis. It controls anti- apoptotic- and pro-apoptotic-related proteins (PARP), including B-cell lymphoma 2, and activates caspase-3, causing the degradation of PARP. CD40 could also be activated by chidamide, and its downstream signaling pathways, c-Jun N-terminal kinase and nuclear factor kappa-light-chain-enhancer of activated B, were thus regulated. These results suggest that chidamide might be a novel medication targeting leukemia stem cells.[74,75]

Overall, HDAC inhibitors are a significant group of promising treatment agents for AML. However, the monotherapy of HDAC inhibitors has always presented moderate anti-leukemic effects, which will be discussed in the following sections. Therefore, it is necessary to explore special combination regimens, including HDAC inhibitors and other therapies. HDAC inhibitors have shown synergistic effect when combined with various IC [Figure 1], such as nucleoside analogs (eg, cytarabine), anthracyclines (eg, idarubicin), and topoisomerase inhibitors (eg, etoposide).[76,79] The mechanism of these synergistic effects has not been completely elucidated, but it is assumed that HDAC inhibitors help formulating a more open chromatin structure that probably allows for more extensive access of topoisomerase inhibitors to the DNA structure and thereby causing higher therapeutic effects of chemotherapies.[79] Other studies, exploring this synergistic influence, for instance, between panobinostat and doxorubicin, implicated DNA double-strand breaks and activated the caspase-dependent apoptosis pathways, showing anti-leukemic efficacy.[78] These results are the foundation of clinical trials combining HDAC inhibitors and IC.

Selected clinical trials of HDAC inhibitors in AML

A series of clinical trials have been performed with HDAC inhibitors in AML cohorts. Generally, HDAC inhibitor monotherapy achieved low response rates (10%–20%).[53]

In a phase II trial in 37 AML patients treated with vorinostat, only one case had hematologic improvement.[80] In another phase III study, 42 patients with high-risk MDS (n = 5) or AML (n = 37) treated with panobinostat after allogenic-HSCT, received one of two schedules: schedule A of 10 mg weekly or schedule B of 20 mg every other week, using a “+ 3 + 4” design. At least one G3/4 AE occurred in 35 out of the 42 cases, of which 22 cases were considered panobinostat-related. Incidence rates of G3/4 AE were not different between the two schedules (A: n = 12, B: n = 10; A vs. B, 57% vs. 48%). After 2-year treatment with panobinostat, the relapse and non-relapse mortality across all doses was 20% and 5%, respectively, and survival probabilities of 2-year OS and relapse free survival (RFS) were 81% and 75%, respectively.[81] In a phase II study of VPA alone or VPA combined with all-trans retinoic acid (ATRA), 75 cases with MDS or refractory and relapsed AML received either of the VPA regimens. 24% of the patients responded to the treatments[82] [Table 2]. Other studies in adverse-risk AML and MDS, in which patients were treated with VPA, either as a monotherapy or combined with ATRA, have also revealed limited effects.[83–85] Due to these moderate or limited results of HDAC inhibitors monotherapy in AML, clinical trials have been designed to combine HDAC inhibitors with other therapies, such as IC or HMAs.[53]

Phase I trial of vorinostat combined with cytaraebine for r/r AML patients showed interesting outcomes: six of 17 (35%) cases had CR, although five of these patients later relapsed and died.[86] In a larger phase II trial, testing a combination of vorinostat, cytaraebine and idarubicin in 75 cases with AML or high-risk MDS, ORR was up to 85% and the addition of vorinostat did not cause increased toxicity.[87] Interestingly, in this study, all patients with FLT3-ITD mutation responded positively, and NRB2 and CYBB mutations were considered as biomarkers related to higher survival rate.[87] In a phase III trial (SWOG 1203), comparing between idarubicin plus high-dose cytaraebine, IC, and vorinostat plus idarubicin, in 738 AML older patients (>60 years old), CR rates were similar across all group (75%–79%), achieving clearly better outcomes for cancers harboring favorable karyotypes in the “7 + 3” group.[88] However, in another cohort that included r/r or secondary AML patients, the combination regimen of...
### Table 2: Selected clinical trials for HDAC inhibitors with or without IC in AML treatment.

| References | Phase | Inclusion criteria | Treatment | Response outcomes | Survival outcomes |
|------------|-------|--------------------|-----------|-------------------|-------------------|
| 80         | II    | Relapsed AML, or untreated AML if (i) patients ≥65 years old, 2) AML with antecedent MDS (AML with trilineage dysplasia), or 3) AML with poor-risk cytogenetics. | Vorinostat: arm A, the dose regimen was 400 mg; arm B, the dose regimen was 200 mg three times daily for 14 days followed by 1 week rest. | In arm A (n = 15), the CR rate was 0% (95% CI, 0% to 23%); In arm B (n = 22), the CR rate was 4.5% (1 response; 95% CI, 0.4%–24%). | In arm B (n = 22), a duration of response exceeding 398 days. |
| 81         | I/II  | AML n = 37, MDS n = 5 | Patients had to be in CR post-HSCT, and fulfill ≥1 of the following criteria: (i) r/r AML; (ii) adverse risk cytogenetics; (iii) secondary to MDS or radio/chemotherapy; (iv) MDS intermediate-2 or high risk or MDS refractory anemia with excess blasts. | Panobinostat: given orally thrice weekly: schedule A, starting dose 10 mg, given weekly; schedule B, starting dose 20 mg using a “3+3” design, given every other week. | Thirty-five of 42 patients (83%) experienced at least one G3/4 AEs, considered panobinostat-related in 22 patients (52%). Rates of G3/4 AEs did not differ significantly between schedules (A: n = 12, 57%; B: n = 10, 48%). | At 2 years after the first panobinostat dose, the cumulative incidence of relapse and non-relapse mortality across all dose levels was 20% (95% CI, 7%–33%) and 5% (95% CI, 0%–11%). |
| 82         | II    | AML n = 24, MDS n = 51 | Patients were started on VPA monotherapy. Addition of ATRA was planned for patients who did not respond or who relapsed after an initial response to VPA. | Eighteen patients (24%) achieved responses, The response rate was 30% in MDS (n = 43) and 16% in AML (n = 32). | Median time to response was 28 days (range 14–96). The median duration of response was 4 months (range 2–27) and 5 months including second responses. Mean response duration was 7 months (8 months including second responses). | All patients with CR on study except one eventually relapsed and succumbed to complications of their underlying disease including patients who underwent allogeneic transplantation. EFS was 47 weeks (range, 3–134 weeks), and OS was 82 weeks (range, 3–134 weeks). |
| 86         | I     | Adults (≥18 and <60 years) with r/r AML with adequate organ function | Decitabine in combination with vorinostat and cytarabine | The ORR: 35%. | |
| 87         | II    | Patients with previously untreated AML or higher-risk MDS age 15 to 65 years with appropriate organ function and no core-binding factor abnormality were candidates. | Vorinostat combined with idarubicin and cytarabine | The ORR was 85%, including 76% in CR and 9% in CRi. ORR was 93% in diploid patients and 100% in FLT3-ITD patients. | |
### Table 2: Selected clinical trials for HDAC inhibitors with or without IC in AML treatment.

| References | Phase | n | Inclusion criteria | Treatment | Response outcomes | Survival outcomes |
|------------|-------|---|--------------------|-----------|------------------|-------------------|
| 88 III     | 738   | Newly diagnosed adults aged 18 to 60 years with AML. | Arm I (standard dose cytarabine, daunorubicin hydrochloride, DA); Arm II (high-dose cytarabine, idarubicin, IA); Arm III (vorinostat, high-dose cytarabine, idarubicin, IA + V) | CR rates were similar among all three treatment arms: 75% for 7 + 3, 79% for IA, and 77% for IA + V (P = 0.58). | There were no differences in outcomes for any standard-risk group; however, in patients with favorable cytogenetics, outcomes were significantly better with 7 + 3 than with IA or IA + V. |
| 89 I       | 21    | Newly diagnosed AML and r/r AML. | Vorinostat was given in combination with fixed doses of cytarabine and etoposide. The study used a standard 3 + 3 dose escalation design | Of the 21 patients enrolled, five achieved CR and two CRp, giving an ORR of 33%. | The median OS in days (95% CI) is 193 (96–515); The median PFS in days (95% CI) is 45.5 (42–342). |
| 90 III     | 38    | Elderly patients with newly diagnosed AML. | Idarubicin plus cytarabine and panobinostat. | The CR rate was 64%. | The time to relapse of 17.0 months (12.8–21.1). Median OS for the whole series was 17 months (5.5–28.4). The 1 year EFS was 78.3%. |
| 91 I       | 46    | Patients aged 18 to ≤ 65 years with newly diagnosed primary or secondary high-risk AML (defined as intermediate, intermediate II, or adverse cytogenetic subsets). | Fixed dose idarubicin and cytarabine and escalating oral doses of panobinostat at 15 mg, 20 mg, and 25 mg | The ORR was 60.9%, 43.5% achieved a CR, and 17.4% achieved CR with incomplete count recovery. |
| 92 I       | 25    | Patients ≥ 60 years old with untreated AML, advanced MDS (defined as intermediate-2 or high risk) or therapy-related myeloid neoplasm. | Dose escalation of panobinostat followed standard “3 + 3” design. Induction therapy consisted of panobinostat, daunorubicin and cytarabine. | The CR/CRi rate was 32%. | Median OS was 10 months; 23 months with CR/CRi vs. 7.8 months without CR/CRi (P = 0.02); Median RFS was 8.2 months. |
| 93 III     | 186   | Patients ≥ 60 years old with newly diagnosed AML, including de novo AML, secondary AML with a preceding history of myelodysplastic or myeloproliferative disorder, and therapy-related AML after treatment of a primary malignancy. | two induction cycles with idarubicin, cytarabine, and all-trans retinoic acid either with VPA or without (STANDARD). | CR rates after induction tended to be lower in VPA compared with STANDARD (40% vs. 52%; P = 0.14) as a result of a higher early death rate (26% vs. 14%; P = 0.06). | EFS and OS were not different between the 2 groups (P = 0.95 and P = 0.57, respectively). RFS was significantly superior in VPA compared with STANDARD (24.4% vs. 6.4% at 5 years; P = 0.02). |

AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; r/r AML: Refractory and relapsed AML; CMML: Chronic myelomonocytic leukemia; AE: Adverse event; HSCT: Hematopoietic stem cell transplant; WHO: World Health Organization; VPA: Valproic acid; ATRA: All-trans retinoic acid; CR: Complete remission; CRi: Complete remission with incomplete blood count recovery; ORR: Overall response rate; OS: Overall survival; EFS: Event-free survival; CI: Confidence interval; RFS: Relapse-free survival.
histone acetylation induced by panobinostat was a patients was tolerable and that the improved level of NPM1 analyses suggested that tails and cytosine methylation in the gene promoter after the treatment with panobinostat was remarkably lower response rate (33%) [89] [Table 2].

In another clinical trial exploring panobinostat combined with cytarabine and idarubicin in older AML patients, the addition of panobinostat caused dose-limiting degree of toxicity but a longer RFS was also observed. In a phase II study evaluating the safety and efficacy of the same drug combination in 46 older AML patients with adverse-risk cytogenetics, the doses of panobinostat escalated at 15, 20, and 25 mg, given thrice weekly from the second week of a 4-week course. The ORR was 60.9%, including 43.5% of CR and 17.4% of Complete remission with incomplete blood count recovery (CRi). The event free survival (EFS) probability at 1-year was 78.3%. These results also revealed that a combination of panobinostat with IC demonstrated tolerable safety and efficacy in younger patients with high-risk AML. In another phase I study combining panobinostat and IC in older AML patients (60–85 years old), patients were treated daily with oral administration of 20 to 60 mg panobinostat, and on day 1, 3, day 5, and day 8 patients also received fixed doses of daunorubicin and cytarabine. The CR/CRi was up to 32%, without dose-limited toxicity occurring in the dose escalation arms. The median OS was 10 months, which represents 23 months with CR/CRi vs. 7.8 months without CR/CRi (P = 0.02). Improved level of histone acetylation after the treatment with panobinostat was remarkably related to CR/CRi rates. These results illustrate that the combination of panobinostat with “+3” for older AML patients was tolerable and that the improved level of histone acetylation induced by panobinostat was a predictor for CR/CRi [92] [Table 2].

In a phase III randomized German-Austrian AML Study (Group 06-04), 186 AML patients randomly received two induction courses of a combination of idarubicin, cytarabine, and ATRA, either with VPA or without (regarded as the standard therapy). After induction chemotherapy, the VPA arm showed a tendency of lower CR rates when compared to the standard therapy (40% vs. 52%; P = 0.14), resulting from a tendency of higher early mortality in the VPA arm (26% vs. 14%; P = 0.06). As a result, VPA was restricted to the first induction course and the dose of idarubicin was reduced. Toxicities after this change dropped to rates comparable to those in the standard therapy. The two arms did not differ in EFS and OS (P = 0.95 and P = 0.57, respectively). However, RFS markedly increased in the VPA arm (5-year survival probability: 24.4% vs. 6.4%; P = 0.02). Subsequent analyses suggested that NPM1 mutation-positive AML patients might particularly benefit from the VPA regimen [93] [Table 2].

**Combination of HMAs and HDAC Inhibitors**

**Mechanisms of HMAs in combination with HDAC inhibitors in AML**

Gene silencing is usually related to modification of histone tails and cytosine methylation in the gene promoter regions. There might be a synergistic effect between HDAC inhibitors and HMAs to relieve transcriptional repression from tumor suppressor genes. In vitro studies have also demonstrated the presence of a synergistic influence between HMAs and HDAC inhibitors [Figure 1].

Transcriptome analyses in a reported study showed that a combination of DAC with panobinostat or VPA resulted in significantly higher transcripts than either treatment alone, illustrating a quantitative synergistic influence on genome-wide expression in U937 cells. This combined treatment particularly affected a mass of genes down-regulation, including epigenetic modifiers (eg, KDM2B) and onco-genes (eg, MYC) that are always overexpressed in solid and hematological malignancies. Gene body DNA demethylation and changes in acH3K9/27 could also be observed.

In another study, chidamide, combined with DAC, showed a noticeable synergistic anti-leukemic effect. Chidamide and this combination could both inhibit the proliferation of HL60 and NB4 cells. The G0/G1 phase could be blocked by chidamide alone, while the combination of chidamide and DAC mainly blocked the cycle at the G2/M phase, accompanied by higher p21 expression. In both chidamide monotherapy or the combination treatment, apoptosis of leukemic cells was induced through up-regulation of Bax and Caspase-3, and down-regulation of BCL-2, showing synergistic cytotoxicity.

Decitabine, combined with vorinostat, also showed a synergistic induction of cell apoptosis, inhibition of cell proliferation, promotion of histone acetylation, and further inhibition of the enzyme DNMT1 in HL60 cells. This combination therapy also caused reprogramming of unique biomarkers, including the receptor tyrosine kinase (AXL) gene that is known to be associated with cell survival and an adverse prognosis in AML. Based on this information, a combination of GSB324 (an AXL-specific inhibitor), DAC, and vorinostat increased the sensitivity of OCI-AML3 cells and significantly reduced disease burden and prolonged survival of murine models injected with pretreated OCI-AML3 cells.

The combined influence of 5-AZA + HDAC1/2 inhibitors (ACY-1035, ACY-957) was also explored in HL60 and MV-4-11 cell lines. This combined treatment significantly reduced 5 phase cells, increased the number of CD11b-positive cells, and induced higher rate of cell apoptosis, when compared with 5-AZA or HDAC1/2 inhibitors alone. These results indicate the presence of a synergistic effect between 5-AZA and HDAC1/2 inhibitors in inhibiting primary AML blasts proliferation. More specifically, gene set enrichment analysis demonstrated that when compared to 5-AZA monotherapy, the combination of 5-AZA and HDAC1/2 inhibitors helped the enrichment of genes, including GATA binding sites within their promoter regions, showing GATA2 gene might be critical for the synergistic influence of HDAC1/2 inhibitors and 5-AZA.

**Selected clinical trials of HMAs combined with HDAC inhibitors in AML**

The combined trials of HMAs combined with HDAC inhibitors in AML and MDS patients, in which the combined
Table 3: Selected clinical trials for HMAs plus HDAC inhibitors in AML treatment.

| References | Phase | n | Inclusion criteria | Treatment | Response outcomes | Survival outcomes |
|------------|-------|---|--------------------|-----------|-------------------|-------------------|
| 106        | II    |   | AML \( n = 217 \), MDS \( n = 42 \) | Control arm: azacitidine alone; Combination arm: azacitidine in conjunction with additional sodium valproate and vorinostat (VOR); | There was no difference in either ORR (41% vs. 42%; RR, 1.05; 95% CI, 0.64–1.72; \( P = 0.84 \)) or CR/CRi/mCR rate (22% and 26%; RR, 0.82; 95% CI, 0.46–1.45; \( P = 0.49 \)) between the control and combination therapy arms. Time to first response and duration of response at 1 year was similar in the AZA and combination arm (6.2 months vs. 5.7 months and 67% vs. 58%); No difference was observed in OS between patients treated with AZA monotherapy (median OS = 9.6 months; 95% CI, 7.9 –12.7) and patients in the AZA/VOR arm (median OS = 11.0 months; 95% CI, 8.5 –12.0; HR, 1.15; 95% CI, 0.87 –1.51; \( P = 0.32 \)). There was no difference in OS between treatment arms in patients with newly diagnosed or r/r AML. | |
| 105        | II    |   | AML \( n = 97 \), MDS \( n = 52 \) | Arm A: azacitidine alone, Arm B: azacitidine ± entinostat. | The HN rates were in Arm A: 32% (95% CI, 22%–44%, including 12% CR, 8% PR, and 12% TL); and in arm B: 27% (95% CI, 17%–39%, including 8% CR, 7% PR, 12% TL) | With a median follow-up of 30 months, 21 patients were alive and 128 had died. The median OS was 18 months in arm A and 13 months in arm B. For patients with MDS and CMML, the median OS was 21.2 months in arm A and 14.7 months in arm B. For patients with AML, the median OS was 7.1 months in arm A and 5.3 months in arm B. The median OS for the entire cohort was 17 months: 15 months for Arm A; 19 months for Arm B (\( P = 0.14 \) Arm A vs. B); and 27% for Arm C (\( P = 0.16 \) Arm A vs. C); Rates of CR/PR/HI and marrow CR were not significantly different across groups, |
| 107        | II/III| 282 | Adults patients with higher-risk MDS (intermediate-2 or high and/or bone marrow blasts \( \geq 5\% \)) or CMML with <20% blasts. | Arm A: azacitidine monotherapy; Arm B: azacitidine plus lenalidomide; Arm C: azacitidine plus vorinostat. | The ORR for the entire cohort was 38%; 38% for Arm A; 49% for Arm B (\( P = 0.14 \) Arm A vs. B); and 27% for Arm C (\( P = 0.16 \) Arm A vs. C); Rates of CR/PR/HI and marrow CR were not significantly different across groups, | |
although within HI, patients in Arm B had higher rates of HI-neutrophil than Arm A (19% vs. 5%; \(P = 0.007\)).

Among previously untreated patients, there was a trend toward improved ORR for Arm B vs. Arm A (\(n = 79\); 49% vs. 35%; \(P = 0.08\)).

For patients remaining on therapy for \(\geq 6\) months, Arm B had a higher ORR (87%) vs. Arm A (62%; \(P = 0.01\)), although no difference in response duration (\(P = 0.98\)).

Median OS and PFS were 19.1 months (95% CI, 10–26.5 months) and 12.6 months (95% CI, 10–17.7 months), respectively, with a 1-year OS rate of 62%.

The OS of patients who achieved CR/CRi was significantly longer than that for patients who failed to achieve remission. 55.6% patients with mutations in epigenetic and transcription factor related gene, but without FLT3-ITD mutation, achieved CR/CRi, whereas the ORR was 36.7% for patients with mutations in other genes.

AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; CMML: Chronic myelomonocytic leukemia; r/r AML: Refractory and relapsed AML; G-CSF: Granulocyte colony-stimulating factor; HN: Hematological normalization; TL: Trilineage; RR: relative risk; CR: Complete remission; CRi: Complete remission with incomplete blood count recovery; HI: Hematologic improvement; MLFS: Morphologic leukemia-free state; ORR: Overall response rate; OS: Overall survival; EFS: Event-free survival; CI: Confidence incidence; PFS: Progression-free survival.
HMAs and HDAC inhibitors were tested. Most studies showing a synergy were single-arm trials. Sequential multi-arm studies, comparing therapeutic effects between the combination of HMAs with HDAC inhibitors and monotherapy of HMA, yielded disappointing results. In a multi-center, open-label, prospective, randomized phase II trial, 259 patients with AML (n = 217) and MDS (n = 42) randomly received either 5-AZA alone (75 mg/m² × 7 days, every 4 weeks) or 5-AZA in combination with vorinostat (300 mg orally, twice a day from day 3 to day 9). The combination regimen did not improve the ORR (P = 0.84) or OS (P = 0.32). Two larger phase II clinical trials that compared combined treatment with 5-AZA and HDAC inhibitors (entinostat or vorinostat) to 5-AZA monotherapy, also showed disappointing survival benefits for the combination therapy.

In addition, more hematologic side effects occurred in the combined regimen, leading to earlier termination of this treatment. A molecular basis for the lower response rate in the combination regimen showed lower reversal of methylation in promoter regions that were more prominent in the combined regimen than in the 5-AZA monotherapy. In addition, the therapeutic schedule of HDAC inhibitors in these trials was heterogenous in terms of the molecular targets. The pleotropic influence of these drugs might have cause the excessive cell toxicity, leading to reduced treatment duration and insufficient exposure time to drugs. Therefore, molecular biomarkers that can predict responses are highly desirable. Future challenges for the combination regimen of HDAC inhibitors and HMAs are optimization of therapeutic schedules, including sequence of administration, dose of each drug, and drug administration time. Elucidating these aspects is important as pharmacodynamic antagonism between the drugs that were included in various regimens has been a critical problem, and must be settled in future clinical trials. Choosing more selective HDAC inhibitors is also of importance. Entinostat particularly targeting HDACs and vorinostat with less selection acting on other protein deacetylases have demonstrated similar results in MDS patients. But their utility in treating AML patients remains to be explored. It is also important to explored whether novel HDAC inhibitors, such as belinostat, pracinostat, or panobinostat can show any additional therapeutic benefit. To date, only one single-arm multicenter phase II study has evaluated the safety and efficacy of pracinostat in combination with 5-AZA in AML patients ≥65 years old who were ineligible for IC. The patients received pracinostat 60 mg/day, 3 days/week, for 3 consecutive weeks, plus 5-AZA at a daily dose of 75 mg/m² for one week, in a 4-week course. Twenty-six of the fifty patients (52%) responded to the treatment, including 42% of CR, 4% of CRi, and 6% of morphologic leukemia-free state. These results are higher than historical data for 5-AZA alone. Median OS and PFS were 19.1 and 12.6 months, respectively, with a 1-year OS probability of 62%. When treated with the combined regimen, 43 cases (86%) had at least one grade 3 or worse therapy-related unfavorable events. Mortality rates in the 30 and 60-day treatments were 2% and 10%, respectively. It was concluded that pracinostat, combined with 5-AZA, was tolerable and active in the first-line treatment of older AML patients ineligible for IC, which contributed to a phase III clinical trial of 5-AZA with/without pracinostat.

Furthermore, due to the limited response to combination of HDAC inhibitors and HMAs, and the synergistic effects between ICs and either HDAC inhibitors or HMAs in experiments and clinical trials, a phase III multi-center clinical trial has been performed to investigate the safety and efficacy of epigenetic modifiers (chidamide and DAC) plus aclarubicin, cytarabine, and G-CSF, in 93 r/r AML patients. In total, 24 r/r AML patients achieved CR and 19 cases obtained CRi, with 46% of ORR. The OS of the 43 responders was remarkably prolonged. 55.6% of patients with mutations involved in transcription factors and epigenetics but without FLT3-ITD mutation had CR/Cri, whereas the ORR was 36.7% for the remaining patients. It was therefore clear that the combined CDCAG regimen was well-tolerated and effective in r/r AML patients. Patients with gene aberrations associated with epigenetics and transcription factors, but without FLT3-ITD, might benefit from this novel regimen.

Conclusions

Epigenetic therapy in AML treatment is still in its infancy, but has developed quickly, holding great promise as a new therapeutic approach. In some early trials, treatment of AML patients with HMAs or HDAC inhibitors alone achieved modest or even disappointing therapeutic effects. Combination regimens, such as co-treatment with HMAs/HDAC inhibitors and IC, resulted in better response rates and prolonged survival, when compared to HMAs or HDAC inhibitors monotherapy. However, the therapeutic effects of combination of HMAs with HDAC inhibitors still need to be further explored from various aspects. Further research is also necessary to identify the therapeutic effect of the triple combination of HMAs, HDAC inhibitors, and IC.

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Conflicts of interest

None.

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