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Epigenetic Modulation in Hematologic Malignancies: Challenges and Progress

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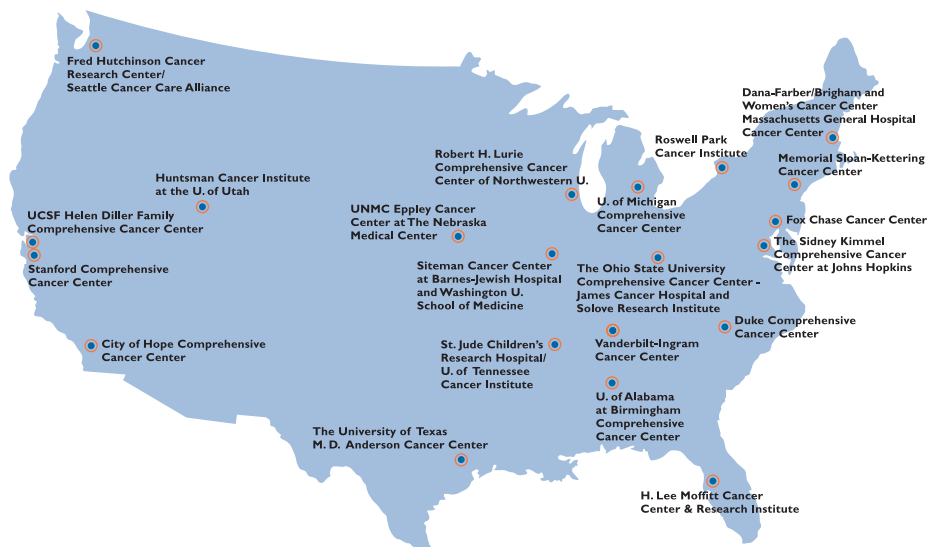
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Epigenetic Modulation in Hematologic Malignancies: Challenges and Progress

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Target Audience

This activity is intended for hematologists and oncologists involved in the diagnosis and management of hematologic malignancies.

Educational Objectives

After completion of this CME activity, participants should be able to:

- Describe the role of epigenetics in the development and progression of hematologic malignancies.
- Discuss DNA methylation and histone deacetylation and the role of therapies in targeting these pathways.
- Review the latest data on the use of epigenetic therapies to treat hematologic malignancies, including treatment-related adverse events.

Release and Expiration Dates

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Participants will read all portions of this monograph, including all tables, figures, and references. A post-test and an evaluation form follow this activity, both of which require completion. To receive your continuing education certificate, you will need a score of at least 70% on the post-test. The post-test and evaluation form must be completed and returned by November 30, 2010. It should take approximately 0.5 hours to complete this activity as designed. There are no registration fees for this activity. Certificates will be mailed within 3 to 4 weeks of receipt of the post-test.

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Constantine S. Mitsiades, MD, PhD, and Kenneth C. Anderson, MD

Key Words

Epigenetics, hematologic malignancy

Abstract

Genetic alterations, including gene mutations, and chromosomal amplifications, deletions, inversions, and translocations, are hallmarks of the molecular biology of cancer. These events lead to oncogene activation, formation of chimeric oncoproteins, and/or inactivation of tumor suppressor genes. Such genetic changes contribute to the neoplastic transformation of cells, as well as the eventual acquisition by malignant cells of a more aggressive biologic and clinical behavior. However, in recent years, it has become apparent that these genetic events are not the sole determinants of the biologic behavior of tumor cells. Indeed, it is becoming increasingly apparent that tumor cells with a given genotype exhibit a differential phenotype depending on the microenvironment in which they reside. Furthermore, extensive data have shown that derivative daughter cells of neoplastic, as well as normal cells, inherit changes in the patterns of gene expression that are not associated with changes in the primary DNA sequence but are instead related to changes in chromatin structure and its accessibility for transcriptional activity. These heritable gene expression changes that are not associated with changes in the primary nucleotide sequence are referred to as *epigenetic changes*. This review provides an overview of the regulation of the “epigenome” in neoplastic cells, with particular emphasis on DNA methylation and histone acetylation as therapeutic targets for hematologic malignancies. (*JNCCN* 2009;7[Suppl 8]:S1–S12)

Genetic alterations, including gene mutations, and chromosomal amplifications, deletions, inversions, and translocations are hallmarks of the molecular biology of cancer.^{1,2} These events lead to oncogene activation, formation of chimeric oncoproteins, and/or inactivation of tumor suppressor genes. Such genetic changes contribute to the neoplastic transformation of cells, as well as the eventual acquisition by malignant cells of a more aggressive biologic and clinical behavior. However, in recent years, it has become apparent that these genetic events are not the sole determinants of the

biologic behavior of tumor cells.³ Indeed, it is becoming increasingly apparent that tumor cells with a given genotype exhibit a differential phenotype depending on the microenvironment in which they reside.⁴ Furthermore, an extensive body of data has shown that derivative daughter cells of neoplastic, as well as normal cells, inherit changes in the patterns of gene expression that are not associated with changes in the primary DNA sequence but instead are related to changes in chromatin structure and its accessibility for transcriptional activity.^{5,6} Such heritable gene expression changes that are not associated with changes in the primary nucleotide sequence are referred to as *epigenetic changes*.

This review provides an overview of the regulation of the “epigenome” in neoplastic cells, with particular emphasis on DNA methylation and histone acetylation as therapeutic targets for hematologic malignancies.

Role of Epigenetics in Human Neoplasias

The DNA inside the nucleus of both normal and malignant cells is organized into nucleosomes, which are the basic functional units of chromatin. Each nucleosome corresponds to 147 base pairs of DNA wrapped around a multimeric complex of histones H2A, H2B, H3, and H4 (2 copies of each histone per nucleosome).⁷ Nucleosomes are linked to each other by DNA sequences (approximately 160–240 base pairs in length), which interact with histone H1 and can be further organized to form more compact helical structures that determine the degree of packaging of the chromatin. The concept of epigenetic regulation of gene transcription was based on observations that covalent chemical post-translational modifications of the tails of histone molecules lead to changes in gene transcription as a result of structural changes in the 3-dimensional conformation of chromatin.⁶ These posttranslational changes, which

include methylation, acetylation, phosphorylation, ubiquitination, and SUMOylation, modulate the accessibility of DNA to transcription factors and other regulators of gene expression.⁶ In addition, these posttranslational modifications of histones interact with another important component of epigenetic modification, namely, the methylation of DNA at CpG dinucleotide sites, to determine the state of transcriptional activity of corresponding genes.

In CpG dinucleotide sites, where a cytosine residue precedes guanosine, the C5 position of the pyrimidine ring of cytosine can receive a methyl group, donated by S-adenosyl methionine, in a biochemical reaction catalyzed by DNA methyltransferases. CpG dinucleotides are not uniformly distributed in the human genome. Instead, they appear to be over-represented in the so-called CpG islands, which are relatively short DNA regions (0.5 kb to a few kb) that are frequently located near promoter sequences of different genes.⁸ Cytosine residues in CpG islands, particularly those present within promoter regions, are typically not methylated in nonmalignant cells, while CpG dinucleotides in downstream sequences within the body of the gene and in the 3' untranslated region are typically present in lower density compared with CpG islands, and are predominantly methylated in nonmalignant cells. The hypomethylation of the promoter region and the presence of CpG methylation in the body of the gene facilitate the recruitment of transcription factors, transcriptional activators, and histone acetyltransferases (HATs) in the promoter region, while methylcytosine-binding proteins and histone deacetylases (HDACs) are recruited to the methylated CpG dinucleotides in the body of the gene. This pattern of differential recruitment of these factors is conducive to active transcription of the corresponding gene.⁵ Notable exceptions to this pattern for nonmalignant cells have been described in the context of gene imprinting and in genes within the regions of X-chromosome inactivation.^{9,10} In contrast to the DNA methylation pattern observed in normal cells, malignant cells exhibit a reversal in the distribution of CpG methylation,^{11,12} which involves 1) widespread hypomethylation in the body of genes, within intronic sequences, and within repetitive DNA sequences, and 2) hypermethylation in CpG islands within promoter regions.^{5,13-17}

Both of these events are considered capable of contributing to the neoplastic phenotype through

different, and potentially overlapping, mechanisms. Global hypomethylation has been proposed to have a negative overall effect on structural stability of chromosomal material because of increased aneuploidy (attributed to hypomethylation in centromeres),^{18,19} increased mitotic recombination (which predisposes to loss of heterozygosity and chromosomal rearrangements), and loss of normal imprinting patterns.^{20,21} In contrast, increased methylation in promoter-associated CpG islands is considered a key mechanism for silencing of tumor suppressor genes, such as p21^{22,23} or Rb.^{24,25} In fact, there are data to suggest that in many tumor models the frequency of hypermethylation of CpG islands in promoter regions of tumor suppressor genes is similar to the frequency of deletions or inactivating mutations of these genes.⁵ The precise mechanisms for hypermethylation of promoter region-associated CpG islands in tumor cells or for the apparent predilection of tumor suppressor gene promoters for this hypermethylation are not completely understood.³

Among the diverse posttranslational histone modifications that can influence gene transcription, acetylation and methylation have been studied in the greatest detail. The acetylation status of histones is regulated by the functionally opposing activities of HDACs and HATs. The latter transfer acetyl groups from acetyl-CoA to lysine residues in the histone tail, while the former facilitate the removal of these groups. The hyperacetylated state of histones has historically been thought to be associated with a structurally open chromatin and active transcription of corresponding genes, whereas histone deacetylation has been associated with suppression of gene expression and/or heterochromatin formation.^{26,27} This has been attributed to the fact that increased acetylation of histones neutralizes the positive charge of their lysine residues, thereby attenuating the electrostatic interaction of the nucleosome histone core with the negatively charged DNA backbone. Furthermore, it has been proposed that acetylated histones constitute sites for docking of bromodomain-containing regulatory factors necessary for transcriptional activation.²⁸ However, the relationship between histone acetylation status and transcription of individual genes is more complex. The activation of gene transcription is determined by several different functionally opposing epigenetic events. For instance, the processes of DNA methylation and histone acetylation seemed

to be functionally opposing, because DNA methyltransferases recruit HDACs to methylated CpG sites of gene promoters, further contributing to a transcriptionally inactive chromatin state.²⁹ Interestingly, there are data to suggest that, in the context of this functionally opposing role of DNA methylation and histone acetylation, the former appears to play a functionally dominant role, which can keep the corresponding genes in a transcriptionally silent status, despite increased histone acetylation.^{14,30,31} Other posttranslational modifications of histones can influence the state of transcriptional activity of genes. For instance, methylation of histones can either activate or suppress gene transcription, depending on the particular site on the *N*-terminal histone tail, where methylation takes place. The methylation of lysine residues 9 and 27 of histone H3 (H3K9 and H2K27) is typically associated with suppression of transcription, in contrast to the methylation of lysine 4 of histone H3 (H3K4), which is associated with transcriptional activation.^{32,33} To further underscore the complexity of this regulatory system, acetylation of H3K9 facilitates the methylation of H3K4, especially in genes with unmethylated CpG islands.^{17,34} Figure 1 illustrates epigenetic inactivation of tumor-suppressor genes and agents that may be able to partially restore this distorted epigenetic picture.³⁵

A growing number of pathways and molecular mediators critical for tumor cell proliferation, survival, drug resistance, metastatic potential, and other aspects of tumor cell biology are regulated at an epigenetic level. Many studies have been able to validate a direct relationship between increased DNA methylation and transcriptional silencing of genes known to have a negative regulatory role on cell cycle progression (e.g., *Rb*, p14, p15, p16, p57, p73);^{13,36-42} proapoptotic signaling (caspase-8, *DAPK1*, *TMS-1*);⁴³⁻⁴⁷ growth factor signaling (*SOCS1*, *SOCS3*, *CRBP1*, *RARbeta2*);^{39,44,48-52} repair of DNA damage (Fanconi anemia-BRCA pathway members, *GSTπ*, *hMLH1*, *O⁶MGMT*);^{13,39,53-56} inhibition of angiogenesis (*VHL*, *EFEMP1*, *BNIP3*, *BNIP3L*, *IGFBP3*, and *EGLN2*);⁵⁷⁻⁵⁹ or inhibition of metastatic potential (*TIMP3*, E-cadherin).^{44,54,60} In these cases, the transcriptional silencing appears to provide an advantage to the tumor cell by abrogating the expression and function of mediators that negatively affect the proliferative capacity, drug resistance, and metastatic potential of the tumor cell.

The main targets of DNA methylation in cancer cells appear to fall into 2 large categories: 1) targets of epigenetic silencing that seem to play a role in a broad spectrum of tumor neoplasias (e.g., *p16*, *Rb*),^{13,36-39,41,54,61,62} and 2) genes silenced in a tumor type-specific manner, reflecting putative tumor suppressive roles in select tissues.^{5,14,15}

Posttranslational modifications of histones also affect the expression and/or function of a pleiotropic spectrum of molecular pathways important for cancer cell biology. HATs and HDACs can regulate gene expression not only by affecting chromatin structure, but also by affecting the DNA binding and transcriptional activity of key transcription factors, such as *p53*, *STAT3*, *ETS*, and *RUNX1*.^{63,64}

Furthermore, histone methylation provides an additional level of regulation of gene expression through a complex system that involves methylation of arginine (either mono- or dimethylation) or lysine (mono-, di-, or trimethylation) residues. The methylation state of histones is regulated by the opposing action of histone methyltransferases and histone demethylases. Arginine methylation is typically associated with transcriptional activation, whereas lysine methylation can be associated with transcriptional repression (when the methylation involves residues H3K9, H3K27, and H3K20) or conversely constitutational activation (when the methylation involves residues H3K4, H3K36, and H3K79).^{65,66}

Epigenetic Changes as Prognostic Markers

The significance of epigenetic changes for neoplastic cells of hematologic malignancies is underscored by 2 observations: agents targeting epigenetic regulation have shown clinical activity for diverse hematologic malignancies; aberrations in epigenetic markers have been found to correlate with clinical outcome in several clinical settings of patients with hematologic malignancies. Perhaps the most robust evidence for the prognostic significance of epigenetic changes in hematologic malignancies has come from the setting of diffuse large B-cell lymphoma, where hypomethylation of the promoter region for the DNA repair enzyme *O⁶MGMT*⁶⁷ has been associated with significantly shorter progression-free and overall survival times among patients receiving cyclophosphamide-based chemotherapy for this disease. This observation

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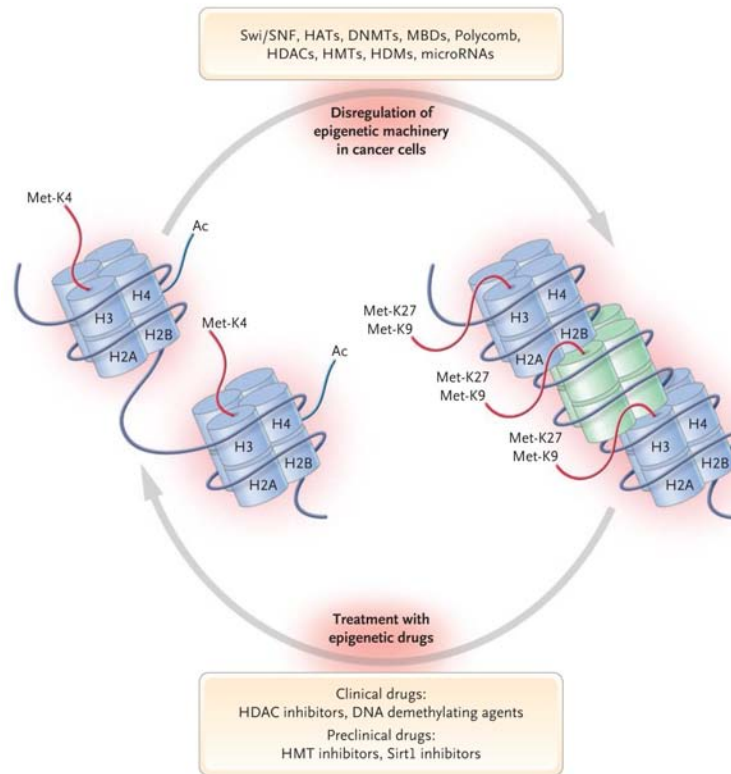


Figure 1 Epigenetic inactivation of tumor-suppressor genes.³⁵ In a normal cell, expression of the mRNA of a tumor-suppressor gene occurs in the context of an unmethylated promoter CpG island and histone modification, such as hyperacetylation and methylation of lysine 4 of histone H3. Gray cylinders indicate octamers of histones, consisting of histones H2A, H2B, H3, and H4. They form the nucleosomes, and the double strand of DNA is wrapped around them. A combination of selection and targeted disruption of the DNA methylation and histone-modifier proteins disrupts the epigenetic circumstances in the cancer cell. Epigenetic inactivation of tumor-suppressor genes is associated with dense CpG-island promoter hypermethylation and the appearance of repressive histone markers such as methylation of lysines 9 and 27 of histone H3. Epigenetic drugs can partially restore the distorted epigenetic picture by removing inactivation markers (e.g., DNA methylation) and inducing the presence of active markers (e.g., histone acetylation). Abbreviations: AC, acetylation; DNMTs, DNA methyltransferases; HATs, histone acetyltransferases; HDAC, histone deacetylase; HDMs, histone demethylases; HMTs, histone methyltransferases; MBDs, methyl-CpG-binding domain proteins; Met-K4, methylation of lysine 4; Met-K9, methylation of lysine 9; Met-K27, methylation of lysine 27; Sirt1, sirtuin 1; Swi/SNF, switching/sucrose nonfermenting chromatin-remodeling complex.

From Esteller M. Epigenetics in cancer. *N Engl J Med* 2008;358:1148–1159. Copyright © 2008. Massachusetts Medical Society. All rights reserved.

is compatible with similar data pertaining to response of glioma to carmustine⁶⁸ and other alkylating agents (alone or with radiation therapy).^{55,56,69,70} In the context of leukemias, p15 methylation was shown to be an independent predictor of inferior disease-free survival in acute promyelocytic leukemia,⁷¹ while patients with acute lymphoblastic leukemia with a higher number of hypermethylated tumor suppressor genes have a worse prognosis.⁷² Lastly, patients with myeloma harboring hypermethylation of the promoter regions for *p16*^{44,73,74} or *DAPK1*⁷⁵ have been reported to have an unfavorable prognosis after treatment with conventional antimyeloma therapies.

Pharmacologic Modulators of Epigenetic Regulation as Therapeutics for Hematologic Neoplasias

Currently, DNA methyltransferase inhibitors and HDAC inhibitors constitute the 2 classes of epigenetic agents that are most advanced in terms of clinical applications. Indeed, members of these 2 classes have already been approved by the FDA for various indications in hematologic malignancies. Further studies may reveal whether these classes of agents may also have activity in the context of solid tumors.

DNA Methyltransferase Inhibitors

Azacitidine (5-azacytidine) and decitabine (2'-deoxy-5-azacytidine) are the 2 DNA methyltransferase inhibitors currently approved by the FDA. Other DNA methyltransferase inhibitors currently in development include zebularine, SGI-110, and RG108. The 2 FDA-approved DNA methyltransferase inhibitors, as well as several of the currently developed compounds in this class, are nucleoside analogs (one exception is the RG108 compound, which is an active site inhibitor). Azacitidine, the prototypical member of this drug class, was initially studied in the 1960s for its properties as a classical cytotoxic chemotherapeutic agent. Indeed, being an analog of cytosine, azacitidine is incorporated into the DNA and, at high doses, inhibits DNA synthesis. However, it was noted that at lower doses (1–2 logs lower than the directly cytotoxic doses of the compound) azacitidine treatment is incorporated not only into the DNA during DNA synthesis,⁷⁶ but in that context, azacitidine also functions to inhibit DNA methyltransferase activity.

A phase III, international, randomized, open-label trial compared subcutaneous azacitidine treatment versus conventional care in patients with higher-risk myelodysplastic syndromes (MDS).⁷⁷ Conventional care was defined as best supportive care, low-dose cytarabine, or intensive chemotherapy, as selected by investigators before randomization. Median overall survival was 24.5 months for the azacitidine group versus 15.0 months for the conventional care group (hazard ratio, 0.58; 95% CI, 0.43–0.77; $P = .0001$). At last follow-up, 82 patients in the azacitidine group had died compared with 113 in the conventional care group. At 2 years, 50.8% of patients in the azacitidine group were alive compared with 26.2% in the conventional care group ($P < .0001$).⁷⁷ Subsequent studies of azacitidine and other DNA methyltransferase inhibitors have confirmed these observations and extended them to other clinical settings of MDS, such as studies showing that decitabine offers a survival advantage compared with intensive chemotherapy in patients with higher-risk MDS.⁷⁸ Studies exploring alternative dosing and schedules of administration,^{79,80} as well as combination regimens with other agents,^{81–83} have been performed or are ongoing. Furthermore, the feasibility of stem cell transplant after treatment with DNA methyltransferase inhibitors has been documented.⁸⁴

DNA methyltransferase inhibitors have also been

tested in leukemias and myeloproliferative disorders, including combinations with imatinib in patients with chronic myeloid leukemia;⁸⁵ combinations with HDAC inhibitors, such as valproic acid^{86,87} or phenylbutyrate,⁸¹ in acute myeloid leukemia (AML); combinations with all-trans retinoic acid;⁸⁷ and combinations with hydroxyurea and gemtuzumab ozogamicin in patients with previously untreated non-M3 AML;⁸² as well as studies in patients with myelofibrosis⁸⁸ or chronic myelomonocytic leukemia.⁸⁹ The aggregate experience from these trials is that DNA methyltransferase inhibitors can be safely administered in combination with other antileukemic agents. While in the setting of MDS, DNA methyltransferase inhibitors are, as single agents, capable of improving the natural history of MDS, and have in fact become a standard of care for patients with higher-risk MDS,⁹⁰ more studies are needed to evaluate whether the anti-leukemic clinical activity of combinations incorporating this drug class is superior to that of conventional regimens.

HDAC Inhibitors

The study of HDAC inhibitors in hematologic malignancies and solid tumors was preceded by early observations in the 1980s that proposed a link between the ability of sodium butyrate to induce cell differentiation in erythroleukemia cells^{91,92} and the compound's ability to trigger hyperacetylation of histones.^{93–95} Sodium butyrate did not receive FDA approval, but anecdotal evidence of clinical response in a butyrate-treated patient with acute myelocytic leukemia⁹⁶ provided impetus for further study of other short-chain fatty acids with HDAC inhibitory activity (e.g., valproic acid). Furthermore, it provided a first level of supportive evidence for the development of new classes of HDAC inhibitors, including hydroxamates, such as vorinostat (suberoylanilide hydroxamic acid), panobinostat (LBH589), belinostat, cyclic peptides (such as FK228, also known as depsipeptide or romidepsin), or benzamides.⁹⁷

The Role of HDAC Inhibitors in Cutaneous and Peripheral T-Cell Lymphoma: T-cell lymphomas were among the first clinical settings in which HDAC inhibitors were shown to be active. In a phase I trial of depsipeptide (romidepsin) conducted at the National Cancer Institute, 3 patients with cutaneous T-cell lymphoma (CTCL) had a partial response, and 1 with peripheral T-cell lymphoma (PTCL) had a complete response.⁹⁸ These observations provided the impetus for extensive clinical testing of romidep-

sin and other HDAC inhibitors in patients with T-cell lymphoma. In a phase II trial of oral vorinostat for refractory CTCL, 8 of 33 patients experienced partial response, including 7 with advanced disease and 4 with Sézary syndrome, while the time-to-disease progression was 30 weeks.⁹⁹ The most common grade 3 or 4 drug-related adverse events were thrombocytopenia and dehydration. These results solidified the notion that vorinostat was active in heavily pretreated CTCL patients and identified the 400-mg daily regimen as the one with the most favorable safety profile. A multicenter phase IIb trial of vorinostat in persistent, progressive, or treatment-refractory CTCL,¹⁰⁰ enrolled 64 patients who had undergone at least 2 prior systemic therapies (with at least 1 of which included bexarotene, unless intolerable) and showed an overall response rate of 29.7% and a median time-to-disease progression of 4.9 months. The results of these studies^{99,100} provided the basis for FDA approval of vorinostat for treating cutaneous manifestations of CTCL in patients with progressive, persistent, or recurrent disease while undergoing or after 2 systemic therapies.¹⁰¹ This effect of vorinostat on CTCL seems to be a class effect, as other HDAC inhibitors also seem to have clinical activity in that setting. For instance, panobinostat (LBH589) has also been shown to be active in this patient population.¹⁰²

HDAC Inhibitors in Multiple Myeloma: The study of HDAC inhibitors in multiple myeloma (MM) was informed by preclinical data that showed that the HDAC inhibitor vorinostat (suberoylanilide hydroxamic acid) induces a constellation of antiproliferative and/or proapoptotic molecular events, including downregulation of transcripts for members of growth factor receptor signaling cascades, antiapoptotic molecules (e.g., caspase inhibitors), oncogenic kinases, DNA synthesis/repair enzymes, and transcription factors implicated in MM pathophysiology.^{103,104} These pleiotropic molecular events are associated with the ability of HDAC inhibitors to exhibit potent antiproliferative/proapoptotic activity against human MM cells, overcome the protective effect that bone marrow stromal cells have on MM cells, and enhance the response of MM cells to other anti-MM agents, including the proteasome inhibitor bortezomib.^{103,104}

Based on these results and the favorable safety profile of oral administration of vorinostat in other disease settings, a phase I trial evaluated the safety and efficacy of oral vorinostat (200, 250, or 300 mg twice

daily for 5 days per week in 4-week cycles; or 200, 300, or 400 mg twice daily for 14 days in 3-week cycles) in patients with MM. In that trial, 13 patients (with a median of 3 lines of prior therapy) were evaluated. Treatment was continued until disease progression or intolerable toxicity was observed.¹⁰⁵ Drug-related adverse events, which included fatigue, anorexia, dehydration, diarrhea, and nausea, were mostly grade 2 or below. Among 10 evaluable patients, 1 case of minimal response and 9 cases of stable disease were observed. Maximum tolerated doses were not determined due to early study termination by the sponsor. Although these observations might seem to indicate only modest single-agent activity of vorinostat in advanced MM, it is notable that the schedule of administration in this MM-specific phase I study was twice-daily. Clinical trials that led to vorinostat approval in CTCL showed that daily dosing is more active and better tolerated⁹⁹ than twice-daily administration.

Given the preclinical observations regarding the anti-MM activity of the combination of vorinostat plus bortezomib, this combination was evaluated in clinical studies in advanced MM, including 2 separate multi-institutional phase I trials.^{106,107} Both studies confirmed clinical activity (with stable or decreasing M-protein in 13 of 16 and 17 of 17 patients, respectively) despite the heavily pretreated patient populations, which included most patients whose disease was refractory to the previous therapy; had received single or double autologous stem cell transplantation; and had previously received thalidomide-, lenalidomide-, or bortezomib-based therapies, including patients who had relapsed or whose disease was refractory to multiple lines of bortezomib-based treatment. Ongoing studies in the MM field are further evaluating the role of this combination in patients with bortezomib-refractory disease, whereas a randomized phase III trial is currently comparing vorinostat plus bortezomib versus bortezomib alone.

The preclinical and clinical observations regarding vorinostat activity in MM triggered interest for studies of other members of the HDAC inhibitor class in this setting. For instance, other hydroxamic acid HDAC inhibitors, such as LAQ824¹⁰⁸ and panobinostat (LBH589),¹⁰⁹ were studied preclinically, while clinical studies of panobinostat in combination with bortezomib are also underway. A related line of research has involved the development of HDAC6-selective inhibitors, such as tubacin.¹¹⁰ This

compound inhibits the tubulin deacetylation mediated by HDAC6. This cytoplasmic deacetylase helps transport misfolded proteins to the aggresome. Single-agent tubacin has limited in vitro anti-MM activity, but enhances the anti-MM activity of bortezomib.¹¹⁰ This effect is at least partly related to inhibition of aggresome function as a result of HDAC6 inhibition. Therefore, in contrast to hydroxamic acid inhibitors (vorinostat, LAQ824), tubacin primarily serves as a “cytosolic deacetylase” rather than a “nuclear deacetylase.”¹¹¹ Therefore, despite some overlap between these 2 groups of functional activities (e.g., through LBH589-mediated inhibition of cytoplasmic deacetylation),^{109,112} it is conceivable that tubacin and other HDAC6-selective inhibitors primarily target cytoplasmic protein homeostasis, rather than functioning as classical examples of therapeutic targeting of epigenetic regulation at the level of chromatin remodeling.

HDAC Inhibitors in Other Hematologic Malignancies and MDS: HDAC inhibitors have been tested in clinical trials for patients with AML,^{113–115} chronic myeloid leukemia,¹¹³ acute lymphoblastic leukemia,^{113,115} chronic lymphocytic leukemia,^{113,116} MDS,^{113,117} and in lymphomas other than CTCL/PTCL.¹¹⁸ These studies have shown that HDAC inhibitors do not generally exhibit substantial single-agent clinical activity (i.e., not to the level observed in CTCL/PTCL). Of note, several of these studies showed that HDAC inhibitor administration was associated with inhibition of the intended targets, as evidenced by hyperacetylation of histones,¹¹³ whereas in some cases differentiation or apoptosis of leukemic cells was also observed.¹¹⁶ The different pattern of responses to HDAC inhibition in CTCL/PTCL versus other hematologic malignancies, remains to be explained at the molecular level. It is conceivable that within each classically defined group of hematologic malignancies there are specific molecularly defined subtypes that are highly responsive to HDAC inhibition. Clinical trials with small numbers of patients may not be able to detect these specific molecularly defined subtypes. Modifications of dose/schedule, as well as rational design of combination regimens, similar to the experience in the MM field, could conceivably extend the spectrum of activity of HDAC inhibitors.^{119,120}

Nonchromatin-Related Sequelae of HDAC Inhibitors: HDAC inhibitors are a prime example of a therapeutic strategy that targets the aberrant epigenome of neoplastic cells. However, the molecular sequelae

of HDAC inhibition extend well beyond the modulation of histone acetylation. In fact, there is now extensive evidence that agents such as vorinostat, panobinostat, and other HDAC inhibitors inhibit acetylation of a diverse range of nonhistone proteins implicated in proliferation, survival, and drug resistance of neoplastic cells (Figure 2).¹²¹

For instance, the function of HSP90,¹²² HIF-1 α ,¹²³ STAT3,¹²⁴ p53,^{125,126} NF- κ B subunits,¹²⁷ and steroid hormone receptors^{128–130} is regulated by their acetylation status and is therefore influenced by the activity of HDACs. Consequently, HDAC inhibitors can mediate their antitumor effects through not only modulation of the histone code but also modifications in the activity of signaling cascades, the components of which are regulated by HDAC activity. Therefore, the term *deacetylase inhibitors* perhaps more accurately describes the properties of this drug class.

Current Challenges and Future Directions

A fundamental reason why targeting the epigenome represents an attractive anticancer strategy for diverse neoplasias is the pleiotropic range of molecular sequelae triggered by these therapies. Specifically, it is hoped that such strategies will be able to counteract the pronounced genetic complexity and heterogeneity of neoplastic cells. This appeal is further enhanced by the increasing number of “druggable” or potentially “druggable” therapeutic targets involved in the regulation of the epigenome in cancer cells. Although DNA methyltransferase inhibitors and HDAC inhibitors are already FDA approved for treatment of different types of neoplasias, mostly in the field of hematologic malignancies, other potentially druggable targets, including HAT or methyltransferase inhibitors, have also emerged in recent years.

However, a fundamental concern related to the pleiotropic nature of epigenetic therapies for cancer is that their molecular sequelae may not only occasionally include the derepression of tumor suppressor genes and/or inactivation of oncogenic transcripts, but also may involve upregulation of some genes that could promote cell survival.³ One notable example of this concern involves the observation that ABC transporter genes (e.g., MDR1-P glycoprotein, ABCG2) are often transcriptionally activated in different types of tumor cells after exposure to HDAC inhibitors.^{131–136} This raises concerns about potential

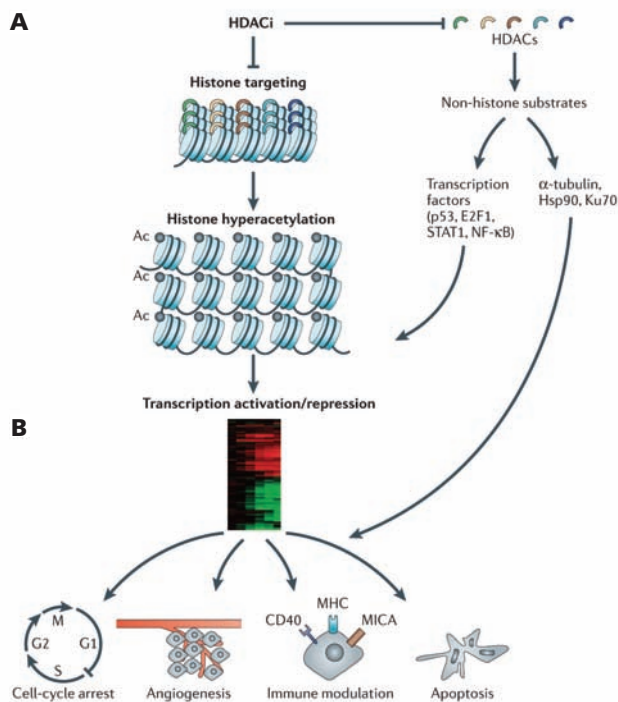


Figure 2 Effects of HDAC inhibition on nonhistone proteins. A) In addition to regulating the acetylation state of histones, HDAC can bind to, deacetylate, and regulate the activity of several other proteins, including transcription factors (i.e., p53, E2F transcription factor 1 [E2F1], and nuclear factor- κ B [NF- κ B]) and proteins with diverse biologic functions (i.e., α -tubulin, Ku70, heat-shock protein 90 [hsp90]). Hyperacetylation of transcription factors with HDAC inhibitors (HDACis) can augment their gene-regulatory activities and contribute to the changes in gene expression observed after direct HDACi-mediated histone hyperacetylation. Hyperacetylation of proteins such as Ku70 and Hsp90, or disruption of protein phosphatase 1 (PP1)–HDAC interactions by HDACi might have no direct or indirect effect on gene expression but could be important for certain biologic effects of HDACi, in particular the induction of apoptosis and cell-cycle arrest. B) Antitumor effects of HDACi. HDACi can affect tumor cell growth and survival through multiple biologic effects. HDACi induce cell-cycle arrest at the G1/S boundary through upregulation of CDKN1A (encoding p21^{WAF1/CIP1}) and/or through downregulation of cyclins. HDACi can suppress angiogenesis through decreased expression of proangiogenic factors, such as vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1- α (HIF1 α), and chemokine (C-X-C motif) receptor 4 (CXCR4). HDACi can also have immunomodulatory effects by enhancing tumor cell antigenicity (upregulation of major histocompatibility complex class I and II, MICA, CD40) and by altering the expression of key cytokines, including tumor necrosis factor α , interleukin-1, and interferon-gamma. Bolden JE, Peart MJ, Johnstone RW. *Nat Rev Drug Disc* 2006;5:769–784, copyright 2006. Reprinted by permission from Macmillan Publishers Ltd.

combinations of this drug class with conventional cytotoxic chemotherapy. Another example is the observation that, in biopsy specimens from 5-azacitidine-treated patients with Epstein-Barr virus-associated tumors, significant demethylation was detected in all latent and lytic Epstein-Barr virus promoters examined within 72 hours of the conclusion of the last infusion of the first cycle of therapy, compared with pretreatment specimens.¹³⁷ This experience, combined with anecdotal immunohistochemical evidence regarding activation of previously silent viral antigen expression, as well as case reports of Epstein-Barr virus or hepatitis B virus reactivation in patients treated with romidepsin,¹³⁸ suggests that more work may be necessary to exclude safety issues that may be caused by reactivation of latent viral infections.

Modifications of dose/schedule, as well as rational design of combination regimens are important strategies that can help expand the spectrum of activity of HDAC inhibitors and other epigenetic therapies.^{119,120} Combining these therapies with other existing drug classes requires careful study to avoid antagonistic effects that may be mediated by some of the many molecular sequelae triggered by pharmacologic modulators of epigenetic regulation of gene expression. This has been proposed as one of the reasons why it is important to develop epigenetic therapies with a higher degree of specificity towards individual target genes, as opposed to the currently available epigenetic therapies, the activity of which is considered to involve a more ubiquitous effect on the epigenome.

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Notes

Post-test

Please circle the correct answer on the enclosed answer sheet.

1. Which of the following properly categorizes the DNA methylation pattern in malignant cells?
 - a. Widespread hypomethylation in the body of genes
 - b. Hypomethylation in CpG islands within the promoter regions
 - c. Hypermethylation within intronic sequences and within repetitive DNA sequences
 - d. None of the above
2. All of the following statements regarding epigenetics and gene transcription are true except:
 - a. The activation of gene transcription is determined by several different functionally opposing epigenetic events
 - b. Methylation of histones can either activate or suppress gene transcription
 - c. The processes of DNA methylation and histone acetylation appear to be functionally similar and complementary
 - d. None of the above
3. Which of the following statements regarding the use of azacitidine and other DNA methyltransferase inhibitors in hematologic malignancies is true?
 - a. Patients with higher risk myelodysplastic syndrome treated with decitabine had similar survival when compared with intensive chemotherapy
 - b. The feasibility of stem cell transplant after treatment with DNA methyltransferase inhibitors has been documented
 - c. DNA methyltransferase inhibitors are not a standard of care for patients with higher risk myelodysplastic syndrome
 - d. All of the above
4. Which of the following statements most accurately describes the action of HDAC inhibitors on multiple myeloma cells?
 - a. Exhibit potent antiproliferative, pro-apoptotic activity against multiple myeloma (MM) cells
 - b. Overcome the protective effect that bone marrow stromal cells have on MM cells
 - c. Enhance the response of MM cells to other anti-MM agents
 - d. All of the above
5. All of the following statements regarding the use of HDAC inhibitors in hematologic malignancies are true except:
 - a. Studies have shown that HDAC inhibitors generally exhibit substantial single-agent clinical activity
 - b. HDAC inhibitors inhibit acetylation of a diverse range of nonhistone proteins implicated in proliferation, survival, and drug resistance of neoplastic cells
 - c. HDAC6-selective inhibitors primarily target cytoplasmic protein homeostasis
 - d. All of the above

Please circle the correct answer below.

Post-Test Answer Sheet										
Please circle one answer per question. A score of at least 70% on the post-test is required.										
1.	a	b	c	d		4.	a	b	c	d
2.	a	b	c	d		5.	a	b	c	d
3.	a	b	c	d						

<p>The activity content helped me to achieve the following objectives: (1 = Strongly disagree; 3 = Not sure; 5 = Strongly agree)</p> <p>Describe the role of epigenetics in the development and progression of hematologic malignancies</p> <p style="text-align: center;">1 2 3 4 5</p> <p>Discuss DNA methylation and histone deacetylation and the role of therapies in targeting these pathways</p> <p style="text-align: center;">1 2 3 4 5</p> <p>Review the latest data on the use of epigenetic therapies to treat hematologic malignancies, including treatment-related adverse events</p> <p style="text-align: center;">1 2 3 4 5</p> <p>Please indicate the extent to which you agree or disagree with the following statements:</p> <p>You were satisfied with the overall quality of this activity.</p> <p style="text-align: center;">Strongly agree Agree Undecided Disagree Strongly disagree</p> <p>Participation in this activity changed your knowledge/attitudes.</p> <p style="text-align: center;">Strongly agree Agree Undecided Disagree Strongly disagree</p> <p>You will make a change in your practice as a result of participation in this activity.</p> <p style="text-align: center;">Strongly agree Agree Undecided Disagree Strongly disagree</p> <p>The activity presented scientifically rigorous, unbiased, and balanced information.</p> <p style="text-align: center;">Strongly agree Agree Undecided Disagree Strongly disagree</p> <p>Individual presentations were free of commercial bias.</p> <p style="text-align: center;">Strongly agree Agree Undecided Disagree Strongly disagree</p>				
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Epigenetic Modulation in Hematologic Malignancies: Challenges and Progress

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