Abstract Epigenetics refers to a stable, mitotically perpetuated regulatory mechanism of gene expression without an alteration of the coding sequence. Epigenetic mechanisms include DNA methylation and histone tail modifications. Epigenetic regulation is part of physiologic development and becomes abnormal in neoplasia, where silencing of critical genes by DNA methylation or histone deacetylation can contribute to leukemogenesis as an alternative to deletion or loss-of-function mutation. In acute myelogenous leukemia (AML), aberrant DNA methylation can be observed in multiple functionally relevant genes such as \( p15, p73, E-cadherin, ID4, RAR\beta2 \). Abnormal activities of histone tail-modifying enzymes have also been seen in AML, frequently as a direct result of chromosomal translocations. It is now clear that these epigenetic changes play a significant role in development and progression of AML, and thus constitute important targets of therapy. The aim of targeting epigenetic effector protein or “epigenetic therapy” is to reverse epigenetic silencing and reactivate various genes to induce a therapeutic effect such as differentiation, growth arrest, or apoptosis. Recent clinical studies have shown the relative safety and efficacy of such epigenetic therapies.

Introduction

Carcinogenesis is a multistep process at the molecular level [64], driven by genetic alterations such as gene mutation and deletion, resulting in activation of oncogenes or inactivation of tumor suppressor genes [64]. Epigenetic changes have also been shown to play a significant role in the malignant transformation of cells [84, 12]. Epigenetics refers to a stable, mitotically perpetuated regulatory mechanism of gene expression without an alteration of the gene coding
sequence. Epigenetic mechanisms include DNA methylation and histone tail modifications such as acetylation and methylation [84, 12]. Epigenetic changes can lead to carcinogenesis by silencing critical genes [84, 12]. DNA methylation is very stable and maintained once established, except in special states such as embryogenesis [146, 81]. Histone modifications are more dynamic biochemical changes in the context of expression regulation [146, 81] but are also involved in stable gene silencing. Another, more flexible mechanism of epigenetic regulation is through small regulatory non-coding antisense RNAs, which can achieve transcriptional or post-transcriptional gene silencing [9], although the role of these processes in carcinogenesis is unknown. Over the past decades, alterations in DNA methylation and histone modifications in leukemogenesis have been well described and are now recognized as targets of therapy for AML and other hematological malignancies.

**DNA Methylation and DNA Methyltransferase (DNMT)**

The addition of a methyl-group to cytosine forming 5-methylcytosine in DNA has genetic and epigenetic effects on cellular development, differentiation, and carcinogenesis [84, 12]. In mammalian DNA, cytosine methylation is restricted to cytosine followed by guanosine (the CpG dinucleotide) [84, 12]. DNA methylation is accomplished by DNA methyltransferases (DNMTs), which catalyze the covalent addition of a methyl group to the 5' position of cytosine from a donor S-adenosylmethionine [67]. Three different proteins, DNMT1, DNMT3A, and DNMT3B, have been shown to have DNA methyltransferase catalytic activity in mammalian cells [123, 122, 9]. In general, DNMT1 serves as a maintenance DNMT, while DNMT3A and 3B serve as de novo DNMTs introducing methyl groups to previously unmethylated CpG sites [123, 122]. DNMT3L, another type of DNMT, does not have catalytic activity but has been identified as a stimulator of the catalytic activity of DNMT3A and DNMT3B [21, 147, 11]. Once DNA methylation is established in a CpG nucleotide, it is maintained after cell division through the activity of DNMTs, which localize to replication foci to work on newly synthesized hemi-methylated DNA [132, 102]. Recent studies suggest that DNA methylation status is determined via complex mechanisms where DNMTs interact with each other and with other proteins to induce DNA methylation [134, 53]. The major target of DNA methylation in normal mammalian cells is repeated transposable sequences, but it also plays a key role in imprinting and X chromosome inactivation in women [84, 12].

CpG sites are rare in the human genome relative to their predicted frequency, presumably because they were eliminated during evolution through C to T mutations of methylcytosine [13]. On the other hand, the human genome contains small regions with clusters of CpG sites, called “CpG islands,” where the frequency of CpG is higher than expected [13]. About half of all human
genes have CpG islands in their promoter regions, and these are not usually methylated in normal tissues, regardless of the transcriptional status of the gene. Methylation in a CpG island is associated with changes in chromatin organization and consequent repression of gene transcription (Fig. 1). One mechanism by which gene silencing is achieved is that of methylation of cytosine residues in CpG dinucleotides triggers the binding of methyl-binding proteins to DNA, which attracts histone deacetylases and histone methylases that eventually modify the structure of histones into a condensed chromatin state [119]. Condensation of the chromatin prevents specific transcription factors or DNA-dependent RNA polymerase from having access to the promoter region to cause gene silencing [84, 12]. Histone H3 lysine 9 (H3K9) methylation appears to trigger further DNA methylation through a feedback loop, thus reinforcing gene silencing [7, 149]. Since CpG methylation is maintained after cell division, gene silencing by DNA methylation is also maintained and is essentially stable once it is established. It can be only reversed physiologically and reset in early embryogenesis [116]. About half of human genes do not have CpG islands in their promoters. In these cases, DNA methylation can mark the silenced state but can be reversed physiologically by activation of gene expression [16]. The extent to which non-CpG island methylation plays a role in carcinogenesis remains unclear.

**Fig. 1** DNA methylation and gene silencing. Each circle indicates CpG sites, filled circle being methylated CpG, open circle unmethylated CpG, and white box indicates exon 1. TSS, transcription start site.

**DNA Methylation in AML**

Cancers have altered patterns of DNA methylation. The global DNA methylation level is often decreased in malignant cells. Simultaneously, hypermethylation also occurs in specific regions of the genome [84, 12]. Hypomethylation was initially postulated as a mechanism of carcinogenesis through activation of oncogenes [50]. It is also known that hypomethylation is associated with chromosomal instability in vitro, and this may play a role in carcinogenesis [24]. On the other hand, aberrant DNA hypermethylation can clearly contribute to
carcinogenesis by silencing tumor suppressor genes, and thus play an important role as an alternative mechanism to deletion or loss-of-function mutation for eliminating expression of functional proteins [84, 12].

Hypermethylation in some genes can be observed in normal tissues during the process of aging [75]. Other genes are methylated exclusively in cancer cells and they may arise from a combination of gene-specific predisposition to methylation and rare chance events that lead to gene silencing and a selective advantage for affected cells [75]. Different malignant diseases are associated with unique DNA methylation patterns. For example, the RB1 gene can be found methylated primarily in retinoblastoma [135], and VHL gene methylation mainly occurs in renal cell carcinoma [69]. In AML, promoter methylation of p15INK4B has long been known [68], and the methylation of this gene in MDS appears to be associated with poor prognosis and a higher chance of developing AML [154]. Other frequently methylated genes in AML are summarized in Table 1. It should be noted that only a few of those genes have known tumor suppressor function and that some are not expressed in normal hematopoietic cells [151]. Promoter hypermethylation in cancer therefore is not necessarily limited to silencing of critical genes. Also, in some cases, methylation can be detected experimentally, but does not lead to significant gene silencing because of low density of methylation or because the involved CpG island is not in the promoter region of the genes. Therefore, only some of the methylated CpG islands are pathophysiologically significant to the neoplastic process. Nevertheless, frequent gene-specific methylation is of potential value for disease classification and/or prognostication [151]. Many cancers are characterized by intense methylation of multiple genes simultaneously, a phenomenon termed CpG island methylator phenotype [76, 91, 133], and this characterizes AML as well. Moreover, frequent aberrant gene methylation is generally associated with poor prognosis [76, 91, 133], including in AML [151].

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Methylation prevalence</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>p15</td>
<td>3171%</td>
<td>Cyclin-dependent kinase inhibitor, TGF-beta-induced growth arrest.</td>
<td>[151, 77, 125, 54, 80, 45, 27, 28, 111]</td>
</tr>
<tr>
<td>p16</td>
<td>0–38%</td>
<td>Cyclin-dependent kinase inhibitor. Tumor suppressor</td>
<td>[151, 62]</td>
</tr>
<tr>
<td>p73</td>
<td>10–13%</td>
<td>Participates in the apoptotic response to DNA damage. Putative tumor suppressor</td>
<td>[54, 45]</td>
</tr>
<tr>
<td>HIC1</td>
<td>83% at intron 2, 0% at promoter</td>
<td>Transcription factor. Putative tumor suppressor</td>
<td>[110]</td>
</tr>
<tr>
<td>ID4</td>
<td>87%</td>
<td>DNA–binding inhibitor. Putative tumor suppressor</td>
<td>[169]</td>
</tr>
</tbody>
</table>
The causes of abnormal methylation in cancer remain poorly defined. High levels of DNMT activity in primary cancer cells have been reported in many studies [46, 79, 120, 38, 57, 82] but not in all [99, 43]. In one study, high expression of \textit{DNMT3a} and \textit{DNMT3b} genes in AML analyzed by gene expression profiling was associated with poor prognosis [17]. When interpreting these results, it is important to be aware that DNMT expression is regulated with the growth state of cells and that rapidly dividing cells have high levels of expression of DNMT [148]. Furthermore, it is not yet clear whether overexpression of DNMT is responsible for increased promoter methylation leading to transcriptional repression.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Methylation prevalence</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{RAR\beta2}</td>
<td>18–20%</td>
<td>Nuclear transcriptional regulator, which limits growth of many cell types by regulating gene expression</td>
<td>[54, 45]</td>
</tr>
<tr>
<td>\textit{DAPK}</td>
<td>3–61%</td>
<td>Serine/threonine kinase which acts as a positive regulator of apoptosis.</td>
<td>[54, 45]</td>
</tr>
<tr>
<td>\textit{CDH1} (\textit{E-cadherin})</td>
<td>13–69%</td>
<td>Calcium-dependent cell adhesion protein and a potent invasion suppressor role</td>
<td>[125, 54, 45, 111]</td>
</tr>
<tr>
<td>\textit{SHP1}</td>
<td>52%</td>
<td>Negative regulator of Jak/STAT signaling pathway</td>
<td>[26]</td>
</tr>
<tr>
<td>\textit{MGMT}</td>
<td>5%</td>
<td>Repair alkylated guanine in DNA</td>
<td>[54]</td>
</tr>
<tr>
<td>\textit{FHIT}</td>
<td>14%</td>
<td>Involved in purine metabolism. Putative tumor suppressor</td>
<td>[80]</td>
</tr>
<tr>
<td>\textit{CRBP1}</td>
<td>28%</td>
<td>Carrier protein involved in the transport of retinol. Putative tumor suppressor</td>
<td>[48]</td>
</tr>
<tr>
<td>\textit{ER}</td>
<td>40–54%</td>
<td>Estrogen receptor</td>
<td>[151, 125, 45, 111]</td>
</tr>
<tr>
<td>\textit{SOCS1}</td>
<td>39%</td>
<td>Negative regulator of cytokine signaling</td>
<td>[45]</td>
</tr>
<tr>
<td>\textit{WIT-1}</td>
<td>49%</td>
<td>Unknown</td>
<td>[128]</td>
</tr>
<tr>
<td>\textit{MYOD1}</td>
<td>61%</td>
<td>Transcription factor which regulates muscle cell differentiation by inducing cell cycle arrest</td>
<td>[151]</td>
</tr>
<tr>
<td>\textit{PITX2}</td>
<td>64%</td>
<td>Transcription factor that regulates procollagen lysyl hydroxylase gene expression</td>
<td>[151]</td>
</tr>
<tr>
<td>\textit{GPR37}</td>
<td>47%</td>
<td>G-protein-coupled receptor 37 precursor</td>
<td>[151]</td>
</tr>
<tr>
<td>\textit{SDC4}</td>
<td>56%</td>
<td>Cell surface proteoglycan that bears heparan sulfate</td>
<td>[151]</td>
</tr>
<tr>
<td>\textit{MEIS1}</td>
<td>64% of AML1-ETO AML</td>
<td>Homeodomain genes which cooperates with HoxA9</td>
<td>[95]</td>
</tr>
<tr>
<td>\textit{THBS1}</td>
<td>25%</td>
<td>Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions</td>
<td>[151]</td>
</tr>
<tr>
<td>\textit{Calcitonin}</td>
<td>71%</td>
<td>Peptide hormone that reduces serum calcium</td>
<td>[111]</td>
</tr>
</tbody>
</table>

The causes of abnormal methylation in cancer remain poorly defined. High levels of DNMT activity in primary cancer cells have been reported in many studies [46, 79, 120, 38, 57, 82] but not in all [99, 43]. In one study, high expression of \textit{DNMT3a} and \textit{DNMT3b} genes in AML analyzed by gene expression profiling was associated with poor prognosis [17]. When interpreting these results, it is important to be aware that DNMT expression is regulated with the growth state of cells and that rapidly dividing cells have high levels of expression of DNMT [148]. Furthermore, it is not yet clear whether overexpression of DNMT is responsible for increased promoter methylation leading to transcriptional repression. In
one study in AML, overexpression of DNMT1 and 3b was associated with \(p15^{INK4B}\) gene hypermethylation [114]. In another study, however, high levels of expression of DNMT1 in MDS were not associated with \(p15^{INK4B}\) hypermethylation [3]. Thus, it remains unclear whether DNMT overexpression contributes functionally to malignant transformation.

**Histone Modifications**

The DNA of eukaryotic cells is packaged into chromatin. The primary subunit of chromatin, the nucleosome, consists of an octamer of core histone proteins, i.e., H3/H4 tetramer and two H2A/H2B dimers, surrounded by 146 bp of DNA [51]. A change in local chromatin architecture alters the accessibility of transcription factors to DNA (Fig. 2) [51]. The chromatin structure is largely determined by posttranslational modifications of core histone proteins, which in turn affect gene expression [51]. In general, increased acetylation is associated

![Fig. 2](image-url)  
**Fig. 2** Histone modifications and chromatin condensation (color). (A) Open chromatin. Histone H3 lysine 9 is acetylated and histone H3 lysine 4 is methylated. Nucleosomes (green ovals) are loosely spaced, and DNA (black strings) is accessible to transcription factor and RNA polymerase. The gene therefore is transcriptionally active. (B) Condensed chromatin. Lysine 9 is deacetylated, and methylated. Nucleosomes are tightly packed, and the gene becomes transcriptionally inactive. Ac indicates acetylation; K4M, lysine 4 methylation; K9Ac, lysine 9 acetylation; K9M, lysine 9 methylation; HAT, histone acetyltransferase; HDAC, histone deacetylase; K4HMT, lysine 4 histone methyltransferase; K9HMT, lysine 9 histone methyltransferase; RNA-poly, RNA polymerase; and TF, transcription factor.
with increased transcriptional activity, whereas decreased acetylation is associated with repression of gene expression [51]. Recent studies have revealed that histone tails undergo many other modifications such as methylation, phosphorylation, ubiquitination, and sumoylation [51, 90]. Such histone tail modifications are referred to as “histone code,” which collectively can characterize the transcriptional status of the gene [81].

Histone deacetylases (HDACs) and the family of histone acetyl transferases (HATs) are involved in determining the state of acetylation of histones [138, 137, 152]. In addition to deacetylation of histones, HDACs regulate physiology by deacetylating transcription factors such as p53, E2F1, and others, [106, 83, 109] or other proteins such as α-tubulin, importin-a7, and others [63, 164, 8, 14, 34, 52, 72]. Histone methylases and demethylases regulate methylation of specific residues resulting in activation of gene expression (histone H3 lysine 4 [H3K4]) or repression of gene expression (histone H3 lysine 9 and 27) [173, 131, 100]. The polycomb group family of proteins, which are essential to the stem cell phenotype, are also involved in determining chromatin structure through interactions with H3K27 [100].

**Histone Alterations in AML**

Histones can be altered in AML through three general mechanisms: (i) direct genetic alterations in histone modifiers, (ii) recruitment of HDAC by mutated proteins or fusion proteins, and (iii) recruitment by DNA methylation (Table 2).

(i) Direct genetic alterations in histone modifiers

A histone methyltransferase, MLL (also known as HRX or ALL1), on chromosome 11q23 is often fused with other genes leading to leukemogenesis
through chromatin modulation [32, 174, 150, 41]. The N-terminal fragment of MLL contains a DNA-binding site [168] and a transcriptional repression domain [172]. The repression domain consists of two different subdomains, one containing the DNA methyltransferase homology domain, which recruits repressor complexes including polycomb group proteins such as HPC2, Bmi1, and the corepressor CtBP [53, 36]. The other repression domain recruits HDAC1 and HDAC2 [165]. The N-terminal fragment of MLL also contains plant homeodomain (PHD) zinc fingers, which are involved in transcriptional regulation and chromatin-remodeling activity through interaction with Cyp33, a known suppressor of HoxA9 and HoxC8 transcription [49]. One of the critical functions of MLL is the maintenance of expression of HoxA9 and HoxC8 by binding to their promoter regions and keeping the chromatin open for transcription [113, 118]. The C-terminus of MLL contains a transcriptional-activation domain that binds to a HAT, CBP [47]. Furthermore, the C-terminal SET domain has histone H3 lysine 4 (H3K4)-specific histone methyltransferase (HMT) activity, and trimethylation of H3K4 is associated with transcriptional activation [113, 118]. In general, the different MLL chimeric proteins include the DNA-binding site and MT domains at the N-terminus but lack the SET domain [172]. It is thought that these result in inappropriately maintained expression of HOX genes involved in self-renewal, leading to leukemogenesis.

A histone acetyltransferase, CBP can be directly disrupted by chromosomal translocations in AML, resulting in the formation of fusion oncoprotein transcription factors. A rare chromosomal translocation observed in AML, t(8;16)(p11;p13) produces a fusion protein of CBP with the MOZ (monocytic leukemia zinc finger) gene [15]. This fusion results in a small deletion of the N-terminal 266 amino acids of CBP leaving the rest of the molecule intact [15]. Interestingly, the MOZ gene also has a putative acetyltransferase domain that is retained in the MOZ–CBP fusion [15]. It is believed that the leukemogenic effect is not merely from the disruption of the normal function of CBP but that the fusion protein results in aberrant recruitment of CBP to MOZ-regulated genes’ promoters leading to abnormal expression of those genes.

Another histone acetyltransferase, p300, was found to be fused to the MLL gene in an AML patient carrying a t(11;22)(q23; q13) translocation [74]. This results in a fusion product that preserves most of the p300 molecules. The authors suggested that the basis for the leukemogenesis of t(11; 22)-AML is the inability of p300 to regulate cell cycle and cell differentiation after fusion with MLL.

(ii) Genetic alterations leading to abnormal recruitment of histone modifiers

Common translocations in AML result in fusion proteins that affect gene transcription by HAT/HDAC recruitment. For example, translocations involving the AML1 gene (also known as RUNX1 or CBFA2), such as t(8;21)(q22;q22), result in a fusion with other genes (ETO in the case of t(8;21)) [136, 42]. The N-terminus of AML1 binds to the promoter region of its target genes such as IL3 [153], GM-CSF [144], and MPO [6, 157, 112]. The C-terminus
of AML1 normally interacts with a co-activator complex containing p300, which has HAT activity, and will result in histone acetylation and transcriptional activation [136, 42]. In AML with an AML1/ETO translocation, the N-terminus of AML1 is maintained and can interact with DNA, but the p300 binding site of the C-terminus is replaced by ETO, which attracts a co-repressor complex containing N-CoR/Sin3/HDACs instead of HAT, resulting in transcriptional repression and a block of myeloid differentiation [136, 42]. Other AML1 partner genes such as TEL and MTG16 also mediate transcription repression [70, 73]. Similarly in AML with inv(16)(p13q22), the beta subunit of core-binding factor (CBFβ), which partners with AML1, is fused with smooth muscle myosin heavy chain gene (MYH11), leading to transcriptional repression of AML1-transactivated genes and suppression of myeloid differentiation [136].

Another translocation that results in altered histone modifications is t(15;17)(q21;q21), characteristic of acute promyelocytic leukemia. This translocation fuses PML with RARα (retinoic acid receptor alpha). RARα is a ligand-dependent transcriptional activator that binds as a heterodimer with members of the RXR (retinoid X receptor) family of nuclear receptors [170]. In the absence of a ligand, the heterodimer binds either N-CoR [93, 71] or SMRT [23, 22], which then form a co-repressor complex containing Sin3 and HDAC [2, 94, 66]. When bound to a ligand (retinoic acid), RARα functions as a transcription activator. The fusion PML–RARα is constitutively complexed with co-repressor molecules at physiologic levels of retinoic acid resulting in repression of target gene expression through histone deacetylases [61, 105, 65].

(iii) Histone modification by DNA methylation

Histone alterations can also be recruited by DNA methylation. Methylated CpG sites trigger the binding of methyl-binding proteins to DNA, which attracts histone deacetylases and histone methylases. This leads to H3K9 deacetylation and methylation, as well as demethylation of H3K4 [119].

Epigenetic Therapy

The aim of epigenetic therapy is to reverse epigenetic silencing and reactivate various genes hoping for a therapeutic effect such as differentiation, growth arrest, or apoptosis. Indeed, pharmacologic inhibition of DNMT or HDAC in vitro has been shown to result in reactivation of gene expression for genes silenced either physiologically or pathologically [11, 108]. The concentrations of these agents required for this effect can readily be achieved in vivo [11, 108]. There have been extensive clinical studies in this field over the past decade, focusing on inhibitors of DNMT and HDAC.
**DNMT Inhibitors**

5-Azacytidine (azacitidine) and its deoxy analogue 5-aza-2’-deoxycytidine (decitabine) are the two nucleoside analogues that have been studied most extensively as DNMT inhibitors (Table 3). Both have been recently approved by the Food and Drug Administration (FDA) for the treatment of MDS. Decitabine is initially phosphorylated by deoxycytidine kinase, and eventually becomes decitabine triphosphate, which is incorporated into DNA. Azacitidine is phosphorylated and activated by uridine-cytidine kinase and is mainly incorporated into RNA and markedly inhibits protein synthesis [29]. Azacitidine diphosphate is also reduced by ribonucleotide reductase to the corresponding deoxynucleotide diphosphate, decitabine diphosphate, which is further phosphorylated by nucleoside diphosphate kinases to decitabine triphosphate [29]. Incorporation of a high concentration of decitabine triphosphate into DNA can inhibit DNA synthesis [104, 103, 31]. At lower concentrations, decitabine triphosphate covalently binds to DNMT after it has been incorporated into DNA, which eventually causes degradation of DNMT without DNA synthesis arrest [1, 35]. DNA replication in the absence of DNMT leads to hypomethylation induction and gene reactivation [29].

### Table 3  Summary of selected clinical trials of azacitidine and decitabine in AML and MDS

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Dose</th>
<th>Disease (N)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azacitidine</td>
<td>1000–1500 mg/m²/course</td>
<td>Relapsed AML (&gt;200)</td>
<td>15–30</td>
<td>2–10</td>
<td>Reviewed in [58]</td>
</tr>
<tr>
<td></td>
<td>Combination with other chemotherapies</td>
<td>Relapsed AML (66)</td>
<td>40</td>
<td>12</td>
<td>Reviewed in [156]</td>
</tr>
<tr>
<td></td>
<td>250 mg/m² × 2 days with etoposide and amsacrine</td>
<td>Relapsed AML (17)</td>
<td>39</td>
<td>–</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>75 mg/m²/d SQ</td>
<td>MDS (270)</td>
<td>6</td>
<td>9</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>(summary of phase II and III studies)</td>
<td>MDS (16)</td>
<td>19</td>
<td>6</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>350–700 mg/m²/course followed by butyrate</td>
<td>Pediatric-relapsed AML (6)</td>
<td>33</td>
<td>17</td>
<td>[115]</td>
</tr>
<tr>
<td>Decitabine</td>
<td>37–67 mg/kg at 1 mg/kg/h</td>
<td>Pediatric-relapsed AML (6)</td>
<td>33</td>
<td>17</td>
<td>[115]</td>
</tr>
<tr>
<td></td>
<td>90–120 mg/m² Q8H for 3 days</td>
<td>Untreated high-risk AML (12)</td>
<td>25</td>
<td>8</td>
<td>[126]</td>
</tr>
<tr>
<td></td>
<td>90 mg/m² Q8H for 3 days</td>
<td>Relapsed AML (8)</td>
<td>13</td>
<td>–</td>
<td>[89]</td>
</tr>
</tbody>
</table>
5-Azacytidine (Azacitidine)

During the 1970s and 1980s, azacitidine was investigated as a treatment for various solid tumors [156, 158] and hematologic malignancies [139, 101, 155, 10]. In these early clinical trials, azacitidine was mostly used as a cytotoxic agent. Single-agent azacitidine used at relatively high dose (600–1500 mg/m$^2$/course) in refractory AML resulted in about 45% overall responses [58]. Combination therapy with other chemotherapeutic agents resulted in an overall response rate of 30–60% [156, 145]. High-dose azacitidine was toxic, however, and this approach was abandoned.

In parallel, in vitro studies have shown that a lower dose of azacitidine induces cell differentiation and apoptosis by promoting the expression of genes that are silenced by hypermethylation [85]. Low-dose azacitidine has been applied to clinical settings, mostly in MDS. A phase III study where patients with MDS were randomly assigned to receive 75 mg/m$^2$ subcutaneously for 7 days, repeated on a 28-day cycle, or supportive care only [140] yielded a response rate of 16% (CR 6%, PR 10%). This led to approval of azacitidine by the FDA for treatment of MDS.

5-Aza-2'-deoxycytidine (Decitabine)

Decitabine was initially evaluated two decades ago in cancer treatment and has shown significant antitumor activity in hematologic malignancies [4]. In an early study by Momparler et al. [115], the overall response rate of single-agent decitabine (37–81 mg/kg over 40–60 h; the length of infusion was increased stepwise) in relapsed pediatric leukemia was 37%, including a CR of 22%. Petti et al. [126] reported a 25% CR rate in a pilot study of single-agent decitabine (90–120 mg/m$^2$ every 8 h for 3 days) in the treatment of 12 untreated patients

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Table 3 (continued)

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Dose</th>
<th>Disease (N)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapsed AML (30 and 33)</td>
<td>125 mg/m$^2$ Q12H × 6 days with amsacrine or idarubicin</td>
<td>26 and 45</td>
<td>–</td>
<td></td>
<td>[163]</td>
</tr>
<tr>
<td>Relapsed AML (35 and MDS</td>
<td>5–20 mg/m$^2$ IV × 5–10 days</td>
<td>14 and 29</td>
<td>–</td>
<td></td>
<td>[77]</td>
</tr>
<tr>
<td>MDS (66)</td>
<td>45 mg/m$^2$ daily × 3 days</td>
<td>20</td>
<td>29</td>
<td></td>
<td>[161]</td>
</tr>
<tr>
<td>MDS (89 versus 81)</td>
<td>15 mg/m$^2$ Q8H for 3 days versus supportive care</td>
<td>9 versus 0</td>
<td>8 versus 0</td>
<td></td>
<td>[87]</td>
</tr>
<tr>
<td>MDS (96)</td>
<td>100 mg/m$^2$/course over 5 or 10 days</td>
<td>34</td>
<td>38 (including hematologic improvement)</td>
<td>[88]</td>
<td></td>
</tr>
</tbody>
</table>
with AML with poor prognosis. In relapsed and refractory adult AML, 90 mg/m² of single-agent decitabine every 8 h for 3 days resulted in only one CR among eight patients treated [89]. Combination therapy studies of decitabine against refractory or relapsed AML were performed by the EORTC, with a regimen of 125 mg/m² of decitabine every 12 h for 6 days (total 1500 mg/m²) with amsacrine (120 mg/m² on days 6 and 7; in 30 patients), or idarubicin (12 mg/m² on days 5–7; in 33 patients) [163]. The CR rates were 26 and 45%, respectively. The median disease-free survival was 8 months.

On the basis of in vitro studies showing that lower-dose decitabine produced more hypomethylation than high doses [85], low doses of decitabine have been evaluated in hematological malignancies [171, 162, 161]. Following promising results in early phase trials, a randomized phase III study was performed in patients with MDS [87], yielding an overall response rate in the decitabine group of 17% including 9% CR. This trial led to the recent approval of decitabine in MDS.

Although low-dose decitabine is clinically active, its optimal dosing schedule is not known. In a phase I trial in relapsed or refractory hematologic malignancies, decitabine activity was found to be significant at low doses, but response were lost with dose escalation, consistent with its mechanism of action [77].

**Mechanisms of Response to Hypomethylating Agents**

Given the dual nature of this class of agents (cytotoxicity and hypomethylation), the in vivo mechanisms of responses need to be elucidated to guide further trials. Yang et al. examined global DNA methylation changes surrogated by LINE and Alu methylation [167] in the peripheral blood of patients with leukemia treated with decitabine [166]. There was a dose-dependent linear decrease in methylation on day 5 at low doses of 5–20 mg/m²/day with no significant increase in hypomethylation beyond 20 mg/m²/day suggesting a plateau effect. In another study where global methylation changes were analyzed during decitabine treatment in patients with MDS [124], LINE hypomethylation was a good pharmacodynamic surrogate of decitabine’s hypomethylating activity but was not connected with clinical activity. Thus, while global methylation decreases after decitabine therapy, it is not a good predictor of responses.

Daskalakis et al. showed in patients with MDS that p15INK4B hypomethylation induction several weeks after therapy was associated with a clinical response [37]. However, in other studies [166, 124, 78], immediate induction of p15INK4B hypomethylation (i.e., day 5–10 after treatment) was not correlated with subsequent response.

To determine whether hypomethylation leads to gene induction, p15INK4B expression levels were analyzed in patients with MDS enrolled on a study of decitabine in three dosing schedules [124]. The expression levels of p15INK4B were induced significantly after treatment, and the induction was higher in
responders than non-responders. It is thus possible that the key to decitabine responses is not hypomethylation per se, but sustained tumor suppressor gene hypomethylation and activation. This deserves confirmation in larger trials.

**HDAC Inhibitors**

HDAC inhibitors reverse the deacetylation of histone tails and activate the expression of selected genes [108]. They were originally discovered based on screens for agent that induce cellular differentiation in vitro. Several structural classes of HDAC inhibitors have now been identified, and some have been evaluated in clinical trials.

Vorinostat (suberoylanilide hydroxamic acid, SAHA) is a hydroxamic acid that is highly potent in vitro. In a recent phase I trial of oral vorinostat for hematologic malignancies [56] several responses were observed in AML (9 of 31 patients, 29%), including 1 CR (duration, >6 months), 2 CRs without platelet recovery (duration, 4–6 weeks), 1 PR, and 5 complete marrow responses (blasts less than 5%).

Depsipeptide is a cyclic tetrapeptide that is also a potent HDAC inhibitor in vitro [117]. It has significant clinical activity in cutaneous T-cell lymphoma [127]. In a multicenter phase II trial [121], depsipeptide was administered to 18 patients with refractory or relapsed AML. Two patients had disappearance of bone marrow blasts in the setting of a normocellular marrow, with concomitant recovery of near-normal hematopoiesis following 1–2 cycles of therapy. The responses, however, were short lived.

Valproic acid (VPA) is an antiepileptic agent that has been shown to inhibit HDAC activity at low levels [60, 160, 33]. In a phase I study, high doses of VPA were administered to result in serum concentrations of 50–100 µg/mL in patients with MDS, with, or without ATRA [92]. Responses were observed in 8 (44%) of 18 patients given VPA monotherapy. More limited activity was seen in AML [129]. Thus, HDAC inhibitors form a class of epigenetic acting agents that have promising activity in AML. However, though histone acetylation has been demonstrated in vivo [83], it has been shown no correlation with response, and there remains a distinct possibility that responses to HDAC inhibitors are related to non-histone acetylation or to other mechanisms [83].

**Combination Epigenetic Therapy**

Elucidation of multiple interacting mechanisms of gene silencing has led to an interest in combining drugs that affect multiple epigenetic pathways. For example, DNA methylation inhibitors and HDAC inhibitors are synergistic in activating gene expression [20] and clinical trials of this approach have started. A combination of decitabine with VPA has shown promising activity in AML, with a response rate of 22% in patients with AML and MDS [55]. There is also
interest in developing drugs that affect other epigenetic pathways such as methyl binding proteins, histone methylation, and other histone deacetylases such as SIRT1. It is likely that such inhibitors will enter the clinic in next few years, and it will be interesting to combine them with existing drugs.

Conclusions

It is now clear that epigenetic changes play a significant role in the development and progression of AML. These epigenetic changes can be important targets of treatment, and recent clinical studies have shown the relative safety and efficacy of such epigenetic therapies. Although epigenetic modulation is effective in the treatment of AML, precise in vivo effects of these drugs have not been well described. Also, events downstream of gene expression induction such as apoptosis, senescence, and immunomodulation remain to be clarified. Further basic, translational, and clinical studies are essential to move the field forward.

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