REVIEW ARTICLE

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Epigenetic Therapies for Cancer

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An illustrated glossary is available at NEJM.org

Chromatin is one of the earliest identified targets for cancer therapeutics. Drug development aimed at altering chromatin can be traced to the differentiating agents of the 1970s and their link to DNA methylation.[1] A more precise understanding of the complexity of chromatin and its role in oncogenesis began to emerge when sequencing of the cancer genome revealed mutations in numerous genes encoding proteins that regulate chromatin. In many cases, these mutations proved to be critical in maintaining the malignant process, an observation that led to new therapeutics. This review summarizes approved agents and their clinical activity, describes therapies in development, and delineates challenges in the field of epigenetics.

Epigenetics begins with DNA and histone proteins — two macromolecules structurally and functionally intertwined in chromatin. The basic chromatin unit, the nucleosome, comprises recurrent 146-bp segments of DNA wrapped around an octamer of histone proteins. The family of histone proteins includes H2A, H2B, H3, H4, and multiple variants, many with unique functions. DNA and histone proteins are modified in ways that regulate accessibility and function, and an alteration in these modifications is one hallmark of cancer. Epigenetic therapies seek to normalize DNA methylation patterns and post-translational modifications on histones that promote or maintain a malignant phenotype.

Enzymes that regulate the methylation of cytosines on DNA, plus a diverse array of post-translational histone modifications, are the machinery that regulates DNA replication and repair and RNA transcription. Post-translational modifications of lysine-rich N-terminal histone "tails" include acetylation and methylation, as well as ubiquitination, phosphorylation, and sumoylation. Despite this diversity, histone modifications and their regulating enzymes are remarkably specific in terms of the histone protein that is modified, the residues affected, and the role in the epigenome — so specific, in fact, that a nomenclature describes the modifications. For example, H3K9ac indicates that an acetyl group (ac) has been added to the amino acid lysine (K), positioned as the ninth residue of histone H3. H3K9me3 indicates that three methyl groups (me3) have been added to the same amino acid as an alternative modification. Acetylation and methylation are often referred to as histone marks rather than post-translational modifications. Acetylation leads to an open, active chromatin state, whereas methylation is more complex and has diverse effects, depending on the residue modified. At some sites, such as H3K9, methylation is associated with a repressed chromatin state. At others, such as H3K4, methylation is associated with gene transcription.

The enzymes regulating the post-translational epigenetic modifications on histones have been categorized as writers, erasers, readers, or movers as a way of classifying the effects of more than 700 proteins that regulate chromatin function.[2-5] Writers, as the name implies, add modifications and include DNA methyltransferases (DNMTs), histone lysine methyltransferases (KMTs), and histone acetyltransferases (HATs), whereas erasers, including histone lysine demethylases

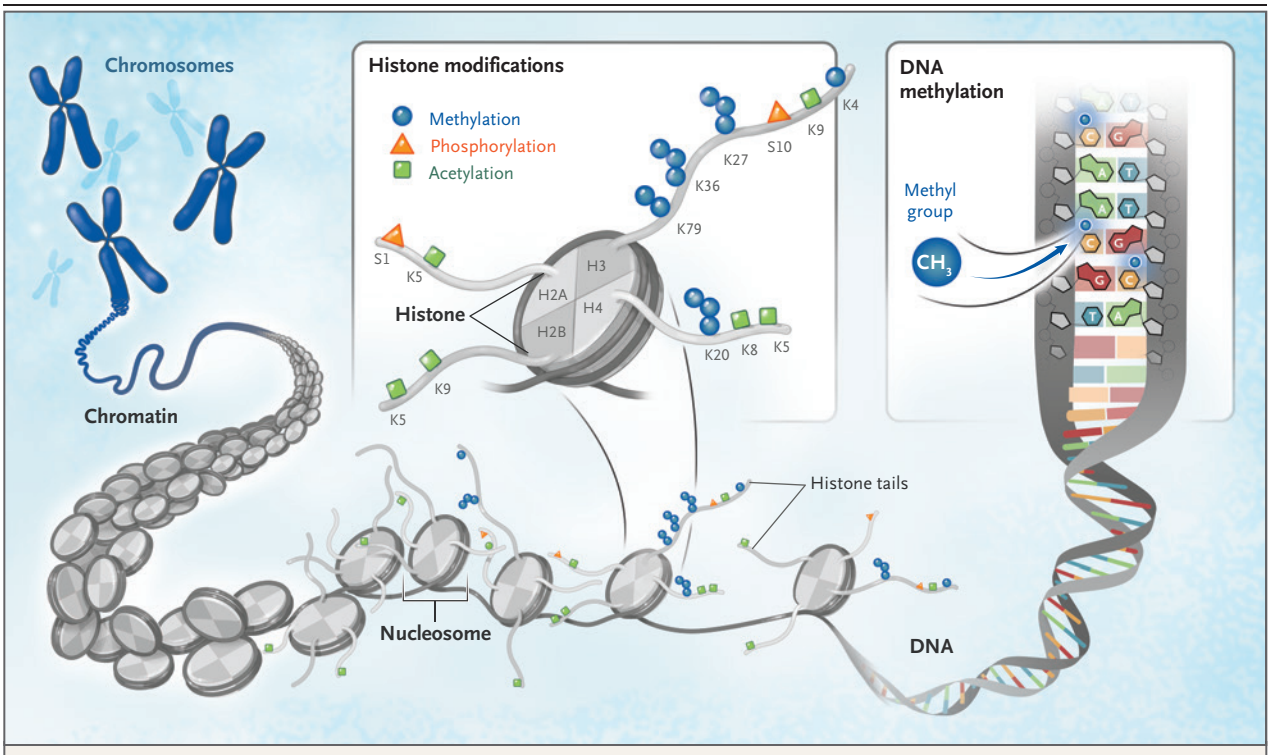


Figure 1. Chromatin.

Comprising 146 bp of DNA wrapped around an octamer of histone proteins, the nucleosome plays a key part in regulating the replication, transcription, and repair of DNA. Post-translational modifications on histone proteins orchestrate chromatin opening for gene transcription or closing for gene silencing. Some of the key modifications are shown — for example, H3K9 acetylation and H3K4 methylation, associated with open chromatin and gene expression, and H3K27 and H4K20 trimethylation, associated with gene silencing. A large array of proteins is involved in regulating and interpreting these post-translational modifications, including readers, writers, and erasers.

(KDMs) and histone deacetylases (HDACs), remove post-translational modifications. Recognition that bromodomain and chromodomain proteins "read" acetylated or methylated residues, respectively, led to the term readers; movers are chromatin-remodeling proteins that move nucleosomes and allow gene transcription. The terms shapers and insulators capture other functions (Figs. 1 and 2).

MUTATIONS IN EPIGENETIC REGULATORS AND THEIR VALIDATION AS TARGETS

Our understanding of the complexity of chromatin and its role in oncogenesis increased as thousands of cancer genomes were sequenced and mutations were found in virtually every epigenetic regulator.[4] However, mutations alone do not invariably have functional consequences, nor do they ensure an epigenetic therapeutic target. A protein-modifying mutation can give rise to an oncogenic driver or impair a tumor suppressor, or it may be a passenger mutation without a key role. With time, an increasing number of mutations have been validated as important in cancer (Fig. 2). Although a handful of mutations in epigenetic regulators result in a gain or change of function, the majority of mutations disrupt conformations or create new stop codons, resulting in loss of function. Gene fusions and alterations in expression add another, perhaps greater, dimension of complexity. All this variation highlights one theme of epigenetics: the importance of the right epigenetic regulator being in the right place at the right time. Too much or too little regulator can be oncogenic.

DIVERGENT ROUTES TO ABERRANT DNA METHYLATION

Aberrant DNA methylation has been associated with oncogenesis in a number of tumor types. In cancers with aberrant methylation, CpG islands (regions of DNA with a high density of cytosine–guanine dinucleotides) in or near promoter re-

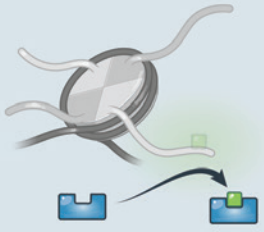
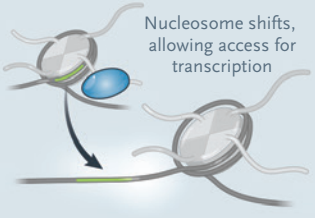
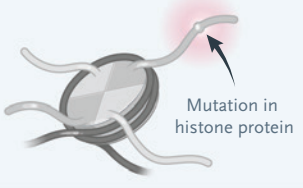
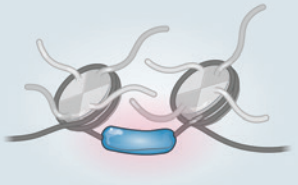
Category	Epigenetic Regulators	Function	FDA-Approved Drug
Writers 	**DNMT1, 3A, and 3B**	Methylates cytosines on DNA, and mutation can lead to aberrant methylation	Azacitidine, decitabine
	EZH2	Methylates histone H3K27	Tazemetostat
	DOT1L	Methylates histone H3K79	
	KMT2A–D, SETD2, NSD1	Methylate histone lysines	
	EP300, CREBBP	Acetylate histone lysines	
Erasers 	**TET2**	Is the first step in cytosine demethylation; is inhibited by 2-hydroxyglutarate (2-HG)	Azacitidine, decitabine
	IDH1, IDH2	Mutated protein produces 2-HG from isocitrate that inhibits TET2 and lysine demethylases	Ivosidenib, enasidenib
	HDAC1–3, 8 HDAC6	Deacetylase removes acetyl groups from histone lysines	Vorinostat, belinostat, panobinostat, romidepsin
	KDM1A, **KDM6A (UTX)**	Demethylates histone lysines	
Readers 	BRD4	Bromodomain proteins read acetyl groups on histone lysines	
	CBX family, CHD1	Chromodomain proteins read methyl groups	
Movers 	**ARID1A, ARID1B, ARID2** SMARCA2**, SMARCA4, SMARCB1,** CHD1	Proteins in the chromatin remodeling complex use ATP to move nucleosomes away from DNA; loss-of-function mutations common in cancer	
Shapers 	**HIST1H1B, HIST1H1C, HIST1H3B, H3F3A, H3F3B**	Structural histone proteins acquire mutations that can be oncogenic	
Insulators 	**CTCF, STAG2, RAD21,** CHD8	Normal binding to CTCF sites on DNA defines and protects gene neighborhoods from inappropriate expression	

Figure 2 (facing page). Commonly Altered Epigenetic Regulatory Proteins Implicated in Cancer.

The schematic illustrations are adapted from Tarakhovsky,[2] Ahuja et al.,[3] Plass et al.,[4] and O'Connor et al.[5] Some of these proteins have undergone successful drug development; others have yet to be introduced to clinical use. Those with putative oncogenic mutations are shown in bold. The regulatory proteins are depicted in blue. Writers add modifications; in the example shown here, an acetyl group (green square) is added by a histone acetyltransferase (blue oval) to a histone lysine. Erasers remove post-translational modifications; here, a histone deacetylase (HDAC; blue rectangle) removes the acetyl group. In readers, the presence of an acetyl group is detected by a bromodomain protein (blue trapezoid). In movers, the histone octomer (gray disk) shifts away from the green DNA region. In shapers, a mutation in the histone protein itself alters function, and in insulators, boundaries are lost by mutation in the CTCF protein. DNMT denotes DNA methyltransferase, H3K histone H3 lysine residue, IDH isocitrate dehydrogenase, KDM histone lysine demethylase, and KMT histone lysine methyltransferase.

gions become hypermethylated, whereas gene bodies are hypomethylated.[6] In 2010, mutations in the DNA methyltransferase writer DNMT3A were identified in more than 20% of samples from patients with acute myeloid leukemia (AML), with more than half the mutations at amino acid R882.[7] However, it was subsequently recognized that R882 mutations were dominant negative, caused reduced catalytic activity, and led to focal hypomethylation, in contrast to the hypermethylation pattern seen with wild-type DNMT3A R882 in AML DNA.[8,9] DNMT3A alterations often occur as initiating mutations that create an ancestral or founder preleukemic clone, establishing an environment in which additional mutations may lead to founding malignant clones.[10,11] With additional mutations in oncogenic drivers, subclones of overt leukemia emerge.[12-14] The ancestral clones persist through remission and relapse.[15]

Other molecular alterations highlight the malignant potential of DNA hypermethylation. One example is loss-of-function mutations, particularly in *TET2*, which are common in AML. By catalyzing the first step in demethylation — conversion of methylcytosine to hydroxymethylcytosine — TET family eraser proteins reverse the methylation of cytosine catalyzed by DNMT1, 3A, and 3B[16]; hinder demethylation; and lead to aberrant DNA hypermethylation.[17] TET2 alterations are also common in clonal hematopoiesis of indeterminate potential (CHIP). CHIP involves an altered clone, occurs in about 10% of people older than 65 years of age, and has been linked with hematologic cancer and coronary artery disease.[18] Another example involves mutations in enzymes of the tricarboxylic acid (TCA) cycle, which have been implicated as indirect epigenetic modifiers leading to DNA and histone hypermethylation. Mutations at R132 in isocitrate dehydrogenase 1 (*IDH1*) and at R172 in isocitrate dehydrogenase 2 (*IDH2*) alter enzymatic activity and result in preferential production from isocitrate of the oncometabolite 2-hydroxyglutarate (2-HG) over alpha-ketoglutarate (Fig. 3).[19,20] Inhibition of TET enzymatic activity by 2-HG, like loss-of-function *TET* mutations, results in DNA hypermethylation; simultaneously, inhibition of lysine demethylases by 2-HG leads to increased histone lysine methylation.[21] Similar observations have been made regarding the accumulation of succinate and fumarate that occurs with succinate dehydrogenase and fumarate hydratase loss-of-function mutations, respectively.[22]

Mutations in *DNMT3A*, *TET2*, *IDH1*, and *IDH2* are found in 28%, 14%, 9%, and 10% of AML cases, respectively,[12,23,24] with a similar mutation pattern described in the angioimmunoblastic subtype of peripheral T-cell lymphoma (AILT).[25,26] Identification of mutations in solid tumors that could alter DNA methylation has raised hopes for extending the epigenetic therapeutic portfolio. Apart from AML, however, R882 mutations are rare. With the exclusion of AML, *DNMT3A* mutations were found in only 2% of 10,767 samples in the Pan-Cancer data set from the Cancer Genome Atlas (TCGA), with only two mutations at R882 (Fig. 3).[27,28] *TET2* mutations have been detected in 2% of the Pan-Cancer data set from TCGA, including a subset in solid tumors with loss-of-function due to truncation of the protein.[27,28] Pathogenic R132 *IDH1* mutations have been found in 4% of cases in TCGA's Pan-Cancer data set, including 78% of low-grade gliomas, 14% of cholangiocarcinomas, and 4% of cutaneous melanomas.

THERAPIES FOR ABERRANT DNA METHYLATION

The DNMT inhibitors azacitidine and decitabine were developed and approved well before the

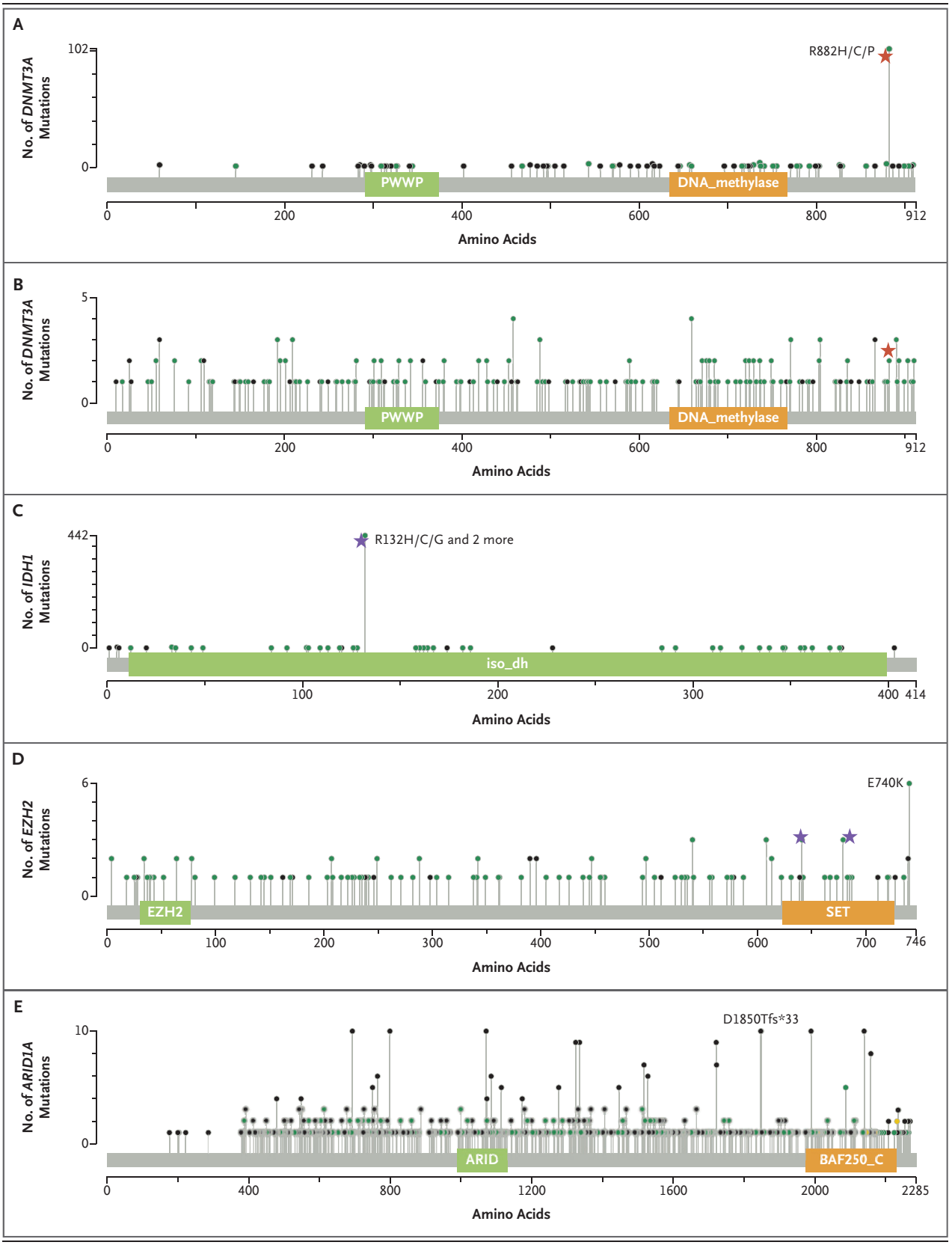


Figure 3 (facing page). Mutations in Genes Encoding Epigenetic Regulators.

Data are from Cerami et al.,[27] Gao et al.,[28] and cBioPortal (www.cbioportal.org). Missense mutations are shown in green, truncating mutations in black, and in-frame insertions or deletions in yellow. Panel A shows 102 R882 *DNMT3A* recurrent mutations (red star) in 1008 samples from three acute myeloid leukemia (AML) and myelodysplastic syndrome data sets (www.cbioportal.org/study/summary?id=mnm_washu_2016, www.cbioportal.org/study/summary?id=aml_ohsu_2018, and www.cbioportal.org/study/summary?id=laml_tcga_pan_can_atlas_2018). Panel B shows the R882 *DNMT3A* mutations (red star) in 10,767 samples (excluding AML samples) from the Cancer Genome Atlas (TCGA) Pan-Cancer data set, which span the gene and can result in either gain or loss of function. Panels C, D, and E show mutations from the 10,967-sample Pan-Cancer data set (32 studies included). In Panel C, the IDH1 hot-spot mutation at R132 (purple star) generates a protein that converts isocitrate to the oncometabolite 2-HG and accounts for the overwhelming majority of mutations; the effects of other mutations are not defined but are likely to be random events. In Panel D, *EZH2* mutations spanning the gene include both missense and truncating mutations, with the effects in most cases unknown or predicted to be loss of function. The Y641 and A687 mutations (purple stars) are known gain-of-function mutations and are oncogenic. In Panel E, *ARID1A* mutations span the gene, many of which are predicted to be loss-of-function mutations that are oncogenic.

complexity of methylation patterns had been discerned.[29,30] Both agents are antimetabolites that inhibit DNMT activity and induce hypomethylation when incorporated into DNA; azacitidine is also incorporated into RNA. With responses observed in 16 to 17% of patients with myelodysplastic syndrome and in 20 to 40% of patients with AML, U.S. regulatory approval was secured for azacitidine (in 2004) and decitabine (in 2006) for treatment of myelodysplastic syndrome and chronic myelomonocytic leukemia. In the European Union, the agents are approved for patients with newly diagnosed or secondary AML who are ineligible for transplantation.[31] Evidence of the activity of monotherapy for solid tumors was not convincing.[32]

Although *TET2*, *IDH1*, and *IDH2* mutations lead to DNA hypermethylation, the activity of DNMT inhibitors in this context is not as striking as would be expected for an effective targeted therapy, and it is not clear that hypomethylation mediates responses.[33-36] In addition, as with all epigenetic therapies, activity is not limited to mutation-bearing cancers, probably because general mechanisms such as DNA damage promote cell death.

Two drugs targeting hot-spot mutations in the genes encoding IDH1 and IDH2 were approved by the Food and Drug Administration (FDA) after a fast-track priority review with an orphan-product designation: enasidenib, targeting IDH2, and ivosidenib, targeting IDH1, were approved in 2017 and 2018, respectively. These were the first approvals for a therapy centered on an oncometabolite. Administered orally on a daily schedule, both drugs markedly reduce blood 2-HG levels. In the first-in-human trial, 38.8% of patients with AML bearing *IDH2* mutations had an objective response to enasidenib.[37] Clearance of circulating mutated *IDH1* or *IDH2* alleles was observed in patients with a response after treatment with ivosidenib or enasidenib.[37,38] Although an observed benefit from a durable partial response represented a paradigm shift for a disease in which only a complete response had previously been shown to extend survival, enthusiasm has been tempered by the emergence of resistance less than a year after treatment. This emergence of resistance is at least in part due to the fact that cells bearing *IDH1* or *IDH2* mutations constitute a subset of leukemic cells rather than the entire cell population.[37] As data mature, it remains unclear whether barriers to activity in solid tumors can be identified and overcome.[39] Although the frequency of *IDH1* mutations is high among patients with glioma, penetration of the blood–brain barrier will be critical in developing IDH1 inhibitors for use in such patients. In an ongoing phase 1 study, vorasidenib showed good brain penetrance and a reduction in 2-HG levels by approximately 93% in patients with glioma.[40]

ABERRANT HISTONE METHYLATION

Methylation of lysine in histone tails, like aberrant DNA methylation, leads to gene silencing and has been linked to cancer. Several KMT writers have been implicated in oncogenesis, including EZH2, NSD2, SETD2, and DOT1L. Gain-of-function mutations in *EZH2* have been described, particularly in follicular lymphoma.[41] Although hot-spot mutations at Y641 reduce methyltransferase activity on unmethylated H3K27, they increase methyltransferase activity on H3K27me2, leading to repressive trimethyl-

ated H3K27me3 and gene silencing.[42,43] Several additional gain-of-function mutations (e.g., A677G and A687V) and loss-of-function mutations have also been classified as oncogenic, but many *EZH2* mutations remain to be fully characterized (Fig. 2).[27,28] Loss-of-function mutations in KDMs can also alter the histone methylation landscape.

KMT INHIBITORS

Interest in EZH2 as a drug target has led to the development of several potential therapeutic strategies. Tazemetostat is currently the most advanced EZH2 inhibitor, with others in trials, including valemetostat, CPI-1205, and CPI-0209. In a phase 2 trial, objective responses were observed with tazemetostat in patients who had relapsed or refractory non-Hodgkin's lymphoma, including 35% and 69% of patients with follicular lymphomas harboring wild-type and mutant *EZH2*, respectively, with corresponding response durations of 13 months and 11 months[44,45]; the results of this trial supported the FDA approval in June 2020 of tazemetostat for follicular lymphoma. Although a similar strategy could be envisioned for solid tumors with hot-spot mutations, these appear to be rare. Among 10,967 TCGA Pan-Cancer samples, the majority of *EZH2* mutations in solid tumors appeared to be loss-of-function mutations.[27,28] Only three Y641 hot-spot mutations were identified, all in cases of cutaneous melanoma, for which in vitro studies suggest that tazemetostat may have activity.[46]

Another use for tazemetostat also supported a regulatory approval. Active gene expression requires movement of nucleosomes away from DNA, a task performed by members of the SMARC family, ATP-dependent enzymes that are part of the SWI/SNF chromatin-remodeling complex. Loss-of-function mutations in some SWI/SNF family proteins create an imbalance that results in unopposed EZH2 activity, with increased H3K27me3 and gene silencing.[41,47] Inhibition of EZH2 disrupts this imbalance, resulting in cell death.[47] This was shown in vitro for several SMARC family members, including *SMARCA2*/*SMARCA4*, *ARID1A*, and *PBRM1*,[48-51] and was validated clinically for *SMARCB1*/*INI1* deletions and loss-of-function mutations, which are prevalent in rhabdoid tumor and epithelioid sarcoma.[45,52] A 15% overall response rate supported the FDA's approval of tazemetostat in the treatment of epithelioid sarcoma. Likewise, the SS18–SSX pathogenic gene fusion in synovial sarcoma leads to SMARCB1 loss from the SWI/SNF complex and also renders cells bearing this alteration sensitive to tazemetostat. Thus, activation of EZH2, loss-of-function mutations in SMARC family members, and SS18–SSX fusions each affect the state of methylation at H3K27, providing another example of multiple alterations leading to the same epigenetic aberration responsible for oncogenesis.[53] Systematic testing is needed to determine whether alterations in other members of the SWI/SNF complex confer sensitivity to EZH2 inhibition.

ABERRANT HISTONE ACETYLATION

Levels of histone acetylation are modulated by HDACs that erase acetyl groups and by the HAT writers that acetylate lysines in histone tails. The HDAC eraser family comprises 11 HDACs grouped into four enzyme classes: I, IIa, IIb, and IV, each variably inhibited by available HDAC inhibitors. The term histone deacetylase is in fact a misnomer in that, with the exception of the class I enzymes that localize to the nucleus, HDACs also deacetylate cytoplasmic proteins and are more appropriately referred to as lysine deacetylases.

Overexpression of deacetylases results in reduced histone acetylation, closed chromatin, and reduced gene expression. Unlike pathogenic mutations in the epigenetic proteins described above, HDACs are seldom mutated; somatic mutations are present in less than 1% of the cases from TCGA's Pan-Cancer database. Overexpression is more common — for example, in neuroendocrine cancers.[54,55] In contrast, HAT mutations are common. Mutations in *EP300* and *CREBBP* are often loss-of-function mutations that reduce histone acetylation and impair interaction with their numerous transcription-factor substrates.[56]

HDAC INHIBITORS

Four HDAC inhibitors — vorinostat, romidepsin, belinostat, and panobinostat — have gained FDA approval, and a fifth, chidamide, has received regulatory approval in China. Global acetylation (e.g., H3K9ac, H3K18ac, H3K23ac, H3K56ac, H4K5ac, H4K8ac, and H4K16ac) results from HDAC inhibition. By binding HDACs directly, inhibitors prevent lysine deacetylation, allowing

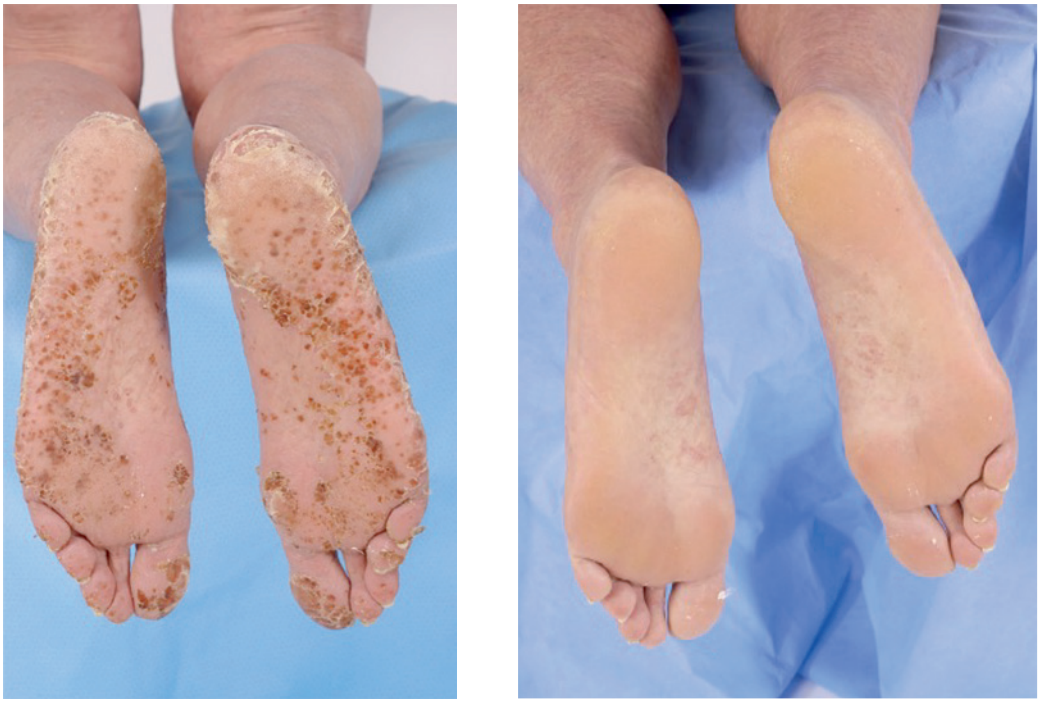


Figure 4. Clinical Images Obtained before and after Treatment with Romidepsin in a Patient with Cutaneous T-Cell Lymphoma (CTCL).

A patient with prior exposure to Agent Orange presented with CTCL lesions on the soles of his feet (left panel). After treatment with romidepsin in a clinical trial, a marked clinical response was noted, with disappearance of the plantar lesions (right panel).

unrestrained HAT activity and hyperacetylation. Acetylation neutralizes positively charged lysines, decreasing their electrostatic attraction to negatively charged DNA, which in turn relaxes chromatin and opens it for transcription of genes that inhibit cell growth or induce a differentiated phenotype. This process has been proposed as the canonical mechanism underlying the action of HDAC inhibitors. What is not known is how gene selection for transcription occurs and whether this is indeed the mechanism by which HDAC inhibitors cause cell death. Most likely, the mechanism of action of HDAC inhibition is more complex, involving DNA and mitochondrial damage.[57] The role of cytoplasmic targets is unclear, particularly for vorinostat, belinostat, and panobinostat, which target class I and II enzymes. Because class II HDACs remove acetyl groups from cytoplasmic proteins as well as from histones, class II inhibitors increase cytoplasmic protein acetylation,[58] facilitating activation of transcription factors such as TP53[59] and TBX5[60] and stabilizing cytoskeletal proteins.[61] The overall effect of altering cytoplasmic protein acetylation, as compared with histone acetylation, is not known.

Vorinostat and romidepsin are approved by the FDA for the treatment of cutaneous T-cell lymphomas, romidepsin and belinostat are approved for the treatment of peripheral T-cell lymphomas, and panobinostat is approved in combination with dexamethasone for the treatment of multiple myeloma. In granting full approval for vorinostat and romidepsin in patients with cutaneous T-cell lymphoma, the FDA considered not only the response rates of 30% and 34%, respectively, but also the clinical benefit afforded to patients with the often painful and disfiguring cutaneous manifestations of the disease (Fig. 4).[62-64] For peripheral T-cell lymphoma, both romidepsin and belinostat received accelerated approval, with responses in one quarter to

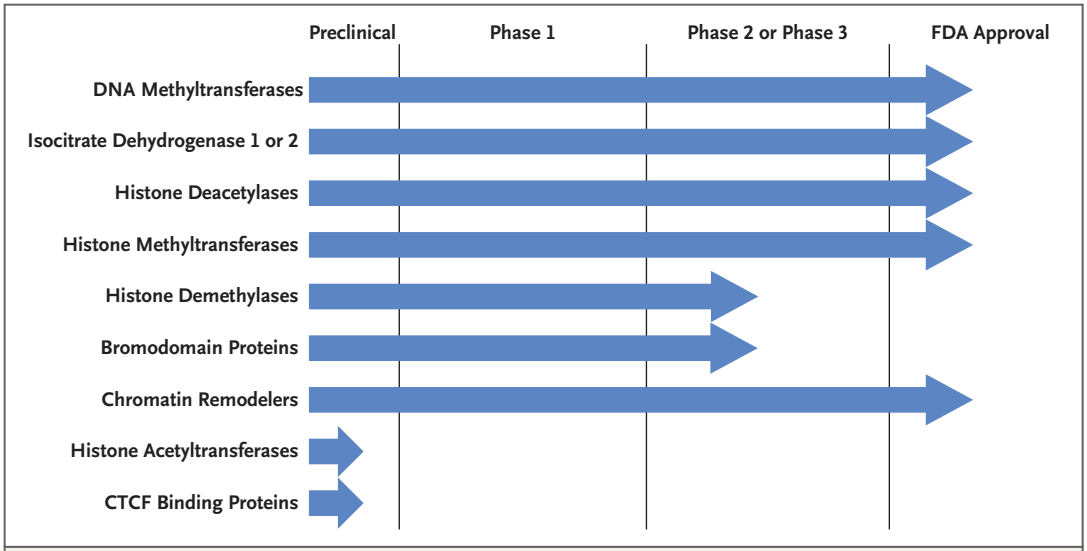


Figure 5. Epigenetic Regulators under Investigation or Approved by the Food and Drug Administration (FDA), According to Protein Family.

Shown is the stage of drug development for key protein families of epigenetic regulators.

one third of patients.[65-67] For romidepsin, the median duration of response exceeded 1 year.

The activity of HDAC inhibitors in T-cell lymphomas, together with the observation that patients with AILT have a high prevalence of mutations in *DNMT3A*, *TET2*, and *IDH2*,[25,26] supports an epigenetic origin of some T-cell lymphomas. So, too, does a response rate of 73% among patients with T-cell lymphomas treated with romidepsin plus azacitidine.[68] However, the recurrent mutations in DNA methylation genes seen in AILT were not observed in cutaneous T-cell lymphoma.[69,70] As in other examples, responses in the absence of mutations confound our understanding of treatment responses as purely epigenetic.

EPIGENETIC AGENTS IN DEVELOPMENT

TARGETING WRITERS, ERASERS, READERS, AND MOVERS

Nine epigenetic agents are currently available for standard-of-care treatment in the United States: two DNMT inhibitors, four HDAC inhibitors, two IDH inhibitors, and the newest, the EZH2 inhibitor tazemetostat (Fig. 5). Many more inhibitors of writers, erasers, and readers are in development (listed in Box 1). An oral DNMT inhibitor, guadecitabine, is being tested in multiple different combinations.[71] Inhibitors of other KMT targets are also in development. One interaction that has received pharmaceutical attention is that of *MLL* (mixed-lineage leukemia gene, also known as *KMT2A*), menin (the MLL scaffold protein), and DOT1L, the sole H3K79 methyltransferase. Gene rearrangements involving *MLL* generate fusion proteins with AF9, AF10, and ENL, which recruit DOT1L to MLL targets, altering the epigenetic landscape.[72-74] Therapeutic strategies have been designed to inhibit DOT1L, the MLL–menin interaction, or both, but the low response rate with the DOT1L inhibitor pinometostat as monotherapy underscores the complexity of this chromatin derangement.[75] Preclinical activity of VTP-50469 suggests that the interaction of MLL with the menin scaffold could be a more central target[76]; a phase 1/2 study of the related compound SNDX-5613 (ClinicalTrials.gov number, NCT04065399) is ongoing.

With regard to histone acetylation, class-specific HDAC inhibitors are in development,[77] including entinostat, which is specific for class I HDACs[78]; NBM-BMX, an HDAC8-selective inhibitor (NCT03726294); and ricolinostat, which is specific for HDAC6.[79] Cells with CREBBP acetyltransferase loss-of-function mutations become dependent on residual p300 acetyltransferase activity, providing a potential therapeutic strategy.[80] The KDM1A (lysine-specific histone de-

Box 1. Epigenetic Therapies in Clinical Development.

DNA methyltransferase (DNMT) inhibitors
Decitabine
Azacitidine
CC-486 (oral azacitidine)
Guadecitabine
ASTX727

Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) inhibitors
Ivosidenib
Enasidenib
Vorasidenib
Olutasidenib

Histone deacetylase (HDAC) inhibitors
Vorinostat
Romidepsin
Belinostat
Panobinostat
Chidamide (also known as tucidinostat)
Entinostat
Mocetinostat
Domatinostat
Pracinostat
OKI-179
Givinostat
Abexinostat
Resminostat
Fimepinostat
NBM-BMX

HDAC6 inhibitors
Ricolinostat
Citarinostat
KA2507

EZH2 methyltransferase inhibitors
Tazemetostat
Valemetostat
CPI-0209
CPI-1205

DOT1L methyltransferase inhibitor
Pinometostat

Lysine-specific demethylase 1 (LSD1) inhibitors
Iadademstat
CC-90011
INCB059872

Bromodomain and extraterminal (BET) inhibitors
Molibresib
Birabresib
ZEN003694
PLX51107

methylase 1 [LSD1]) eraser targets H3K4. Methylation at H3K4me1 or H3K4me2 is a key activating mark in normal cell growth and differentiation. Overexpression of LSD1 in AML and myelodysplastic syndrome reduces H3K4me1 and H3K4me2 and, like other epigenetic aberrations, results in gene repression. Multiple LSD1 inhibitors are in development, including iadademstat, which has led to differentiation of leukemic blasts and occasional AML responses.[81] Two additional LSD1 inhibitors under investigation are INCB059872 (NCT04061421) and CC-90011 (NCT03850067), each evaluated in combination with other agents.

Bromodomain and chromodomain readers recognize histone acetylation and methylation targets, respectively. BRD4, a member of the bromodomain and extraterminal (BET) family, reads hyperacetylated regions of chromatin. The BET inhibitors molibresib and birabresib have shown activity in patients with NUT (nuclear protein in testis) midline carcinoma, a rare cancer with the pathognomonic *BRD4–NUT* fusion gene, providing proof of concept for BRD4 inhibition.[82,83] The observation that BET family proteins are involved in numerous transcription complexes that play a part in overexpression of oncogenes[84] has led to the development of multiple BET inhibitors.

TARGETING SHAPERS AND INSULATORS

Agents for newer targets — shapers and insulators — are also in development[3] (Fig. 2). Histones, shapers of the nucleosome and the protein complexes that regulate chromatin, can acquire mutations that mimic or alter protein–protein interactions regulated by post-translational modification. Mutations in histone proteins occur in cancer, with histone H3.3 a target for epigenetic drug development. Ninety percent of chondroblastomas and 20% of pediatric glioblastomas have a methionine at the lysine 36 position in histone H3.3, which inhibits the H3K36 methyltransferases, MMSET and SETD2, reducing H3K36 methylation and altering gene expression.[85]

Finally, a new group of epigenetic regulators has recently been identified as important in cancer. Insulators are DNA–protein complexes that shield one DNA region from another, preventing inappropriate promoter–enhancer interactions and the spread of chromatin silencing.[86,87] The role of these proteins has been investigated in the context of three-dimensional genomic structure. The insulator protein CTCF binds specific DNA sequences, creating neighborhoods of interacting sequences in normal cells. Aberrant gene expression in cancer can result from alterations in CTCF, in DNA sequences, or in associated proteins such as STAG2, RAD21, or CHD8.[86-89] The CTCF mutation rate in the Pan-Cancer data set is 2% overall, with the mutation considered to be oncogenic in half the cases.

EPIGENETIC THERAPIES IN COMBINATIONS

Epigenetic therapeutics have been combined with classic chemotherapies, targeted therapies, other epigenetic agents, and immune checkpoint inhibitors to broaden response rates among patients with hematologic cancers and to extend the reach of such treatments to solid tumors (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Although synergy is easy to demonstrate in vitro, it is context-dependent, and clinical results have often been disappointing. To date, only the combination of panobinostat, bortezomib, and dexamethasone has received accelerated FDA approval.[90] The hope is that deeper epigenetic insights may eventually guide the development of agents and combinations of agents that can be used in a precision medicine strategy. DNMT–HDAC inhibitor combinations were identified as synergistic as early as 1983,[91] but there has been no consistent evidence of a benefit in AML, despite extensive studies.[92] Investigations involving careful scheduling, different diseases, new targets, and different agents may avoid the pitfalls of earlier studies. Given its long half-life, entinostat may be especially well suited to combination therapy. Finally, numerous combinations with immune checkpoint inhibitors are in progress, on the basis of the ability of DNMT and HDAC inhibitors,[32,93,94] and now EZH2 inhibitors,[95] to induce the expression of genes encoding proteins directly involved in the immune response and to induce immunogenic cancer–testis antigens or endogenous retroviral sequences.

LESSONS LEARNED AND THE WAY FORWARD

In 2020, despite the availability of nine approved epigenetic agents, most advances in the epigenetic treatment of hematologic cancers and solid tumors remain a work in progress. Epigenetics is a field approaching its 50th anniversary, and the magnitude of its import and its redundancy, interconnectedness, and vulnerabilities are gradually coming into focus; gaps in knowledge are increasingly evident, even as some are filled. Our definition of cancer in terms of hallmarks implies an acknowledgment of a finite set of keystone characteristics, and this is likely to be just as true in epigenetics. Aberrant DNA methylation, for example, has emerged as a recurring oncogenic event,[6] with the identification of at least three different paths to the phenotype: mutations in the genes encoding DNMT, TET, and several enzymes in the TCA cycle. This underscores the need to identify the molecular underpinnings in order to target the responsible genetic aberration rather than the phenotype, DNA methylation. As a case in point, despite increased DNA methylation, DNMT inhibitors have only limited value in the context of *TET* and *IDH* mutations.[33-36] Furthermore, although normalization of the epigenome is considered a therapeutic strategy, it has not been proved that this represents the predominant mechanism in our therapies.

The lens of precision medicine is critical to the future development of epigenetic therapies. With hundreds of potential targets,[4] epigenetic alterations are among the most commonly observed aberrations in cancer. They may arise early as foundational mutations, or they may arise late and drive clonal subsets. Understanding the interplay of these alterations may help us discern which mutations cause actionable vulnerabilities. But context matters, and mutations in one context may not have the same effect as mutations in another. In AML, inhibition of IDH1 was a paradigm shift. In cholangiocarcinoma, ivosidenib has a low response rate, although a median overall survival of 14 months led to a pivotal randomized trial, which is ongoing.[39] Cell type also matters: although epigenetic alterations are often residue-specific, they may also affect the expression of hundreds of genes and occur on a preexisting landscape that varies widely according to cell type, in both malignant and normal cells. Thus, resulting net changes from epigenetic alterations are inevitably context- and cell-dependent.

Another challenge stems from the fact that, unlike oncogenes, for which inhibitors can be designed, the vast majority of "epigene" alterations are loss-of-function mutations, which are inherently difficult to treat. It will be necessary to design drugs that interfere with adaptive mechanisms, an increasingly validated approach. FDA approval of tazemetostat is based on the unopposed EZH2 activity and vulnerability to tazemetostat that results from loss of SMARCB1 from the SWI/SNF complex. Similar strategies

may soon be available for loss-of-function mutations in acetyltransferases.

Although epigenetic therapy is likely to be less effective when foundational alterations are superseded by oncogenic drivers, it may succeed earlier in the evolution of a tumor or in tumors with fewer acquired mutations. For germline epigenetic aberrations, such as those that occur with succinate dehydrogenase and other enzymes of the TCA cycle, treatment may be both preventive and therapeutic.

Finally, epigenetic therapies, including inhibitors of DNMT, HDAC, and EZH2, are sometimes active against both mutant and wild-type genotypes. Rather than invest in expanding indications for existing therapies, we should focus on achieving a deeper understanding of epigenetic mechanisms in order to develop better therapies.

CONCLUSIONS

Chromatin remains an important therapeutic target. The activity of established and investigational epigenetic therapies in well-defined clinical contexts has provided evidence that this strategy can be effective. Given the sheer number of potential targets, a systematic approach that identifies and validates potential drug targets is needed to focus drug development and achieve the promise of this strategy.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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