EPIGENETICS

Targeting the cancer epigenome for therapy

Peter A. Jones[1], Jean-Pierre J. Issa[2] and Stephen Baylin[1,3]

Abstract | Next-generation sequencing has revealed that more than 50% of human cancers harbour mutations in enzymes that are involved in chromatin organization. Tumour cells not only are activated by genetic and epigenetic alterations, but also routinely use epigenetic processes to ensure their escape from chemotherapy and host immune surveillance. Hence, a growing emphasis of recent drug discovery efforts has been on targeting the epigenome, including DNA methylation and histone modifications, with several new drugs being tested and some already approved by the US Food and Drug Administration (FDA). The future will see the increasing success of combining epigenetic drugs with other therapies. As epigenetic drugs target the epigenome as a whole, these true 'genomic medicines' lessen the need for precision approaches to individualized therapies.

Writers
Enzymes that apply covalent modifications, such as methyl or acetyl groups, to specific amino acids on histones.

Readers
Proteins that can recognize specific modifications on histones at defined positions in the protein backbone.

Plasticity
The reversibility of epigenetic marks on DNA and proteins.

[1]*Van Andel Research Institute, Grand Rapids, Michigan 49503, USA.*
[2]*Fels Institute for Cancer Research, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania 19140, USA.*
[3]*Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21287, USA.*

Correspondence to P.A.J.
peter.jones@vai.org

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No multicellular organism can develop and function without the interaction between the genome and the epigenome. The epigenome consists of specific covalent modifications of chromatin components — which include DNA, RNA and proteins (such as histones) — that ensure the somatic inheritance of differentiated states. The structure and function of the epigenome are controlled by these covalent marks, which are applied by enzymes (writers) to the 147 bp of DNA and the eight histone components of a nucleosome[1]. These marks instruct the proteins that recognize them (readers) to identify and remodel particular genomic regions to modulate gene expression. The functions of the best-studied marks are remarkably context dependent, and they can have apparently opposing roles depending on these contexts (BOX 1; FIG. 1). The plasticity of the epigenome owes much to the existence of erasers; that is, enzymes capable of removing active and repressive marks.

The past few years have seen the fruition of many national and international mapping projects, such as those conducted by the US National Institutes of Health (NIH) Roadmap Epigenomics Mapping Consortium[2], the International Human Epigenome Consortium[3], The Cancer Genome Atlas Network[4], BLUEPRINT and the International Cancer Genome Consortium, which have defined the genome-wide distribution of epigenetic marks in many fetal and adult normal and cancerous tissues. In parallel, genome sequencing efforts of thousands of uncultured tumours have revealed the frequent existence of mutations in writers, readers and erasers, thus establishing a causative role for an altered epigenome in cancer[4–6].

The vast majority of human cancers harbour both genetic and epigenetic abnormalities, with a fascinating interplay between the two[4,6]. For example, childhood tumours seem to be driven by a small number of mutations — quite often in genes encoding chromatin-modifying enzymes — and exhibit profoundly altered DNA methylation patterns[7,8]. Adult tumours also frequently have mutations in genes that encode chromatin-regulating enzymes[9], and there is a growing realization of the profound effects of these mutations on the epigenome. For example, mutations in the isocitrate dehydrogenase-encoding genes *IDH1* and *IDH2* in gliomas and acute myeloid leukaemia (AML) inhibit the activity of histone demethylases and DNA demethylases[10–12], resulting in altered DNA and histone methylation patterns that drive the disease phenotype[13]. Also, mutations in *TET2* (which encodes a member of the TET family of enzymes) are frequently seen in myeloid malignancies and result in hypermethylation of haematopoietic-specific enhancers[14]. By contrast, mutations in *DNMT3A*, the gene encoding DNA methyltransferase 3A, are associated with a specific DNA hypomethylation pattern in AML[15]. Mutations in histone H3 lysine 36 (H3K36) in sarcomas[16] and in H3.3K27 in gliomas[17] exert dominant-negative inhibition of their respective methyltransferases, resulting in global reprogramming of these histone marks. There is now a long list of chromatin-controlling genes that have been found to be mutated in cancer, and determining how these mutations directly, or indirectly, alter the epigenome is an intense research endeavour, which will no doubt influence the crafting of future epigenetic therapeutic strategies.

Box 1 | **Location of chromatin covalent marks determins function**

The activity of chromatin is governed by a set of covalent marks, the locations and functions of which are markedly dependent on the context within the transcriptional unit[140]. Promoters can be divided into those with and without CpG islands (CGIs), and promoters have different marks relative to those on enhancers or gene bodies. Active, poised or inactive promoters, enhancers or gene bodies have specific combinations of covalent modifications associated with them. The location of the mark can be critical; for example, actively transcribed gene bodies have both 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), whereas active promoters are unmethylated[141]. The mechanisms by which DNA methylation patterns can be somatically inherited are understood, but far less is known regarding the copying of histone marks. The marks are applied by enzymes called writers, interpreted by proteins called readers that recognize the modifications, and can be removed by enzymes called erasers. All of these processes are potential drug targets and the subject of active discovery efforts.

Nonetheless, cancer is essentially a disease of gene control, which, until quite recently, was regarded as resulting from the dysregulation of transcription factors, such as cellular tumour antigen p53 and proto-oncogene protein MYC. Transcription factors are undoubtedly of major significance in neoplasia, but the development of small-molecule inhibitors against them represents a conundrum because they are not enzymes and because it is difficult to inhibit protein–protein interactions using drugs. By contrast, readers, writers, erasers and remodellers of the epigenome either are enzymes or recognize small covalent modifications, and thus represent ideal drug targets. Investigations are underway to identify small molecules that alter the structure and accessibility of the diseased epigenome, thus possibly indirectly targeting the effects of aberrant transcriptional circuitry.

In this Review, we first discuss the endogenous and exogenous processes that can lead to epigenetic aberrations that predispose to cancer, and review existing therapeutic strategies (either approved or currently in clinical trials) and the mechanisms by which they target the epigenome. We then review the role of epigenetic marks as biomarkers of drug response, as well as the efficacy of combining epigenetic therapies and other cancer therapeutics. We also discuss existing challenges and emerging strategies to overcome them. As noted earlier, RNA is also a chromatin component with well-known effects on gene regulation but is not discussed here.

Epigenetic pathways to cancer

It is important to realize that epigenetic changes are intrinsically heritable at the somatic cell level[18]. This is essential for stable phenotypic inheritance, and the structure and function of the epigenome can be heritably changed by intrinsic and extrinsic factors such as mutations or ageing (FIG. 2). These changes can fundamentally alter the behaviour of stem cells and their differentiation hierarchies, giving rise to cellular states that are permissive for the expression of mutant oncogenes[19]. For example, recent work has shown that the epigenome of the cell of origin can determine the phenotype of rhabdoid brain tumours in children[20] or of chronic lymphocytic leukaemia in adults[21].

Chromatin modifiers can be mutated in the germ line or in somatic cells either by endogenous or exogenous processes[6]. For instance, a high percentage of point mutations in germ or somatic cells are induced by spontaneous hydrolytic deamination of 5-methylcytosine (5mC) to thymine, which means that the epigenetic mark is contributing directly to genetic changes[22,23].

Ageing and environmental exposure to carcinogens can directly alter the epigenome in a somatically heritable fashion, thus providing a substrate for further changes or serving as driver events[24–26]. Moreover, a potential role for nutrition in altering the epigenome has long been hypothesized, and this field of research may well be stimulated by the recent discovery of vitamin C as an essential cofactor for the TET enzymes[27]. These proteins act as erasers of the 5mC mark on DNA by catalysing its oxidation to 5-hydroxymethylcytosine (5hmC), and TET genes are frequently mutated in human cancer[28]. Vitamin C deficiencies might therefore contribute to increased DNA methylation and aberrant gene expression.

Evidence is also mounting that an important determinant of epigenetic abnormalities in chromatin and DNA methylation that occur during tumour initiation and progression is cell stress associated with chronic DNA damage. Such stress — present during chronic inflammation and during the ageing process — can result in the transient assembly of transcription-repressive complexes that are crucial for DNA repair at damaged loci[29–33].

All of these processes can lead to the undermining of the normal homeostasis of the epigenome and give rise to widespread and pronounced changes in chromatin that are visible to pathologists through the light microscope. Targeting these changes in an attempt to restore a more normal epigenomic configuration therefore seems a viable treatment strategy for cancer.

Drugging the epigenome

There are two classes of drugs in clinical trials that target the epigenome: broad reprogrammers (so-called genomic medicines) and drugs developed to treat specific patient subsets, which represent more classical targeted therapies for precision medicine. At appropriate drug doses, both broad and narrower reprogrammers achieve precise interactions with the epigenetic regulatory proteins that are targeted. Drugs that target the epigenome and have entered clinical trials are listed in TABLE 1.

Broad reprogrammers. Broad reprogrammers include DNMT inhibitors (DNMTi), histone deacetylase inhibitors (HDACi) and inhibitors of the bromodomain and extra-terminal motif proteins (iBETs). These drugs tend to cause large-scale changes in gene expression[34,35], generally reversing cancer-specific gene expression alterations.

DNMTi entered clinical trials as classical anticancer agents more than 40 years ago with little success, but were revived in the past two decades as their mechanism of action was discovered[36]. Clinical trials with the 5-azanucleoside drug azacitidine (also known as

Erasers
Enzymes that can remove specific modifications at defined sites on DNA or histones.

TET family
The ten-eleven translocation family of α-ketoglutarate-dependent dioxygenases catalyse the oxidation of 5-methlcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and further products. Genes encoding these enzymes are frequently mutated in human cancers.

Driver
A gene in which the activation or deactivation of expression is causally related to the establishment of the malignant state.

Genomic medicines
Drugs that have wide-ranging effects on the epigenome.

Precision medicine
The use of drugs to target specific abnormalities identified in a patient.

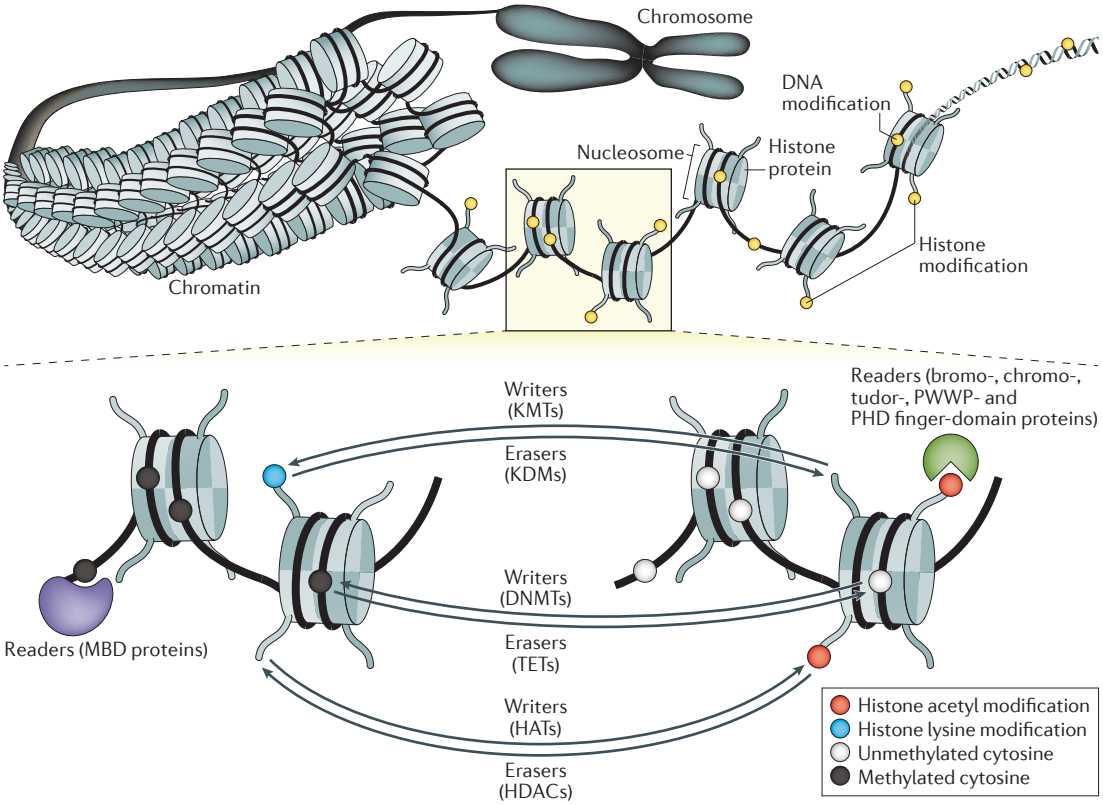

Figure 1 | **Modulation of covalent modifications on chromatin.** A 147 bp sequence of DNA is wrapped around a core of eight histone proteins to compact the genome into nucleosomes and then into chromatin and chromosomes. A subset of covalent modifications (yellow circles) on both the histone and DNA components, which control accessibility of DNA to transcription factors and other regulators, are shown. Covalent marks are established by 'writers', such as histone lysine methyltransferases (KMTs), histone acetyltransferases (HATs) and DNA methyltransferases (DNMTs). These modifications are interpreted by 'readers', including methyl-CpG binding domain (MBD) proteins on the DNA and multiple proteins for the histone marks as shown. Progress over the past decade has shown that almost all of the marks can be removed by 'erasers', such as histone demethylases (KDMs), histone deacetylases (HDACs) and by the ten-eleven translocation (TET) family of 5-methylcytosine oxidases. The interplay between these enzymes helps to establish and maintain cellular identity in addition to the central role of transcription factors by regulating access to the DNA sequence. PHD, plant homeodomain.

5-azacytidine; Vidaza, Celgene) and its deoxy derivative decitabine (also known as 5-aza-2′-deoxycytidine; Dacogen, Otsuka) at doses that were optimized based on epigenetic modulation[37] demonstrated that 15% or more of patients with myelodysplastic syndrome (MDS) or AML respond to epigenetic therapy, as shown by a reduced malignant cell burden, improved blood cell count and improved survival[38,39]. These data led to US Food and Drug Administration (FDA) approval of the inhibitors for the treatment of these conditions.

The activities of DNMTi can be impressive, with long-term responses in individual patients and with occasionally much-delayed responses that suggest indirect effects mediated by reprogramming rather than by direct cytotoxicity[40,41]. However, primary and secondary resistance to these therapies are common[42,43], and activity has been limited in solid tumours, possibly owing to the short half-lives of these S phase-specific drugs[44]. The dinucleotide guadecitabine is a second-generation hypomethylating drug with improved pharmacology and pharmacodynamic effects (compared with first-generation drugs) that has shown promise in early clinical trials[45].

HDACi were initially discovered based on drug screens for differentiation inducers in leukaemias[46] (for a detailed review, see REF. 47). Vorinostat (Zolinza; Merck & Co.), belinostat (Beleodaq; Spectrum Pharmaceuticals) and romidepsin (Istodax; Celgene) have all been approved for the treatment of cutaneous or peripheral T cell lymphomas, and panobinostat (Farydak; Novartis) was recently approved for the treatment of drug-resistant multiple myeloma when used in combination with the proteasome inhibitor bortezomib (Velcade; Millennium Pharmaceuticals)[48]. HDACi have shown limited single-agent activity in other malignancies[49]. iBETs, which reversibly bind to the bromodomains of BET proteins, constitute a third class of broad reprogrammers that was developed to target bromodomain-containing protein 4 (BRD4). *BRD4*, which is translocated in some cancers[50], encodes a reader of the acetylated histone signal that is essential for high-level expression of oncogenes such as *MYC*[51] through the promotion of enhancer activity[52]. iBETs have generated considerable excitement in preclinical studies and have now entered early-stage clinical trials.

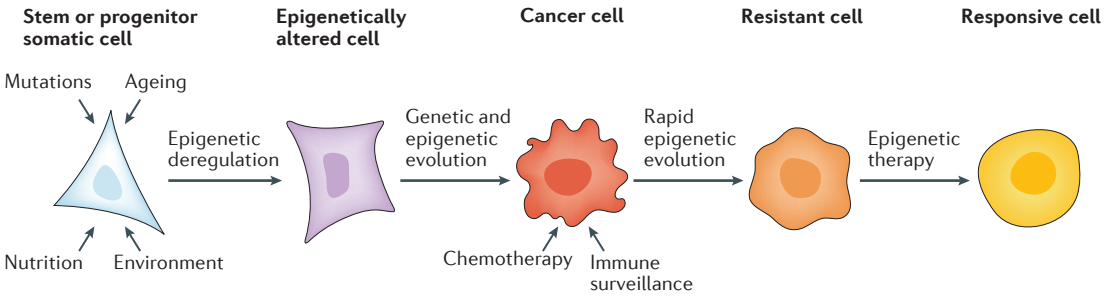

Figure 2 | **Somatic inheritance of acquired traits in cancer.** Normal cells can acquire both genetic and epigenetic alterations induced by endogenous and exogenous sources in addition to mutations inherited through the germ line. Mutations can be clonally selected if they provide a growth advantage and cooperate with epigenetic alterations, which can silence or activate genes to provide tumour cells with essentially two heritable pathways to rapidly evolve under the Darwinian selection pressures operating in multicellular organisms. The interplay between these two processes gives rise to the presence of altered chromatin, which can be recognized by pathologists under the light microscope in almost all human cancers. Further selection can take place following host immune surveillance or chemotherapy and, once again, the availability of both genetic and epigenetic pathways can rapidly speed up the emergence of resistance. Epigenetic therapy has the potential to reverse epigenetic abnormalities, thus restoring sensitivity to treatment.

Targeted therapies. The identification of activating mutations in cancers has also led to the development of therapies targeting many of the specific genetic defects in epigenetic pathways. The H3K27 histone *N*-methyltransferase EZH2 is activated by mutations in lymphomas[53], and use of an EZH2 inhibitor induced selective killing of cell lines carrying such mutations[54]. The tricarboxylic acid (TCA) cycle genes *IDH1* and *IDH2* are mutated in gliomas and AML, resulting in aberrant hypermethylation due to the production of a metabolite that inhibits DNA and histone demethylation[10,11]. First-generation IDH inhibitors have demonstrated activity in early clinical trials in AML[55]. However, not all *IDH*-mutant cell lines show sensitivity to these inhibitors[56], perhaps because DNA methylation is inefficiently reprogrammed by these drugs[57]. Aberrant DNA methylation is a key long-term epigenetic memory signal in cancer cells[58] that is central to the pathobiology of *IDH*-mutant gliomas[13]. Consistent with this finding, DNMT inhibition can be more effective than IDH inhibition in these cases[59].

Another strategy in targeted epigenetic therapy has been to exploit synthetic lethality approaches. Drugs that inhibit the H3K79 *N*-methyltransferase DOT1L appear to be active *in vitro* in leukaemias with activation of MLL (mixed-lineage leukaemia; also known as histone-lysine *N*-methyltransferase 2A (KMT2A))[60], whereas a drug targeting lysine-specific histone demethylase 1 (LSD1; also known as KDM1A) is active *in vitro* in malignancies with specific DNA methylation patterns[61]. These drugs have also recently entered clinical trials (TABLE 1). As expected for targeted therapies, preclinical studies show particular efficacies in specific patient subsets; thus, the clinical trials with these agents are expected to proceed very differently from trials with reprogrammers described earlier. For example, the effects of the LSD1 inhibitor seem particularly robust for small-cell lung carcinoma[61].

The existing excitement in the field of epigenetic therapy is immediately apparent when one considers the number of different drugs currently in clinical trials (>30), as well as the regular identification of new targetable epigenetic pathways including writers (for example, G9A; also known as EHMT2)[62], erasers (for example, JMJD3 (Jumonji domain-containing protein 3; also known as KDM6B))[63] and readers (for example, proteins with a methyl-CpG-binding domain (MBD))[64]. In fact, just as was done for kinases, there is a broad effort currently to develop compounds and drugs that inhibit all targetable epigenetic enzymes, in the hope that some of these will prove to be clinically useful anticancer therapies (FIG. 3).

Mechanisms of response

Clinical activity of a rationally developed drug is usually attributed to the original rationale but could also be related to off-target effects. Distinguishing between these possibilities is especially important for future drug development.

Dynamics of methylation changes. In epigenetic therapy, the best single-agent activity reported to date has been for the DNMTi azacitidine and decitabine in myeloid leukaemias. DNMTi lead to hypomethylation at low doses, but at high doses these agents are also cytotoxic, owing to their direct incorporation into both DNA and RNA (azacitidine) or DNA only (decitabine)[36,65], making it even more relevant to decipher response mechanisms. Studies on samples obtained from patients treated with these drugs demonstrated acute genome-wide demethylation, as measured by analysing the methylation of repetitive elements (such as LINEs or *Alu* elements)[66,67]. Robust demethylation of specific tumour suppressor gene promoters such as *p15* (also known as *CDKN2B*) was also observed[68], as was global gene-specific demethylation[69,70].

Synthetic lethality
A relationship between two genes in which the combined inactivation of the genes results in cell death, whereas the inactivation of either gene alone has no effect. It can also refer to a gene whose perturbation only results in cell death in the presence of a particular cellular feature (for example, a mutation).

LINEs
(Long interspersed nuclear elements). Highly repetitious elements that make up a considerable portion of the human genome; their methylation status can be used as a surrogate for overall genomic methylation.

Alu elements
Interspersed DNA sequences of about 300 bp that belong to the short interspersed element (SINE) family and are found in the genome of primates.

Table 1 | **Epigenome-targeting drugs that are approved or in clinical trials**

Inhibitor	Mechanism	Rationale	Drug	Target	Cancer type	Approval or trial status	Pharmaceutical company
DNMTi	Inhibition of DNA methylation	Removes hypermethylation of tumour suppressor genes	Azacitidine (Vidaza)	Pan-DNMT	MDS	EMA and FDA	Celgene Corporation (and generic)
			Decitabine (Dacogen)	Pan-DNMT	AML MDS	EMA (for AML) and FDA (for MDS)	Otsuka Pharmaceutical (and generic)
			Guadecitabine	Pan-DNMT	AML	Phase III	Astex Pharmaceuticals
HDACi	Inhibition of histone deacetylation	Reduces oncogene transcription and signalling, and promotes cell cycle arrest and apoptosis	Belinostat (Beleodaq)	HDAC class I and class II	Peripheral T cell lymphoma	FDA	Spectrum Pharmaceuticals
			Panobinostat (Farydak)	HDAC class I, class II and class IV	Multiple myeloma	FDA	Novartis
			Romidepsin (Istodax)	HDAC class I	Cutaneous T cell lymphoma	FDA	Celgene
			Vorinostat (Zolinza)	HDAC class I, class II and class IV	Cutaneous T cell lymphoma	FDA	Merck & Co.
			Abexinostat	HDAC class I, class II and class IV	Lymphoma	Phase I and phase II	Pharmacyclics
			ACY-241	HDAC6	Multiple myeloma	Phase I	Acetylon Pharmaceuticals
			AR-42	HDAC class I, class II and class IV	Haematological malignancies	Phase I	Arno Therapeutics
			CUDC-907	HDAC class I and class IIb	Solid tumours and haematological malignancies	Phase I	Curis
			CXD101	HDAC class I	Solid tumours and haematological malignancies	Phase I	Celleron Therapeutics
			Entinostat	HDAC class I	Breast cancer	Phase III	Syndax Pharmaceuticals
			Givinostat	HDAC class I and class II	Haematological malignancies	Phase II	Italfarmaco
			Mocetinostat	HDAC class I	Solid tumours and haematological malignancies	Phase II	Mirati Therapeutics
			Resminostat	HDAC1, HDAC3 and HDAC6	Hepatocellular carcinoma	Phase II	4SC
			Ricolinostat	HDAC6	Solid tumours and haematological malignancies	Phase II	Acetylon
iBET	Inhibition of BET binding to acetylated histones	Reduces oncogene signalling through super-enhancers	CPI-0610	Pan-BET	Haematological malignancies	Phase I	Constellation Pharmaceuticals
			TEN-010	Pan-BET	AML, MDS and solid tumours	Phase I	Tensha Therapeutics
			BAY1238097	Pan-BET	Solid tumours and lymphomas	Phase I	Bayer Corporation
			OTX015	Pan-BET	Haematological malignancies	Phase I and phase II	Merck & Co.
			INCB054329	Pan-BET	Leukaemias and solid tumours	Phase I and phase II	Incyte Corporation
			BMS-986158	Pan-BET	Solid tumours	Phase I and phase II	Bristol-Myers Squibb (BMS)
			FT-1101	Pan-BET	AML and MDS	Phase I	Forma Therapeutics
			GSK525762	Pan-BET	Solid tumours and haematological malignancies	Phase I	GlaxoSmithKline (GSK)

Table 1 (cont.) | **Epigenome-targeting drugs that are approved or in clinical trials**

Inhibitor	Mechanism	Rationale	Drug	Target	Cancer type	Approval or trial status	Pharmaceutical company (USA)
IDH inhibitors	Inhibition of mutant forms of *IDH*, a TCA cycle enzyme; also affect erasers of DNA methylation (TET enzymes) and histone methylation	Inhibits activating mutations	AG-881	IDH1 and IDH2	*IDH* mutant malignancies	Phase I	Agios Pharmaceuticals
			AG-120	IDH1	*IDH1* mutant malignancies	Phase I and phase II	Agios
			IDH305	IDH1	*IDH1*^{R132} mutant malignancies	Phase I	Novartis
			AG-221	IDH2	*IDH2* mutant malignancies	Phase I and phase II	Agios
EZH2 inhibitors	Blockage of H3K27 methylation	Inhibits activating mutations; induces apoptosis or differentiation	CPI-1205	EZH2	Lymphomas	Phase I	Constellation
			Tazemetostat	EZH2	Lymphomas and sarcomas	Phase I and phase II	Epizyme
DOT1 inhibitor	Inhibition of H3K79 methylation	Synthetic lethality of cells with *MLL* rearrangement	EPZ-5676	DOT1L	*MLL*-rearranged leukaemias	Phase I	Epizyme
LSD1 inhibitor	Inhibition of H3K4 and H3K9 demethylation	Promotes expression of tumour suppressor genes	GSK2879552	LSD1	AML and small-cell lung cancer	Phase I and phase II	GSK

AML, acute myeloid leukaemia; DNMTi, DNA methyltransferase inhibitors; EMA, European Medicines Agency; FDA, US Food and Drug Administration; H3K27, histone 3 lysine 27; HDACi, histone deacetylase inhibitors; iBET, inhibitors of the bromodomain and extra-terminal motif proteins; IDH, isocitrate dehydrogenase; LSD1, lysine-specific histone demethylase 1; MDS, myelodysplastic syndrome; *MLL*, mixed-lineage leukaemia; TCA, tricarboxylic acid; TET, ten-eleven translocation.

The dynamics of methylation changes after drug exposure were also examined; repetitive element methylation recovers fairly rapidly over a period of days after the drugs are withdrawn[66], whereas gene-specific remethylation is variable, with a subset of patients showing sustained demethylation over several weeks[67,71]. In terms of clinical correlation, acute demethylation has been found to correlate with clinical responses in some (for an example, see REF. 45) but not all[72] studies, whereas sustained demethylation of genes such as *p15* generally correlates well with responses[67,71]. However, this effect could partially be explained by clonal elimination of aberrantly methylated cells. These data, along with the clinical observations that responses are more consistent at low drug doses (favouring hypomethylation)[37,73] and that some patients respond to these drugs despite resistance to powerful cytotoxic drugs[45], argue convincingly for an epigenetic mechanism of clinical responses.

Variability in response. It has been more challenging to explain the variability in individual responses between or within tumour types. Myeloid malignancies are generally more responsive to hypomethylating drugs than lymphoid leukaemias or solid tumours[74]. This may be in part an artefact of pharmacology and clinical trial testing. Hypomethylating drugs are S phase-dependent and therefore have low incorporation into DNA in some malignancies (including many solid tumours), limiting their demethylating properties[44]. In addition, even in myeloid malignancies, these drugs are most active when used as frontline therapy[74], a strategy that has never been investigated in other cancers in which they have been used exclusively in patients resistant to chemotherapy. Still, it is notable that myeloid leukaemias may have a higher dependence on epigenetic mechanisms than solid tumours, as evidenced by a relatively low mutational burden and a high proportion of mutations in DNA methylation regulators, such as TET2 or DNMT3A[75].

In terms of inter-individual variability, several studies have investigated baseline molecular profiles in patients with MDS or AML treated with hypomethylating drugs, and most found no or relatively weak correlations between cancer genomes or epigenomes and response to therapy[76,77]. For example, genes that are commonly mutated in MDS are not associated with response to DNMTi, with the possible exception of *TET2* mutations[76,77], although even for this gene the effect was not seen in all studies[78]. Similarly, genome-wide promoter DNA methylation studies have not yielded a robust predictive signature for response to the drugs[72]. This is perhaps not surprising given the broad reprogramming potential of these drugs; although specific proteins (such as DNMTs) are targeted, the downstream effects of global hypomethylation are probably pleiotropic and highly variable from patient to patient, making prediction of response very difficult.

Reactivation of abnormally silenced expression. The widespread DNA methylation changes that have been documented in all human tumours have, until very recently, been thought to be the most likely targets for epigenetic therapy given that some of them may well be drivers of the transformed state or responsible for drug resistance. Numerous studies have shown the reactivation of abnormally silenced tumour suppressor

Frontline therapy
The use of a drug early in treatment before other drugs have been used.

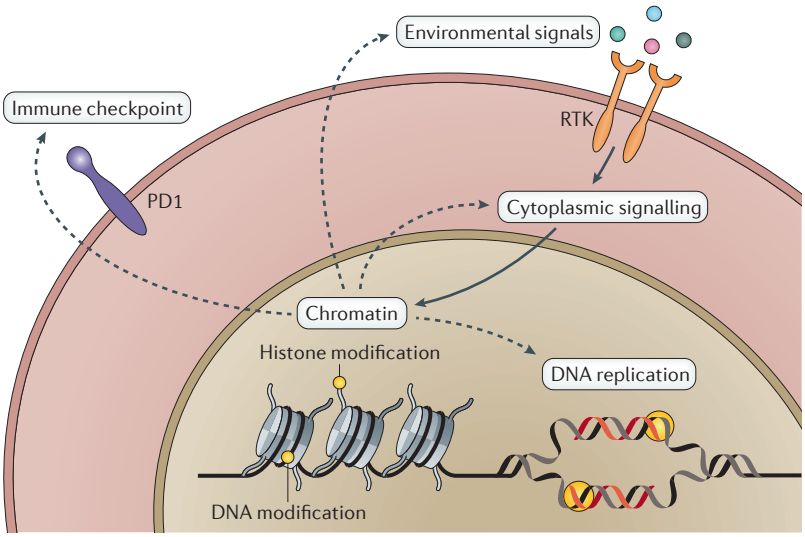

Figure 3 | **Targeting chromatin for therapy.** Cancer drugs have been traditionally developed to target signals received from outside the cell, such as growth factors or hormones (coloured circles), or to interfere with signalling in the cytoplasm. Cytotoxic drugs, which induce DNA damage or interfere with mitotic processes in the nucleus, have also become the mainstay of many chemotherapy regimens. The development of drugs to target chromatin is relatively new and is an area of intense interest as it provides opportunities to develop enzyme inhibitors — which can be less toxic than traditional treatments — and allows for interference of the actions of transcription factors, which are difficult to target directly. Importantly, targeting chromatin can enhance the activities of other drugs in combination therapies (shown by dashed lines). PD1, programmed cell death protein 1; RTK, receptor tyrosine kinase.

genes[79,80], DNA repair genes[41,81] and *de novo* methylated Polycomb-regulated genes[79], as well as microRNAs[82] (FIG. 4), using epigenetic therapeutic agents. The rationale underlying these approaches is the notion that reactivation of expression could normalize growth in treated cells. Restoring the activities of methylated CCCTC-binding factor sites (CTCF sites) could also be relevant, as one study in *IDH*-mutant gliomas showed that reduced CTCF binding disrupts chromosomal topology, resulting in aberrant regulatory element interactions that induce oncogene expression[13].

Another intriguing finding was the demonstration that reduced gene body methylation can attenuate the levels of expression of genes upregulated by MYC[35], which is commonly overexpressed in human cancers. The activation of MYC highlights the fact that epigenetic alterations could be a key driving force for the abnormal self-renewal properties of cancer cells with stem cell-like properties. As mentioned above, multiple genes that are either abnormally activated or silenced in cancer, in association with epigenetic alterations, could be involved[83,84]. A key example is the silencing of the potent tumour suppressor *p16* (also known as *CDKN2A*). Such loss of function allows cells to bypass the signals for senescence, and genetic knockout of this gene in mice results in stem cell expansion[85,86]. A number of genes encoding proteins that normally contribute to the inhibition of the WNT pathway — a key stem and progenitor cell pathway that is overactivated in cancer — are epigenetically silenced in colon cancer and other cancer types[84,87]. Importantly, abnormal stem and progenitor cell expansion potentially contributes to drug resistance in cancer. Epigenetic abnormalities may help to drive this expansion, and reversing this process can block the growth of such cells and restore drug sensitivity[88].

Activation of normally silenced expression. The approaches discussed above all rely on restoring the activities of genes that have become abnormally silenced and might therefore contribute to carcinogenesis. But there is also the possibility that activating genes and repetitive DNA elements that are epigenetically repressed in both normal and cancer cells may significantly enhance patient response to epigenetic therapy. The expression of tumour antigens, such as melanoma-associated antigen 1 (MAGE1)[89] and cancer testis antigens (CTAs), was found to be upregulated by decitabine, and interferon signalling was increased in cells exposed to DNMTi[90,91]. This activation of the host immune response would be expected to increase the visibility of the tumour to the host immune defence mechanisms (as discussed below).

Moreover, recent work from our laboratories and that of others has demonstrated the upregulation of endogenous retrovirus (ERV) transcripts following treatment with DNMTi; these ERVs lead to the formation of cytoplasmic double-stranded RNA and apoptosis[92,93]. Importantly, the sensing of these RNAs by viral defence proteins leads to the death of colon cancer stem cells[93], which may be highly relevant to the role of epigenetic abnormalities in the expansion of cancer stem and progenitor cells[88]. Similar to the activation of CTAs, ERVs can increase immunogenicity. Activation of viral defence pathways in normal cells in the patient might potentially lead to off-target effects, but this risk is possibly mitigated by the fact that 5-azanucleosides have an absolute requirement for incorporation into DNA to function as 'suicide inhibitors' of DNMTs[94]. As most cells in the human body are quiescent, the drugs are highly targeted to S phase cells, thus providing a more focused therapeutic window.

DNMTi therefore have pleiotropic effects on both abnormally silenced genes relevant to cancer and on genes and repetitive DNAs held silent by epigenetic processes. As the drugs inhibit DNA methylation globally, the net effect is to reset the epigenome and activate several pathways at one time, which might increase the efficacy of these drugs. It is this pleiotropic rather than targeted outcome that may be of great therapeutic value. Most of the pathways silenced epigenetically during tumour development would be expected to have been selected on the basis of conferring a growth or survival advantage to the tumour, making it unlikely that the treatment would be potentially counterproductive. Nevertheless, as with all treatment approaches, there is the possibility of worsening the situation.

Combinations of drugs

Pioneering work by DeVita and others introduced the enduring concept that almost all cancers are most efficaciously treated with combinations of drugs[95]. Monotherapies are rarely effective, with the notable

CCCTC-binding factor sites (CTCF sites). Binding sites for the CTCF transcription factor, which is involved in transcriptional activation, insulator activity and regulation of chromatin architecture.

Cancer testis antigens (CTAs). A group of proteins expressed during male germ cell development, which are silenced in normal cells and may become re-expressed ectopically in cancers. Many are highly immunogenic.

Monotherapies The use of a single drug to treat a malignancy.

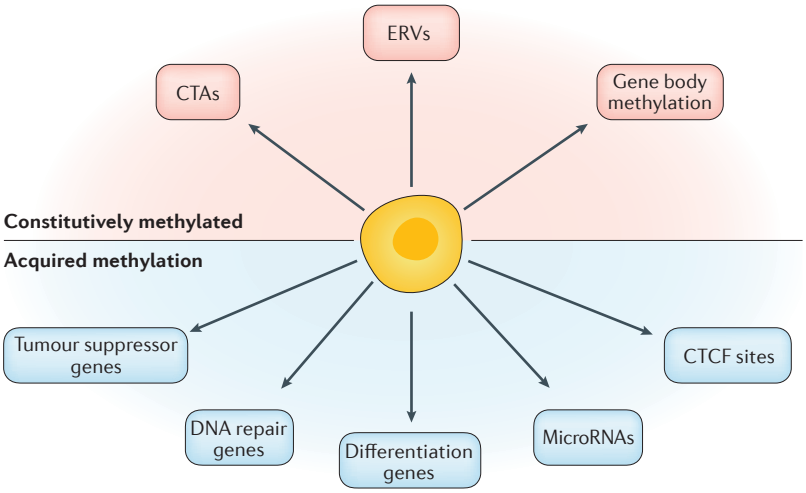

Figure 4 | **Activation of constitutively and *de novo* methylated elements by DNMT inhibitors.** Most of the genome in both normal and cancer cells is constructively methylated, although somewhat less so in cancers, which show global hypomethylation[142]. Removal of the methylation can lead to the upregulation of cancer testis antigens (CTAs) and endogenous retroviruses (ERVs), which might help to increase the immunogenicity of the cells as well as inducing apoptosis of cancer stem cells[91–93]. Decreased gene body methylation can lead to decreased transcription thus inhibiting the action of transcription factors such as the proto-oncogene protein MYC[35]. The DNA methyltransferase inhibitors (DNMTi) also increase the expression of tumour suppressor, DNA repair and differentiation inducing genes, which can help to restore more normal behaviour. In addition, reactivating the expression of microRNAs[82] and recommissioning CCCTC-binding factor sites (CTCF sites) can potentially help in response[13]. The wide-ranging activities of the DNMTi make them true 'genomic medicines'.

exception of targeted tyrosine kinase inhibitors, which give rise to rapid and impressive results in patients with chronic myeloid leukaemia (CML) or gastrointestinal stromal tumour (GIST)[96,97]. Rapid tumour responses are also seen with single-agent serine/threonine-protein kinase BRAF and epidermal growth factor receptor (EGFR) inhibitors in cancers bearing specific mutations[98–100]; however, the marked tumour shrinkages that occur rapidly in patients are almost invariably followed by the emergence of drug-resistant variants.

The future of epigenetic therapies, particularly for the most common solid tumours, is almost assuredly going to depend on rational combinations of drugs that will take advantage of the genomic consequences of targeting chromatin regulation. These strategies can involve hypothesis-driven combinatorial approaches utilizing different epigenetic therapy agents and/or combining these therapies with other cancer treatment approaches. DNA demethylating agents are effective alone in the treatment of haematological malignancies, as evidenced by their FDA approval in this arena, although their efficacy is likely to be enhanced by combinatorial therapy approaches.

Combination of DNMTi and HDACi. The most explored drug combinations are those that simultaneously inhibit DNA methylation (such as DNMTi) and histone deacetylation (such as HDACi)[101–103]. This strategy was initially developed to explore the roles for the interactions of these chromatin-modulating events in maintaining the abnormal silencing of genes with cancer-specific promoter DNA methylation[104]. The idea addressed the fact that densely methylated DNA is usually associated with transcriptionally repressive chromatin, which is generally accompanied by under-acetylated histone lysines[105,106]. Laboratory studies have firmly established that the re-expression of such genes is augmented by initial treatment with low doses of DNMTi followed by application of HDACi[101,102,104,107]. In this paradigm, downstream events coupling histone deacetylation with demethylation amplify the effects[104,108]. The dynamics appear to depend mostly on blocking HDAC1 and HDAC2, which reside in the nucleus[47,109]. Recently, this fact was solidified by the finding that the depletion of chromodomain-helicase-DNA-binding protein 4 (CHD4) — a key component of the nucleosome remodelling and deacetylase (NuRD) repressive transcriptional complex that binds HDAC1 and HDAC2 — robustly augments the effectiveness of low doses of a DNA demethylating agent in the re-expression of hundreds of silenced genes, including known tumour repressors[107].

This combinatorial paradigm has been explored in many preclinical studies, almost always showing increased expression of silenced genes and antitumour responses involving apoptosis[110–113]. A problem for fully understanding the implications of these studies is that a wide range of doses for the various drugs have been used, often high doses that would yield many off-target effects that are not well tolerated in patients. Similarly, multiple trials in patients have or are being conducted that combine DNMTi and HDACi, but whether the combination is beneficial to the outcome remains uncertain. Reports for MDS and AML have been conflicting to date, with one recent large trial showing no evidence of any benefit of the combination[114], whereas other smaller trials show increased efficacy with the combination[115,116]. Exciting possibilities have recently been seen for a small group of patients with advanced non-small-cell lung carcinoma (NSCLC)[117,118]. For example, 2 out of 65 patients treated with low doses of DNMTi combined with HDACi showed robust and durable clinical responses[117]. The implication of this treatment would be to benefit hundreds of thousands of individuals with lung cancer worldwide. This potential treatment awaits evaluation of the contribution of each agent alone versus in combination, as well as investigations into the mechanisms of efficacy and the establishment of biomarkers that can help to personalize the approach by identifying the small percentage of patients that are likely to respond.

Combination of epigenetic therapies and cytotoxic drugs. Preclinical studies are beginning to suggest that DNMTi and HDACi have their greatest efficacy when combined with other cancer therapies. One particularly promising approach that is being tested in clinical trials is combining DNMTi with standard cytotoxic drugs in an attempt to resensitize ovarian cancers to these standard agents[119–124]. The idea is that epigenetic mechanisms may often underlie the acquired resistance to

cytotoxic drugs and thus could be reversible with drugs that inhibit DNA methylation and/or HDACs[88] (FIG. 2). Results in a series of trials exploring combinations of DNMTi and chemotherapy continue to be encouraging[119–124], and this combination will probably continue to be tested for ovarian and other cancers.

Combination of epigenetic agents and immunotherapy. One particularly exciting future use of DNMTi and/or HDACi may be their combination with immune checkpoint therapy, a promising cancer treatment paradigm that has emerged over the past several years and which is now FDA-approved for forms of NSCLC, melanoma and renal cancer[125–127]. This approach is based on the fact that many cancers evolve means to escape from immune detection, so-called immune evasion[128,129]. A major component for this escape is a complex interaction between tumour- and immune-cell ligands and receptors that renders T cells 'tolerant' or incapable of mounting an immune attack against cancer cells[125–127]. A chance occurrence in clinical trials for patients with advanced NSCLC first highlighted the potential of combining epigenetic agents and immunotherapy: a small group of patients whose disease progressed after low-dose treatment with a combination of azacitidine and the class I HDAC inhibitor entinostat (Syndax Pharmaceuticals) had robust and durable tumour responses when they were subsequently enrolled in a trial of immune checkpoint therapy[118]. It remains to be determined whether these results reflect the effects of combined therapy exposures or are due to immunotherapy only. In the interim, the clinical suggestion has led to in-depth, preclinical studies to dissect the mechanisms that might underlie epigenetic therapy-mediated augmentation of immune checkpoint therapy.

Mounting evidence has suggested that either DNMTi or HDACi reverse immune evasion because they increase tumour cell interferon responses and the expression of surface tumour antigens and proteins such as major histocompatibility complex (MHC) molecules, which present these to immune cells[130,131]. As discussed above, recent studies have shown that cancer cells treated with DNMTi produce a state that has been termed 'viral mimicry' (REFS 92,93), resulting in an interferon-producing tumour cell response that would trigger immune attraction. This might be most effective in the setting of reversing immune tolerance for checkpoint therapy[92,93,132,133]. If clinical efficacy is confirmed for combining epigenetic and immune therapies, as is being tested in several trials, it will be important to determine the effects of epigenetic drugs on host immune cells, in addition to their influence on cancer cells.

Combination of different epigenetic therapies. New combinatorial therapies are now possible by combining existing epigenetic therapeutic agents with newly developed small molecules. Some of these are already in clinical trials as single agents (TABLE 1), and the initial results will undoubtedly inform future combinatorial strategies. Examples include combining either DNMTi or HDACi with inhibitors of LSD1, which has induced antitumour responses[134]. The rationale for this combination is that LSD1 is a key player in the stability of transcription-repressive complexes[135].

Triple therapy. Sensitization for immune checkpoint therapy following combined inhibition of DNMT1 and EZH2 induces ovarian cancer cells to express CXC-motif chemokine 9 (CXCL9) and CXCL10, which can stimulate T helper 1 cells[136]. In studies using a humanized immune model in immunocompromised mice, this chemokine upregulation induces the attraction of tumour-infiltrating lymphocytes (TILs), which in turn can kill tumour cells when combined with immune checkpoint therapy[136]. This study is emblematic for showing how multiple combined epigenetic therapy approaches may be considered for sensitization to immune checkpoint and other immunotherapy strategies.

Future combinatorial therapies. The therapies discussed above suggest future strategies for combinatorial epigenetic therapy strategies for cancer management. For example, initial clinical trials are already ongoing with iBETs, which block BRD4-mediated targeting of transcription-activating complexes to super-enhancers and gene promoters[137,138]. A recent preclinical study suggests that combining drugs that block oncogene activation, such as an iBET with an HDAC inhibitor, with newer and older drugs that might reverse abnormal gene silencing could be effective[139]. Similarly, combining inhibitors of abnormal MLL protein function, which fosters abnormal gene activation in leukaemias[60], with drugs that reverse aberrant gene silencing is another concept to consider. These are but a few examples of strategies that are likely to dominate new ways to elevate epigenetic therapy to an important position in cancer therapy.

Conclusions

Cancers subvert both the genome and the epigenome to evolve mechanisms by which tumour cells can escape growth control and surveillance to become increasingly autonomous of the requirements of the host. The involvement of altered chromatin in cancer has been apparent since the early days of pathology diagnosis through light microscopic observations. Given that epigenetic pathways exhibit greater flexibility by several orders of magnitude relative to genetic alterations, it is likely that tumours rely more on epigenetic alterations to escape immune surveillance and develop drug resistance. Epigenetic processes, particularly those involving DNA methylation, are somatically heritable and can contribute to stable but malleable changes in gene expression that interact with gene mutations to give tumours the advantage of evolving at a much more rapid pace than they could achieve by relying on genetic alterations alone.

The focus on drug targets has changed from growth factors and intracellular signalling molecules (particularly kinases) to chromatin, which interprets these upstream signals (FIG. 3). The targeting of chromatin

Immune checkpoint therapy
The use of antibodies that target regulatory pathways in T cells to enhance antitumour immune response.

has pleiotropic consequences for cancer cells and does not always require knowledge of existing mutations to be effective. This strategy also has the possibility of activating non-genic regions of the genome, particularly ERVs, thus increasing the target size. Therefore, epigenetic drugs represent true 'genomic medicines' and will almost certainly exhibit the greatest efficacy when used in combination and when used jointly with other therapies such as standard chemotherapy or immunotherapy.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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