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The roles of DNA, RNA and histone methylation in ageing and cancer

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Abstract

Chromatin is a macromolecular complex predominantly comprising DNA, histones and RNA. Its methylation is highly conserved throughout phylogeny as it provides an instructive template that helps coordinate context dependent access for gene expression, DNA repair and DNA replication. Dynamic changes in chromatin methylation are central to cell fate determination and normal development of the organism. Consequently, inherited or acquired mutations in the major epigenetic protagonists that regulate methylation of DNA, RNA and/or histone proteins are commonly observed in developmental disorders, ageing and cancer. This has provided the impetus for the clinical development of epigenetic therapies aimed to reset the methylation imbalance observed in these conditions. In this review we discuss the key principles of chromatin methylation and focus on how this fundamental biological process is corrupted in cancer. We discuss methylation-based cancer therapies and provide a perspective on the emerging data from early phase clinical trials therapies that target regulators of DNA and histone methylation. We also highlight promising future strategies, including monitoring chromatin methylation for diagnostic purposes and combination epigenetic therapy strategies that may improve immune surveillance in cancer and increase the efficacy of conventional and targeted anti-cancer drugs.

Introduction

The control and adaptability of virtually all biological processes involve post-synthesis chemical modification of macromolecules such as DNA, RNA and proteins. One of the most abundant modifications, methylation, is widespread throughout all kingdoms of life and involves an alkylation reaction where a methyl group replaces a hydrogen atom. Methylation is catalysed by methyltransferase enzymes, all of which utilise S-adenosylmethionine (SAM) as the methyl donor (Figure 1A). These methylation "writers" regulate the function and activity of three classes of fundamental molecules: DNA, RNA and proteins. They co-operate with dedicated "erasers" and "readers" – the protein machinery that removes or recognizes these methylation marks. The importance of the various methylation pathways is highlighted by the fact that their dysregulation is linked to many diseases[1-5].

DNA methylation occurs predominantly at palindromic CpG dinucleotides where a methyl group is added to the 5' position of the cytosine pyrimidine ring to generate 5-methylcytosine (5mC) (Figure 1B). It also occurs, albeit more rarely, in non-CpG contexts. Three enzymes,

DNA methyltransferase (DNMT) 1, 3A and 3B, perform and maintain the patterns of genomic methylation (Figure 1B)[2]. CpGs can undergo further oxidation of the 5-methyl group, catalysed by the ten-eleven translocation (TET) family of dioxygenases, to 5-hydroxymethyl (5hmC), 5-formyl (5fC), or 5-carboxyl (5caC) forms, which has been proposed as the initial step of active DNA demethylation in mammals (Figure 1B)[6]. More recently, 6-methyladenosine (m6A) was also identified in mammalian genomes, although its physiological consequence remains unclear[7,8]. For a more detailed discussion of DNA methylation please refer to *xxx et al in this issue*.

RNA methylation constitutes most of the known RNA modifications (over 70 types of RNA methylation have so far been identified), their biological and molecular functions remain largely unknown (Figure 1C). Indeed, until recently the vast majority of modifications had been ascribed only to ribosomal and/or tRNAs due to substrate abundance. However, pioneering research is now revealing that all classes of RNA are methylated (for examples see REF. [9-13]). Furthermore, demethylation at specific sites is possible, at least for m6A (Figure 1C)[14,15]. The functional role of RNA methylation in development and disease is an exciting and rapidly moving field and will be reviewed in detail by *xx et al in this issue*.

Protein methylation mainly occurs on the side chains of lysines and arginines (Figure 1D), but methylation of histidine, carboxyl methylation of aspartate, glutamate, or carboxyl-terminal residues (leucine and isoprenylcysteine) also occur in mammals[16]. However, histone methylation will be a major focus of this review and we will primarily focus on the involvement of lysine and arginine methylation pathways in disease (Figure 1E). Lysine residues on histones may be mono-, di- or tri-methylated (me1, me2, me3 respectively), whereas arginines may be mono-, symmetrically or asymmetrically di-methylated (me1, me2s, me2as respectively) (Figure 1D)[17]. Unlike acetylation and phosphorylation, histone methylation does not alter the charge of the histone protein. Arginine methyltransferases, the PRMTs, are structurally related, as are the histone lysine methyltransferases, virtually all of which contain a conserved SET domain **[G]** possessing enzymatic activity. One exception to this generality is DOT1L, which methylates H3K79 in eukaryotic genomes. A more detailed description of histone modification sites and the relevant enzymes can be found in Figure 1E and REF. [17].

During the methylation reaction, SAM is converted to S-adenosylhomocysteine (SAH) (Figure 1A), which actually inhibits methyltransferase activity[18]. Thus, methyltransferases are susceptible to events that alter the intracellular SAM/SAH ratio. Interestingly, raising the levels of SAM has been shown to repress proto-oncogenes via CpG methylation of their promoters[19]. Additional methylation pathway complexity exists because certain enzymes methylate multiple classes of macromolecule. For instance, human nucleolar protein 1 (NOP1) catalyses 2'-O-methylation of rRNA as well as methylation of glutamine 105 in histone H2A[20]. In addition, DNMT2 is structurally related to the DNMTs described above, yet it possesses only weak DNA methylation activity but catalyses 5-methylcytosine in tRNA very efficiently[21].

Until relatively recently, the methyl groups on DNA, RNA and proteins were generally considered to be highly stable modifications[22]. However, identification of specific demethylase enzymes (Figure 1) has indicated a more dynamic state of methylation, consistent with regulatory and functional roles[17]. As discussed below, this dynamic behaviour provided the rationale for therapeutic intervention.

In this review, we discuss the cellular functions of DNA, RNA and histone methylation, and the changes observed in these dynamic marks during normal ageing. We also highlight our current knowledge on how mutations in the various writers, readers and erasers of chromatin methylation contribute to cancer and why drugs that alter their function may be important to treat various malignancies. Early clinical trial data with these drugs are beginning to emerge and we provide a perspective on these data and emphasise the key knowledge gained regarding the safety and efficacy of this exciting new class of drugs. Finally, we highlight promising future strategies for monitoring dynamic changes in chromatin methylation for diagnostic and prognostic purposes and discuss potential new avenues whereby manipulating chromatin methylation in combination with established anti-cancer drugs may improve the clinical utility of epigenetic therapies.

[H1] The cellular functions of methylation

Important clues to the functions of particular DNA, RNA or histone methylation marks can be gained from knowledge of their cellular and intramolecular localizations. Thus, much effort has been expended in mapping the modified sites using various biochemical (e.g. chromatin immunoprecipitations) and biophysical techniques (e.g. mass spectroscopy), which together have revealed detailed genetic, epigenetic and epitranscriptomic methylation profiles.

CpG DNA methylation is widespread in mammalian genomes, with around 70% of sites being methylated. The exception to this are CpG-rich regions (CpG islands) within the promoters of active genes, which are characteristically unmethylated[23]. CpG DNA methylation represents a fundamental mechanism of stable repression underlying processes such as X chromosome inactivation **[G]** and gene imprinting **[G]**[23]. It is significantly enriched in heterochromatic regions and many inactive gene promoters. Active genes can also harbour methylated CpGs within their transcribed regions, where they act to regulate mRNA alternative splicing by recruiting methyl-CpG-binding protein 2 (MECP2)[24]. Notably, many tumour suppressor genes are silenced by DNA methylation in cancer[23]. Mammalian genomes mainly consist of repetitive elements and although many are methylated[25], their biological significance is uncertain. Nevertheless, hypomethylation of these elements is observed in cancer cells[26,27] and this can lead to activation and transposition of the endogenous retroviral sequences thereby promoting genetic instability and tumour development[1] (Figure 2).

Regarding histone methylation, no strict rule governs its distribution in chromatin (detailed in REF. [17] & [28]). In loosely packed ***euchromatin***, certain methylations such as H3K4me3 are present in active promoters, whereas H3K36me3 is present within actively transcribed regions where it preferentially mark exons but is present at lower levels in those that are alternatively spliced[29]. Others, such as H3R17me2as, mark the promoters of active hormone-regulated genes. Active genes are also marked by H3K79me, but this methylation is also linked to many other processes including DNA damage response and heterochromatin formation (detailed in REF. [30] & [31]). Specific functional regions of the genome, such as enhancers and origins of replication, are also marked by specific histone methylations, in these cases H3K4me1 and H3K36me1 respectively. Histone methylation in tightly packed ***heterochromatin*** depends on the subtype; *facultative* heterochromatin contains genes that are differentially expressed during development and/or differentiation and which then become silenced. It is characterized by an abundance of the repressive mark H3K27me3, as found on the inactive X-chromosome in mammalian female cells, whereas c*onstitutive* heterochromatin is particularly enriched with the inactive marks H3K9me2 and H3K9me3[17,32]. Finally, certain developmental genes possess so-called 'bivalent' domains that contain both active H3K4me3 and inactive H3K27me3 marks. This 'poised' state is thought to allow them to rapidly respond to differentiation cues[33].

Histone methylation predominantly functions via direct recruitment or inhibition of histone binding proteins. For example, H3K4me3 specifically recruits activatory proteins, such as inhibitor of growth (ING) proteins, to gene promoters whilst inhibiting binding of repressors such as the nucleosome remodeling and deacetylase (NuRD) complex[17,28]. In mammalian heterochromatin, H3K9me2/3 is specifically bound by the chromodomain **[G]** of heterochromatin protein 1 beta (HP1β), a protein important for the higher architecture of heterochromatin[17,28] (Figure 2). Methylation such as H4K20me also underpins genomic integrity[34].

Although it is undeniable that histone modifications robustly modulate key cellular processes and there is compelling evidence for the role of histone methylation in DNA processes, as outlined above, it is difficult to formally prove a direct causative role of any histone modification in mammalian cells (see REF. [35] for discussion). This situation is further compounded by (i) most histone modifying enzymes also methylating non-histone substrates, such as the tumour suppressor p53 protein[36], and (ii) some methyltransferases methylating multiple classes of macromolecule[20], as described above. In the case of RNA methylation, the situation is even more confounded because the modification can occur post-transcriptionally[37], or co-transcriptionally[9]. Furthermore, unlike histone methylation, some methylations of RNA (*e.g.* m7G) can directly affect local secondary structures by interfering with non-canonical base pairing. Other methylations may act as binding platforms for specific binding proteins, as exemplified by m6A, which is bound by YTH-domain proteins[38]. Utilizing these mechanisms, RNA methylation can directly affect processing, stability, translation and localization[39]. The effect may even be *trans*-acting, as exemplified by m6A in *Xist* RNA that is required for it to mediate transcriptional repression[40].

Thus, methylation is widespread throughout the genome and epitranscriptome. This creates an obvious and huge potential for crosstalk between different methylation pathways. This is exemplified in histones, where crosstalk occurs between methylations, not only within the same histone but also between modifications in different histones[17,28]. There can also be cooperation between methylation of histones and DNA. For instance, the RING E3 ubiquitin ligase Ubiquitin Like With PHD And Ring Finger Domains 1 (UHRF1) binds to nucleosomes harbouring H3K9me3, and this is significantly enhanced by CpG methylation of the nucleosomal DNA[41]. Furthermore, the ubiquitin-like domain within the ligase promotes

ubiquitination of histone H3, which recruits DNMT1 to newly replicated chromatin to ensure the inheritance of DNA methylation[42].

As discussed above, distinct general methylation patterns are found in heterochromatin and euchromatin domains. In fact, there are well-defined regions of demarcation, so-called 'boundary elements', between heterochromatin and euchromatin suggesting that methylations may be involved in establishing and/or maintaining the higher order topological structures of the genome. Indeed, specific factors such as CCCTC-binding factor (CTCF), which play a role in maintaining boundaries between distinct chromatin regions, bind to DNA in a methylation-dependent manner[43,44]. CpG methylation prevents CTCF binding, suggesting a role for DNA methylation in regulating these sites (Figure 2).

[H1] Methylation in ageing and cancer

Although the primary focus of this review is on the role of chromatin methylation in cancer, it is important to emphasise that methylation of DNA, RNA and histones has also been widely implicated in developmental diseases, non-malignant acquired diseases such as autoimmunity and ageing. Overall, the key principles of the methylation pathways, in terms of location and regulation of DNA templated processes, are highly conserved throughout mammals. Indeed, their importance is underscored by the fact that disruption of these processes, through inherited or acquired mutations in the main protagonists, results in embryonic lethality or can produce severe disease states[1-5]. Mutations related to developmental diseases have been well described in the literature and the major mutational targets are listed in Supplementary Table 1.

As a cell ages, the methylation landscape changes dynamically (Figure 2). Early studies suggested that global DNA hypomethylation is a hallmark of aging; however, recent work has highlighted that loss of DNA methylation occurs primarily in repetitive regions of the genome that correlate with constitutive heterochromatin, whilst hypermethylation primarily occurs at promoter CpGs[45,46]. As discussed above, in normal tissues repetitive elements are maintained in heterochromatin and highly methylated to prevent their transcription. Ageing is accompanied by selective loss and reorganization of heterochromatin and upregulation of transcripts from repeat regions, in particular retrotransposable elements[47], which are associated with DNA double strand breaks[48,49] and can negatively impact on genome stability and disease[50]. In contrast, a stable epigenome is reported to contribute to longevity and

cancer resistance[51]. In addition, locus-specific DNA hypermethylation has been associated with ageing blood and tissues[52,53] and appears to be conserved between mice, monkeys and humans[54]. DNA methylation tends to increase with age at some CpG islands, particularly at polycomb target genes[55,56] and at promoters of tumour suppressor genes[57]. The sum of these changes in DNA methylation likely lead to an age-related transcriptional programme[53]. In line with this, a number of DNA methylation clocks have been described (discussed in [58]) and genome-wide DNA methylation has been shown to serve as a reliable estimator of age[59] and can predict mortality[60] and lifespan[61,62] in model organisms.

Histone methylation changes with ageing are also context specific and there are conflicting data from different ageing models (detailed in REF. [63] and [64]). A gain of novel bivalent domains is observed in ageing cells[65,66] (Figure 2), and increased levels of the heterochromatin-associated proteins, including histone H2A variant macroH2A (associated with gene repression[67]) and HP1β, were observed in tissues from old versus young mice and primates[68,69]. A general loss of histones and redistribution of methylation marks has also been observed in yeast and human fibroblasts[70,71], suggesting this is an evolutionarily conserved mechanism that regulates replicative lifespan **[G]**, but it remains unclear if replicative senescence also occurs in mammalian cells *in vivo*.

In addition to changes in DNA and histone methylation, certain mRNAs are less m6A methylated in old versus young human blood cells[72]. Thus, biological methylations exist in flux, responding to various pathologies and natural processes such as ageing. It is the existence of such dynamic equilibria that underpins the rationale to therapeutically target these pathways in the hope that pathological perturbation of the methylome can be reversed.

Advancing age sets the platform for the development of a range of acquired age-associated co-morbidities that ultimately shorten the life span of the organism, one of the best studied of which is cancer. Of note, the same changes documented in ageing cells, such as demethylation of retrotransposons and satellite DNA and high levels of their transcription, are also observed in human cancer[73-75]. In recent years, we have begun to gain fascinating insights into the origins of cancers, which are derived from clonal populations of cells, bearing dysregulated chromatin. An excellent example of this is Age Related Clonal Hematopoiesis (ARCH)[61]. Several laboratories have independently demonstrated that in more than 10% of adults aged 60 years or older, steady state haematopoiesis (> 5 x 10^{11} blood cells generated each day)

occurs from a restricted number of clonal haematopoietic stem and progenitor cells harbouring somatic mutations that confer a clonal advantage. Strikingly, ARCH is associated with an increased risk of a subsequent haematological malignancy and with an almost two-fold increased risk of cardiovascular mortality[76-78]. Mutations in genes encoding DNMT3A, TET2 (Supplementary Table 1) and additional sex-combs like 1 (ASXL1), which regulate DNA and histone methylation, account for greater than 70% of cases of ARCH[76-78]. Remarkably, these findings in patients[79] could have been largely predicted from mouse models where these genes have been conditionally deleted in haematopoietic tissues. Haematopoietic stem cells (HSC) from these mice show an increased self-renewal capacity, allowing them to outcompete non-mutated HSC and clonally expand[80-85]. The prognostic significance of diseases such as acute myeloid leukaemia (AML), arising from pre-existing ARCH, is yet to be fully determined. However, the presence of clonal haematopoiesis was reported to negatively impact clinical outcomes in patients receiving treatment for non-haematological cancers[86]. Clonal haematopoiesis does not just influence cancer development and therapeutic response but is also a major risk factor for atherosclerotic disease, which is another feature of ageing[87,88]. There remains much to be learnt about the global changes in chromatin methylation in conditions such as ARCH, and how this has such a profound influence on the cancer and cardiovascular disease. Whilst there is compelling evidence correlating ARCH with a range of pathologies frequently observed in advanced age, if we are to develop strategies to alter the natural history of this process it is imperative that we understand the molecular aetiology behind these associations.

[H2] Methylation deregulation in cancer

Global and regional changes to DNA and histone methylation are a seminal feature of cancer cells (Figure 2). A major finding from the cancer genome efforts has been that pervasive mutations occur in a range of enzymes that methylate and de-methylate DNA and histones[1,89]. Nevertheless, the diverse range of molecular mechanisms employed by cancer cells to dramatically alter chromatin methylation patterns was relatively unexpected. These mechanisms include mutations in metabolic enzymes, resulting in production of oncometabolites **[G]** that essentially poison the iron-dependent dioxygenases that regulate histone and DNA demethylation, and somatic mutations in the core histone genes that lead to a global loss of the histone modifications (Figure 3).

D-2-hydroxyglutarate (D2HG) is one such oncometabolite, which inhibits numerous demethylases, leading to changes in genomic and transcriptomic methylation profiles as well as gene expression and genome topology[32,90-92]. Oncogenesis has been associated with specific mutations in isocitrate dehydrogenases (IDHs), which prevent conversion of isocitrate to α-ketoglutarate (α-KG) and additionally promote the reduction of α-KG to the structural analog D2HG[93,94]. Both the TET family of proteins involved in DNA demethylation and the Jumonji-C domain family of histone demethylases, are examples of iron-dependent dioxygenases whose catalytic activity at chromatin is competitively inhibited by elevated levels of D2HG. Mutations in *IDH1* and *IDH2* are mutually exclusive in AML, and IDH1/2 mutations induce cytosine hypermethylation and inhibit TET2-mediated 5-hydroxymethylation[95,96]. This results in aberrant DNA cytosine methylation (5mC and 5hmC) patterns, including hypermethylation at genes involved in proliferation and differentiation, as well as histone methylation gains. The observation that *IDH1* and *IDH2* mutations can partially phenocopy the loss of TET function may explain the mutual exclusivity of these mutations in cancer[95,97]. This is supported by the clustering of DNA methylation profiles of TET2-mutant AML with *IDH1- and IDH2*-mutant cancers, suggesting several common targets.

In addition to TET enzymes, D2HG can inhibit the N6-methyladenosine (m6A) RNA demethylase fat mass and obesity associated protein (FTO), another α-KG-dependent dioxygenase[15]. This leads to significantly higher m6A levels in IDH1/2-mutant than in IDH1/2-wild-type AML, despite comparable FTO expression[90]. Although evidence points to an oncogenic role of FTO in AML[15], a recent study suggested that inhibition of FTO by accumulated D2HG may have opposing pro- and anti-tumour effects in both AML and glioma, dependent on the mutation status of IDH, and the abundance of FTO and MYC[98]. Deconvoluting the contribution of each enzyme affected by D2HG and understanding what role the observed changes in DNA and histone methylation play in initiating and maintaining these malignancies is the subject of ongoing research and is likely to influence future therapeutic decisions.

One of the best examples of dysregulated histone methylation resulting in changes to gene expression and genome integrity is the extensive loss and gain of H3K27me3. This can occur through multiple distinct mechanisms, including recurrent gain- or loss-of function mutations in enhancer of zeste 2 (EZH2), the primary methyltransferase responsible for this histone modification[99]. More recently, we have also learnt how the catalytic activity of EZH2 can be

compromised by so called 'oncohistones'[100] (Figure 3). Around 30% of all paediatric glioblastomas contain mutations in histone genes resulting in H3K27M, H3K27I and H3G34V/R substitutions (reviewed in REF. [100]). Of these, up to 90% of diffuse intrinsic pontine gliomas (DIPG) harbour a point mutation in canonical (*HIST1H3B* (H3.1)) or variant (*H3F3A* (H3.3)) histone H3, resulting in a lysine 27 to methionine (K27M) substitution[101-103]. While these histone genes are associated with distinct DNA mutations (detailed in REF. [104]) they are maintained throughout the course of the disease, suggesting that they are driver mutations, and required for tumour maintenance[105]. H3K27M mutations in H3.1 or H3.3 are mutually exclusive with each other, and with mutations in H3.3 that result in a glycine 34 to arginine or valine (G34R/V) substitution. Whilst these tumours are histologically indistinguishable, the different histone mutations mark a clinically and epigenetically distinct group of glioblastomas. The H3.3G34R/V mutated tumours are found almost exclusively in the cerebral hemispheres[101,102], whereas H3.1K27M tumours are restricted to an area of the brainstem called the pons and H3.3K27M tumours can also be found along the midline of the brain[106]. This striking anatomical restriction is currently unexplained and potentially suggests a distinct cell of origin or a cell extrinsic factor, such as the surrounding tumour microenvironment that significantly influences the anatomical tropism for these genetically distinct tumours. These tumours promote similar global histone modification changes, most notably H3K27me2/me3 loss leading to derepression of genes, or redistribution of the active mark H3K36me3, together with a general DNA hypomethylation phenotype. Global H3K27me3 loss in H3K27M DIPG was initially proposed due to inactivation of polycomb repressive complex 2 (PRC2), of which EZH2 is a canonical subunit, via sequestration of EZH2 to H3K27M[107]. Despite reports supporting a higher affinity of PRC2 for H3K27M as compared with wild-type H3[108-110], H3K27M was often inversely correlated with PRC2 occupancy, challenging the idea that H3K27M sequesters PRC2 on chromatin[111,112]. A new model, which proposes a dynamic hit-and-run PRC2-H3K27M interaction, may reconcile some of these findings[113]. Several studies have shown that the loss of H3K27 trimethylation leads to major gene expression changes that are accompanied by a concomitant gain of acetylation at H3K27[107,111,114]. These findings have encouraged a number of laboratories to explore the possibility that epigenetic therapies may reset the imbalance caused by the oncohistone[115-119]. Despite these advances, we are still at the early stages of our understanding about the molecular mechanisms by which oncohistones contribute to these anatomically distinct malignancies and how they may be therapeutically targeted.

[H1] Therapeutically targeting methylation

A key rationale for targeting epigenetic regulators in cancer lies in manipulating the oncogenic transcriptional programme to modulate the expression of genes driving malignant progression and in essence reprogramme cancer cells to a more 'normal' differentiation state. An array of novel compounds targeting the enzymatic domains of methyltransferases that 'write' chromatin methylation, demethylases that 'erase' this modification or chromatin proteins that have specialised domains that 'read', or bind, chromatin methylation have entered the preclinical and clinical arena in recent years[120]. Here we will primarily focus on the first-in-class epigenetic therapies that have progressed to evaluation in clinical trials.

[H2] Inhibitors of DNA methyltransferases

Inhibitors of DNMTs in current clinical use, such as azacytidine (5'-azacytidine) and decitabine (5-aza-2'-deoxycytidine), are cytidine analogues that incorporate into replicating DNA and covalently bind and sequester active DNMTs, triggering their degradation by the proteasome. These inhibitors therefore have broad cellular effects due to indiscriminate inhibition of all DNMT-containing complexes, leading to a global loss of DNA methylation[121]. In addition, incorporation of cytidine analogues into RNA and DNA induces DNA damage and inhibition of protein synthesis, leading to cytotoxicity at high doses. Lower sub-cytotoxic doses have received regulatory approval for the treatment of myelodysplastic syndrome (MDS) and AML as agents with demonstrated efficacy for patients unfit for intensive chemotherapy[122-124]. The prolonged time to initial response, which typically occurs around 3 months from treatment onset[125], is consistent with the primary effects of low dose treatment being mediated via DNA demethylation and epigenetic reprogramming as opposed to direct cytotoxicity[126-128]. Whilst response rates of up to 60% to azacytidine have been reported in MDS[125,129], DNMT inhibitors have shown limited clinical efficacy as monotherapy for solid tumours. This has been attributed to a number of factors including limited DNA incorporation as a consequence of slower cell proliferation in solid tumours, poor cellular uptake and metabolic instability. This prompted the development of modified cytidine analogues with improved stability and oral bioavailability[129]. Several of these have shown efficacy in preclinical solid tumour models and are currently in clinical trials in a range of malignancies (Table 1). Despite their limitations, DNMT inhibitors are the most effective epigenetic therapy developed to date and it is hoped that the future development of specific catalytic inhibitors of individual DNMT enzymes, or targeting of specific DNMT-containing complexes, may deliver more potent and specific anti-tumour effects whilst circumventing the dose-limiting toxicities

associated with cytidine analogues. To this end, a reversible non-DNA incorporating selective inhibitor of DNMT1 has recently been developed and shown to elicit global reduction in DNA methylation and inhibit tumour growth in mouse tumour models[130].

[H2] Modulators of histone methylation

A large number of specific inhibitors of different histone methyltransferases have been developed over the last few years (reviewed in [131] and [132]). Inhibitors of EZH2, DOT1-like histone lysine methyltransferase (DOT1L), protein arginine methyltransferase 1 (PRMT1) and 5 (PRMT5) have now entered clinical trials with clinical development being most advanced for inhibitors of EZH2. EZH2 is the enzymatic component of the core PRC2 complex, whose activity is stabilised through binding to suppressor of zeste 12 (SUZ12), embryonic ectoderm development (EED) and retinoblastoma binding protein P46 (RbAp46/48)[133]. EZH2 catalyses mono-, di- and tri-methylation of H3K27, which is linked to transcriptional silencing[99]. Although possessing less potent histone methyltransferase activity, the EZH2 homologue enhancer of zeste 1 (EZH1) may contribute to H3K27 methylation in specific contexts, particularly in the presence of low levels of EZH2[134]. EZH2 plays a critical role during B-cell maturation and is a promising therapeutic target in multiple myeloma, follicular lymphoma and diffuse large B-cell lymphoma (DLBCL)[135]. Around 20-30% of follicular lymphomas and germinal centre-DLBCL harbour heterozygous point mutations in specific residues in the EZH2 SET domain (Y641, A677, A687), which enhance targeting of di- and mono-methylated, rather than unmethylated, H3K27 leading to substantially increased levels of H3K27me3[136-138]. An acquired dependence on EZH2 activity is also seen in BRCA1 associated protein 1 (BAP1)-mutant malignant mesothelioma[139] and in tumours with defects in the chromatin remodelling switch/sucrose non-fermentable (SWI/SNF) complex[140,141], for example SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (SMARCB1 (INI1))-deficient malignant rhabdoid tumours[142]. The SWI/SNF nucleosome remodelling complex antagonises PRC2-mediated gene silencing and has been shown to evict polycomb complex components from chromatin[143,144]. SWI/SNF inactivation is synthetic lethal with EZH2 mutations in a range of cancers. Whilst the simplest model proposes that SWI/SNF mutations drive transformation through gain of PRC2 function and silencing of tumour suppressor genes, non-catalytic activity of EZH2 has also been implicated and it has been suggested that combined loss of both SWI/SNF and PRC2 function could induce cell death due to global transcriptional dysregulation rather than derepression of specific PRC2 targets[134,140,142,145]. BAP1 is a deubiquitinase targeting histone H2AK119 that

opposes polycomb mediated silencing[146], however, sensitisation to EZH2 inhibitors following BAP1 loss appears to be cell type specific and has been observed in mesothelioma but not uveal melanoma[139,147]. PRC2 can also function as a tumour suppressor and recurrent inactivating mutations in EZH2 have been reported in myelodysplastic syndrome and chronic myeloproliferative neoplasms[148-152]. Loss-of-function mutations in other core PRC2 components EED and SUZ12 are also seen in T-cell acute lymphoblastic leukaemia and malignant peripheral nerve sheath tumours[153-155]. The contrasting function of PRC2 in different tumour contexts is thought to reflect the critical role the specific cellular transcriptional programme and chromatin environment plays in determining the genes targeted by PRC2 and highlights the need for careful monitoring of patients treated with EZH2 inhibitors[156].

Aberrant EZH2 activation is a feature of multiple cancers, including breast cancer, castration-resistant prostate cancer, small cell lung cancer (SCLC) and neuroblastoma and has been linked to oncogenesis and acquisition of stem cell-like transcriptional programmes[157-163]. Although EZH2 upregulation may be a consequence rather than a driver of the malignant process in some cancers[134], preclinical studies have nevertheless demonstrated EZH2-dependency in many of these tumour types. Multiple EZH2 inhibitors are currently being evaluated in ongoing phase I/II clinical trials in a range of cancers (Table 1). Interim phase I results have demonstrated a favourable safety and tolerability profile, with dose-limiting toxicities a rare occurrence[164-166]. Encouragingly in follicular lymphoma, 71% of patients with activating EZH2 SET domain mutations responded to EPZ-6438 (Tazemetostat), with 11% of patients achieving a complete response[166]. In contrast, only 33% of the patients without *EZH2* mutations responded and 31% developed progressive disease. Promising clinical results have also been reported in BAP1-inactivated malignant mesothelioma, with 51% of patients achieving disease control at 12 weeks, which was sustained to 24 weeks in 25%[165]. Clinical responses have also been reported in a small number of patients with SMARCB1-mutant tumours[164]. Overall, given that the majority of trials were conducted in heavily pre-treated patients with limited treatment options, it is encouraging that responses to EZH2 inhibitors have been seen. The increased response rates in EZH2-mutant tumours mirrors preclinical studies and highlights the importance of understanding the mechanisms underlying EZH2 dependence in order to target treatment to those patients most likely to respond[135,167-169]. This is particularly important given the highly context-dependent function of EZH2, underscored by the contrasting oncogenic and tumour suppressive roles of EZH2 in different

cancers[156].

DOT1L is an H3K79 methyltransferase that is integral to the initiation and maintenance of MLL fusion-protein driven leukaemia. In a recently reported phase I study, the DOT1L inhibitor EPZ-5676 (Pinometostat) (Table 1) was shown to be well tolerated and induced a complete remission in 2 of 51 patients with MLL fusion-protein leukaemia, providing proof of concept that targeting DOT1L can impact the progression of this aggressive disease[170]. However, response rates were lower than had been hoped given the critical role of DOT1L in preclinical models of MLL fusion driven malignancies. More recently, the arginine methyltransferase PRMT5 has been implicated in driving neoplastic growth of multiple tumours including B cell lymphoma, multiple myeloma, breast cancer and glioblastoma[171-178]. Preclinical studies employing PRMT5 inhibition provided a rationale for targeting this enzyme in both haematological and solid tumours, and on the back of these data specific inhibitors of PRMT5 have recently entered early stage clinical trials[179]. It is currently too early to understand where these drugs will have the greatest efficacy and if biomarkers of response may emerge from the clinical evaluation.

Similar to the context-dependent function of histone methyltransferases, the dynamic association of lysine demethylases with multi-protein complexes governs their stability and substrate specificity[180]. Given its role in promoting growth and inhibiting differentiation in AML and SCLC, lysine-specific demethylase 1A (LSD1) has been the primary focus for pharmacological targeting in the clinical setting to date[132]. Small molecule inhibitors of LSD1, currently in clinical trials (Table 1), irreversibly inhibit LSD1's demethylase activity by covalently binding the FAD cofactor. Preliminary results from a phase I trial of ORY-1001 demonstrated an acceptable safety profile and some evidence of clinical efficacy in AML, with partial responses in 3 of 14 patients[181]. Further results are awaited from ongoing trials in AML, MDS and SCLC.

[H2] Perspective on early clinical trials

Given the recent unprecedented interest in chromatin regulators, we now have a suite of small molecule inhibitors regulating DNA and histone methylation; however, a key challenge facing the field is that our pre-clinical evaluation of these compounds has not equated well to the success of these therapies in the clinical arena. Many of the drugs discussed above such as DOT1L, LSD1 and EZH2 inhibitors showed remarkable promise in pre-clinical studies,

however, their clinical efficacy has been more modest. What does this mean for the field? Far from being unproductive, the clinical studies conducted thus far have been invaluable in highlighting several important messages. Overall, epigenetic therapies are safe and tolerable; when assessing inhibitors to ubiquitously expressed proteins, the importance of this finding should not be underestimated. Epigenetic regulators are not directly involved in cell cycle progression or apoptosis and consequently most of these therapies do not have an immediate cytotoxic effect. Tumour lysis syndrome **[G]** has rarely been observed and most of these drugs have their best clinical response after weeks to months of continuous therapy. Finally, a key lesson learnt is that the value of a specific epigenetic therapy, much like the function of most epigenetic regulators, will be disease-specific and context-dependent, explaining the relatively low complete response rates in most epigenetic therapy trials.

Given the modest outcomes from the early clinical trials, are epigenetic therapies ineffective? One could argue that anyone who had expected a single agent epigenetic therapy to be a panacea for multiply relapsed and refractory cancer had not fully appreciated the primary function of this class of proteins and/or the vast adaptive potential of cancer cells. In many ways it is not surprising that single agent epigenetic therapies have only had a modest response in the clinical setting. In somatic tissues, epigenetic regulators function primarily to nuance access to the DNA template for DNA repair and replication and gene expression. The ambition for many of these drugs had been to target an essential dependency required to sustain a malignant transcription programme. However, targeting epigenetic regulators inevitably induces global changes in chromatin architecture and the cellular transcriptional programme, therefore the ability to link therapeutic effects to regulation of specific genes across different tumours is likely to be the exception rather than the rule. It is also important to appreciate that many of the enzymes targeted, particularly lysine and arginine methyltransferases, have non-histone substrates and the significance of this to the therapeutic and toxic effects of targeting these enzymes is yet to be fully elucidated[182,183].

It is too early to pass judgement on the role of epigenetic therapies in cancer management. There remains much to be learnt about how best to leverage these small molecules in the clinical setting and a better understanding of mechanisms of resistance to these drugs is needed. A consistent theme is that cancer cells adapt to survive the therapeutic challenge by finding an alternative mechanism to sustain the malignant gene expression programme in the presence of the epigenetic therapy. For instance, whilst several epigenetic therapies targeting

the prototypical oncogene *MYC* through transcriptional repression generated much excitement, it is now clear that this is only transiently effective as cancer cells evolve multiple adaptive responses to retain *MYC* expression[184-186]. The mechanisms that underpin these adaptive responses and render epigenetic therapies ineffective are varied and include tumour-intrinsic mechanisms and heterogenous cellular and molecular effects of the drugs[187]. Thus far the tumour-intrinsic mechanisms of resistance appear to be quite different to the paradigm of acquired resistance reported for other targeted therapies such as kinase inhibitors[188]. For example, there have been few descriptions of classical 'gate-keeper' mutations **[G]** in the epigenetic writer, reader and eraser domains targeted by epigenetic drugs. These observations suggest that mutations in the functional domain most likely result in sufficient functional compromise to the epigenetic regulator and probably phenocopy the effects of the drug, consequentially providing no selective advantage to the cancer cell. Pre-clinical modelling of resistance mechanisms has shown that resistance to these therapies largely emerge through cellular reprogramming and transcriptional plasticity[184-186].

The possibility that epigenetic therapies provide a fixed barrier that enforces an adaptive transcriptional response from cancer cells, presents a unique opportunity. Could they be used to homogenise intra-tumour heterogeneity? If the choices of cancer cell adaptation to any therapy are thought of as a large roundabout with several different exits representing alternative routes, including genetic evolution, divergent transcriptional programmes or metabolic pathways, or a phenotypic switch such as epithelial to mesenchymal transition (EMT), could epigenetic therapies be used as an instructive pressure to enforce a homogenous path to escape? If so, this would create a predictable vulnerability that could be targeted with a second drug in combination to achieve maximum therapeutic benefit. It is likely that the adaptive response to the same epigenetic therapy will be different in a cell context-dependent manner. For this strategy to be effective, a more detailed understanding of the shared routes of escape in each cancer will be required.

(H1) Combination and future therapies

The cornerstone of cancer therapy is effective and rational drug combinations. This paradigm is central to the future success of epigenetic therapies. The key issue is how best to combine these drugs and with what?

Numerous different combination strategies are currently being pursued in clinical trials,

encompassing combination of epigenetic therapies with chemotherapy, targeted therapies and immunotherapy (Figure 4 and Table 1). The evidence that cancer cells can escape from selective pressure through transcriptional adaptation provides a molecular rationale for utilising epigenetic therapy to block or reverse resistance. Furthermore, the potential for epigenetic inhibitors to promote tumour immunogenicity has generated excitement about combining these drugs with immunotherapy, particularly antibodies targeting T-cell inhibitory receptors such as anti-PD-1 and anti-CTLA-4 (Table 1). The power of these immune checkpoint inhibitor therapies to induce sustained remission in patients with advanced malignancies has created hope that identifying appropriate therapies to combine with these agents will broaden the therapeutic efficacy to encompass more patients and a broader range of tumour types.

(H2) Immunotherapy

The clear dynamic interplay between modulation of epigenome and downstream effects on cancer immune surveillance is an emerging area of interest. It is recognised that common epigenetic regulators govern both inflammation-induced and oncogenic transcriptional programmes[189]. This is particularly relevant to interactions between tumour and host where, in an ideal scenario, targeting the same chromatin regulator would inactivate an oncogenic pathway, enhance tumour immunogenicity and modify transcriptional programmes in responding immune cells to enhance their anti-tumour function. The concept that modulating the host immune response may contribute to the full potency of epigenetic therapies is supported by several recent reports and could in part account for the slow temporal kinetics of response to these agents[190]. It has been shown that both DNMT and LSD1 inhibitors induce de-repression of endogenous retroviral elements (ERVs) and production of double stranded RNA that activates anti-viral sensing pathways triggering tumour type I interferon production[191-193]. This enhanced tumour interferon signalling is further augmented following combination of DNMT inhibitors with histone deacetylase (HDAC) inhibitors and in preclinical studies these epigenetic agents induced intra-tumoural T-cell infiltration and augmented the response to immune checkpoint inhibitors[194,195]. Chromatin modifiers also have direct context-dependent roles in shaping cytokine responses and orchestrating immune cell differentiation. For example, inhibiting EZH2 activity can either positively or negatively influence tumour-specific immune responses by modulating both tumour immunogenicity and the function of infiltrating T-cells, NK-cells and macrophages[196]. Interestingly, a role for RNA methylation in modulation of immune function was recently reported. Depletion of

YTHDF1 (YTH N6-methyladenosine binding protein 1), which binds m6A-modified mRNA, enhanced cross-presentation of tumour antigens by dendritic cells. This effect was attributed to inhibition of lysosomal protease production and resulted in substantially enhanced CD8+ T-cell mediated anti-tumour immunity in *YTHDF1* knockout mice[197].

(H2) Targeted therapies

High EZH2 activity plays a key role in the pathogenesis of germinal centre-DLBCL and follicular lymphoma though repression of B-cell terminal differentiation genes and provides an example of synergism between EZH2 inhibition and therapeutic targeting of essential B-cell dependencies[135]. EZH2 inhibition in both EZH2-mutant and wildtype DLBCL increases dependence on B cell activation signalling and thus synergises with targeted inhibitors of B-cell receptor signalling such as Bruton's Tyrosine Kinase (BTK) inhibitors[198]. B-cell lymphoma 6 (BCL6) and EZH2 co-operate to maintain stable silencing of B-cell differentiation genes and combination therapy with E2H2 and BCL6 inhibitors more potently inhibits DLBCL growth than single agent treatment in preclinical models[199]. Translocations involving B-cell lymphoma 2 (BCL2) are the key pathogenic driver of follicular lymphoma, and BCL2 is frequently dysregulated in DLBCL. The antiapoptotic function of BCL2 promotes tumour survival and BCL2 inhibitors are currently being evaluated in clinical trials. Combining EZH2 with BCL2 inhibitors more effectively suppresses the growth of DLBCL xenografts[135].

(H2) Chemotherapy

The potential to epigenetically manipulate and revert chemoresistant transcriptional programmes makes combining epigenetic agents with chemotherapy an attractive prospect[126,160,200]. A less explored aspect is the potential for using epigenetic agents to augment cytotoxicity by amplifying the DNA-damaging effects of chemotherapy, radiotherapy or targeted agents. DNA and histone methylation have a critical role in the response to DNA damage and DNA repair pathways. For example, p53-binding protein 1 (53BP1) is recruited to the site of DNA double strand breaks (DSBs) and binds to specific post-translationally modified histone residues, including H4K20me2, and H3K79me via its tandem Tudor domain[31,201]. Blocking H3K79 methylation by depleting DOT1L inhibits 53BP1 recruitment to DSBs[202]. In yeast, Dot1 promotes DNA repair via homologous recombination and in mammalian cells DOT1L depletion leads to defects in DNA repair, enhancing sensitivity to DNA damaging agents[203,204], and also impairs transcriptional recovery following genotoxic stress[205]. Utilising inhibitors of DOT1L or other epigenetic agents to sensitise cancer cells to

DNA damaging agents via dysregulation of DNA repair pathways is an important potential future therapeutic avenue.

(H2) Combining epigenetic therapies

Combinations of two different epigenetic agents are also being explored. Trials combining DNMT with HDAC inhibitors have yielded mixed results at the cost of increased toxicity, likely attributable to the broad specificities of agents in current clinical use[206]. Given that epigenetic proteins frequently participate in multiple chromatin complexes with diverse functions, an important limitation of epigenetic therapies is the broad inhibition of all complexes containing the target protein generating potential unwanted adverse effects. An innovative strategy to more selectively target an individual epigenetic regulatory complex, is the development of inhibitors that concurrently target two epigenetic proteins within the same complex. The feasibility of this approach has been demonstrated by the recent development of dual inhibitors of LSD1 and HDACs, which provide more effective and sustained inhibition of the REST corepressor 1 (CoREST) complex than existing class I HDAC inhibitors, and more potently inhibit melanoma proliferation whilst exhibiting less toxicity to normal melanocytes and keratinocytes[207]. Given the ongoing development and rapid expansion of a library of agents binding to specific epigenetic reader, writer and eraser domains, the prospect of taking an epigenetic complex-focussed approach to the development of targeted therapeutics becomes more tangible, allowing more refined targeting of a specific dysregulated process whilst limiting off-target effects.

[H1] New opportunities for therapeutic intervention and monitoring

The observations that derepression of ERVs can enhance anti-cancer immune surveillance through **viral mimicry [G]** highlight the potential of specifically leveraging the repetitive genome for therapeutic gain (Box 1). Another emerging area with boundless opportunities is the possibility of repurposing sequence specific genome editing strategies as therapeutic agents (Figure 5A). The feasibility of specifically directing either components of the DNA or histone methylation machinery for locus specific gene regulation in human cells *ex vivo* has already been established[208]. Similarly, it has recently been demonstrated that epigenetic therapies can also be localised specifically within the genome using compounds coupled to catalytically deficient Cas9, which functions as a synthetic courier[209]. These tools may allow us to effectively activate or silence specific genes or large stretches of chromatin, and to specifically alter chromatin topology, replication timing and DNA repair. The application of

these strategies to alter disease states in pre-clinical models has yet to be explored and transition into clinical approaches will require significant innovation. However, with future advances in medicinal chemistry coupled to novel delivery modalities these strategies may soon enable us to take precision medicine to a new frontier.

The non-random nature of methylome profiles also suggests a common mechanism, or common cell of origin for distinct tumour subtypes. Consistent with this, genome-wide mapping of DNA methylation changes has proven useful for classification into prognostic subgroups[95,102] and for the development of clinically applicable assays in AML[210]. DNA methylation also poses several advantages as a biomarker for cancer diagnosis, including early and frequent occurrence of DNA methylation changes in cancer and cell-type specificity. DNA methylation is stable in fixed samples and can be easily detected using well-established techniques in a range of bodily fluids (Figure 5B). Despite this, publication of close to 200 biomarkers has yielded only 14 commercially available DNA methylation-based biomarker assays designed to measure the methylation of only 13 genes (for a recent review see REF. [211]), and only one of these is used to guide treatment decisions[212]. There is currently a great interest in developing non-invasive methods to monitor dynamic changes in mutation status and DNA methylation, for diagnosis and prognosis. The potential clinical application of this approach has also recently been highlighted[213]. Similarly, global changes in 5hmC can also be broadly used as a diagnostic biomarker for cancers, and development of protocols for detecting 5hmC are of great interest.

[H1] Conclusions and outlook

The last decade has seen a great convergence of what were previously largely disparate fields of research in genomics, epigenetics and chemistry. The heroic efforts in genomics to document all the recurrent mutations that underpin various developmental disorders and cancers have reinforced the central role of chromatin regulators in these diseases. Concurrently, decades of careful biochemistry, cell and molecular biology by chromatin biologists have provided invaluable insights into the molecular function of these epigenetic regulators. These insights have highlighted the highly dynamic nature of the epigenome and provided the molecular rationale for therapeutically targeting these proteins. This challenge has been widely embraced by medicinal chemists in academia and the pharmaceutical industry. Together, they have produced an unprecedented array of small molecules that target proteins responsible for writing, reading or erasing methylation on DNA and histone proteins.

Several of these epigenetic therapies have rapidly progressed into early phase clinical trials, which have been invaluable to establish the safety of many of these compounds and highlight that even as single agents in relapsed and refractory cancers, they show efficacy.

The platform for cross-disciplinary progress has now been established. It provides a unique opportunity to make dramatic inroads into the understanding and management of various human diseases. The success of this ambition rests on how we tackle the next frontier of challenges. Here we see several opportunities; our understanding of the non-coding genome, particularly the transposable elements, is still rudimentary. Investigating the regulation of the largest fraction of our genome and the role it plays in normal development and pathological processes such as cancer may yield new opportunities for therapeutic intervention. A further opportunity lies in finding innovative methods to use the knowledge gained over the last decade to guide diagnosis and management in everyday clinical practice. Recent efforts to use the highly annotated cancer genome to monitor response and resistance to cancer therapies[214,215] raises the possibility that cancer-specific DNA and RNA modifications may similarly prove effective. Finally, and arguably the most important opportunity, is identifying how best to integrate epigenetic therapies into routine clinical management. The future clearly involves combination strategies, however, given the context-dependent function of epigenetic regulators, it is important to carefully establish the best evidence for combination drug regimens involving an epigenetic therapy. This is not an insignificant task as all animal models of cancer have significant limitations. Genetically engineered mouse models fail to recapitulate the intratumour heterogeneity inherent in human cancer and patient derived xenograft models also have major limitations in clonal representation and lack an effective immune system, which as discussed above, appears to be central to the efficacy of many epigenetic therapies. Therefore, it is important to acknowledge and accept these limitations and not be guided solely by pre-clinical evidence in imperfect animal models of cancer. A detailed understanding of the molecular mechanism that may provide therapeutic benefit may be equally, if not more, valuable than empirical pre-clinical efficacy studies. Despite these challenges, there is much to be optimistic about for the future of epigenetic therapies. The multidisciplinary collaborative approach in recent years involving geneticists, biochemists, medicinal chemists, cell biologists and clinicians has yielded an enormous amount of new knowledge about the fundamental role of epigenetic regulators in diseases associated with advanced age including cancer. Continued collaborative momentum will likely see their

efforts make significant differences in the altering the natural history of these disorders in the very near future.

Box 1. Exploiting the non-coding genome for therapeutic benefit

Transposable elements constitute the largest fraction of the human genome and methylation of DNA and histones plays an integral role in their regulation. Although evolutionarily diverse, these elements have a fundamental role in development and disease[216]. Transposons can be highly cell lineage specific and can regulate tissue gene expression programmes through a number of mechanisms involving the active promotion or repression of transcriptional activity. These elements have been proposed to be critical for establishing the chromatin environment that facilitates cellular plasticity, a feature not just required for normal development but often a central component of oncogenesis. It has been estimated that up to 30% of transcripts for some tissues can originate in repetitive elements, highlighting the major influence these elements have on tissue specific gene expression[217]. Many of these transposable elements have a conserved regulatory structure, such as long terminal repeat (LTR) elements, which provide a promoter at the 5' end, and transcriptional termination and polyadenylation signals at the 3' end of some retrotransposons. Consequently evolutionary pressure has likely evolved distinct mechanisms to tightly control the influence of transposons. For instance, it has recently become apparent that vertebrate specific chromatin complexes such as the HUSH complex can be major regulators of the LINE-1 family of retrotransposons[218,219]. The largest fraction of our genome likely holds many intriguing possibilities and as our knowledge of this area expands, some of these transposon families may be amenable to therapeutic manipulation with epigenetic drugs to effectively manage diseases such as cancer.

Figure legends.

Figure 1: Methylation of DNA, RNA and histones. A. Conversion of S-adenosyl methionine (SAM) to S-adenosyl homocysteine (SAH) occurs during a methylation reaction. **B.** DNA methylation: Formation of 5-methylcytidine (5mC) by DNA methyltransferase (DNMT) enzymes. Further oxidation steps catalysed by Ten-Eleven Translocation (TET) enzymes and potential steps of demethylation are indicated by dashed arrows. The lower cartoon depicts CpG methylation by DNMT enzymes on a DNA duplex. DNMT1 preferentially methylates hemimethylated CpG sites and maintains methylation after cell division, whereas DNMT3A and DNMT3B have an equal preference for unmethylated and hemimethylated sites and they are responsible for *de novo* methylation at the non-CpG sites. Methylation of adenine is shown for completion although the enzyme responsible for its methylation (m6A) is unknown. **C.**

RNA methylation: Two examples of RNA modifications are shown (m6A and m7G). The RNA cartoon shows methyltransferases and a demethylase, together with their target nucleotide (A, G, C or U) and methylated product (in parenthesis). The cartoon represents a generic RNA structure and the exact sites shown are for illustrative purposes only. **D.** Lysine and arginine methylation: Lysine methyltransferases (KMTs) methylate lysines and demethylases (KDMs) reverse the methylation. Lysines may be mono-, di- or trimethylated (Kme1, Kme2 or Kme3 respectively). Arginine methylation is performed by type I and II protein arginine methyltransferases (PRMTs) and leads to mono-, di-symmetric or di-asymmetric methylation (Rme1, Rme2s or Rme2as respectively). **E.** Histone methylation: Schematic showing the principle sites of methylation within histone H3 and H4 N-terminal tails together with the relevant methyltransferases (above) and demethylases (below).
Methyl groups are shown in green. Methyltransferases are in black text whereas demethylases are in red.

Figure 2: Changes in the DNA and histone methylation patterns in cells from young, ageing and tumour-bearing individuals. A. In normal cells, CpG methylation is low at promoter regions, whereas genic and intergenic regions show high methylation levels[220]. Distal regulatory sequences such as enhancers are commonly marked by low levels of 5-methylcytidine (5mC) and higher levels of 5-hydroxymethylcytidine (5hmC). A limited number of genomic loci gain DNA methylation during ageing and in cancer, in particular CpG islands in promoters of tumour suppressor genes (TSGs) and polycomb targets[57]. Changes in enhancer methylation are associated with dysregulated chromatin structure, which is normally maintained in distinct regions by boundary factors such as CCCTC-binding factor (CTCF) and cohesion. **B.** In normal tissues, repetitive elements are maintained in heterochromatin and highly methylated. Demethylation of retrotransposons and satellites and high levels of their transcription are observed in ageing cells[47] and in human cancer[73-75]. **C.** A general loss of histones is observed in replicative senescence[70,71] and ageing cells have an altered heterochromatin composition, including high levels of histone H2A variant macroH2A and heterochromatin binding protein 1 (HP1β)[68,69] Some of these features are also seen in cancer cells. **D**. In ageing cells, a gain of novel bivalent domains is observed, mostly due to gains of repressive H3K27me3 marks on regions that were marked by the active histone modification H3K4me3 in young cells[65,66]. Bivalent domains show the lowest DNA methylation in normal cells[66]. During ageing, DNA hypermethylation occurs at bivalent chromatin domain promoters[55], which is also a key feature of several cancers[221-223]. This may

lead to reduced expression of TSGs. Arrows indicate the strength of gene transcription/repression.

Figure 3: Expression of oncometabolites and oncohistones in cancer lead to changes in DNA and histone methylation. **A.** Mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2) promote the reduction of α -ketoglutarate (α-KG) to the oncometabolite D-2-hydroxyglutarate (D2HG), resulting in inhibition of numerous demethylases. **B.** Mutations in genes encoding histones H1, H2A, H2B and H3 have been reported in cancer (detailed in REF. [224]) which result in expression of oncohistones and marked changes in DNA and histone methylation. H3K27M and H3G34R mutations are two examples. The former leads to inhibition of polycomb repressive complex 2 (PRC2), while some evidence points to inhibition of SET domain containing 2 (SETD2) methyltransferase by H3G34R[225]. H3.1K27M-, H3.3K27M- and H3G34R-mutant glioblastomas are found in distinct parts of the brain (indicated by the circles), suggesting difference in cell or origin, or tumour environment.

Figure 4: Targeting methylation in combination with immunotherapy. Inhibitors of DNA-methyltransferases (DNMT), enhancer of zeste 2 (EZH2) and lysine-specific demethylase 1A (LSD1) are currently being evaluated in clinical trials with immune checkpoint blocking antibodies against PD-1, PD-L1 or CTLA-4. These antibodies block T-cell inhibitory receptors promoting activation of CD8+ T-cells, which secrete pro-inflammatory cytokines interferon-γ and TNF-α and recognise and kill tumour cells displaying foreign antigens in complex with MHC class I. Inhibitors of DNMT, LSD1 and EZH2 can derepress endogenous retroviral elements (ERVs) leading to activation of endogenous anti-viral RNA sensing pathways and tumour type I interferon production, which acts in an autocrine manner to drive expression of interferon stimulated genes (ISGs)[191-193,226]. Enhanced tumour MHC class I antigen presentation in conjunction with increased infiltration of tumours by CD8+ cytotoxic T-cells leads to increased tumour killing. EZH2 inhibition has been shown to augment interferon-induced gene expression in tumour cells[227-229]. DNMT inhibitors can induce aberrant expression of tumour associated antigens such as cancer testis antigens, which provide a source of 'foreign' MHC-I peptides that may promote the development of tumour-reactive T cells. Manipulation of DNA and histone methylation also modulates differentiation and effector function of tumour-infiltrating immune cells. Inhibiting EZH2 in T-regulatory cells, has been shown to reprogramme them to more pro-inflammatory phenotype and to enhance the anti-tumour effects of anti-CTLA-4 therapy[230,231]. i, inhibitor; IFNAR, interferon-α receptor (type I interferon); IFNGR, interferon-γ receptor (type II interferon).

Figure 5. **New opportunities for therapeutic intervention and monitoring A.** Targeted drug delivery may be achieved by directing either components of the DNA or histone methylation machinery or epigenetic drugs to a specific gene locus, for example by coupling them to catalytically deficient (dCas9). **B.** 'Liquid biopsies' are a non-invasive method for collecting circulating tumour DNA, for multiple downstream applications, including monitoring of DNA methylation. sgRNA, single guide RNA; TF, transcription factor; RNA pol II, RNA polymerase II; 5mC, 5-methylcytidine; 5hmC, 5-hydroxymethylcytidine.

Table 1. Selected current clinical trials targeting DNA or histone methylation

Target	Inhibitor (Name)	Malignancy	Phase	Year	Clinical trial number
PRC2 Inhibitors					
EZH2	EPZ-6438 (Tazemetostat)	B cell lymphoma (BCL) with *EZH2* mutation	II	2018	NCT03456726
EZH2	EPZ-6438 (Tazemetostat)	DLBCL, Advanced solid tumours	I/II	2013	NCT01897571
EZH2	EPZ-6438 (Tazemetostat)	Rhabdoid tumours, Synovial Sarcoma, SMARCB1- or EZH2-mutant tumours	II	2015	NCT02601950
EZH2	EPZ-6438 (Tazemetostat)	Paediatric INI1(SMARCB1 negative tumours) or synovial sarcoma	I	2015	NCT02601937
EZH2	EPZ-6438 (Tazemetostat)	Malignant mesothelioma (BAP1 mutant)	II	2016	NCT02860286
EZH2	EPZ-6438 (Tazemetostat)	Recurrent ovarian, primary peritoneal, endometrial carcinoma	II	2019	NCT03348631
EZH2	SHR2554	Mature lymphoid neoplasms	I	2018	NCT03603951
EZH2	PF-06821497	Small cell lung cancer (SCLC), CRPC, DLBCL, Follicular Lymphoma	I	2018	NCT03460977
EZH2	CPI-1205	B-cell lymphoma	II	2015	NCT02395601
EED	MAK683	DLBCL, advanced solid tumours	I/II	2016	NCT02900651
DOT1L Inhibitors					
DOT1L	EPZ-5676 (Pinometostat)	Newly diagnosed AML with MLL rearrangement	Ib/II	2019	NCT03724084
DOT1L	EPZ-5676 (Pinometostat)	Paediatric AML and ALL with MLL rearrangement	Ib	2014	NCT02141828
Arginine Methyltransferase Inhibitors					
PRMT5	GSK3326595	Advanced solid tumours, Non-Hodgkin Lymphoma	I	2016	NCT02783300
PRMT5	JNJ-64619178	B-cell Non-Hodgkin Lymphoma, advanced solid tumours	I	2018	NCT03573310
Demethylase Inhibitors					
LSD1	IMG-7289	Myelofibrosis	I	2017	NCT03136185
LSD1	IMG-7289 ± ATRA	AML, Myelodysplastic syndrome	I	2016	NCT02842827
LSD1	SP-2577 (Seclidemstat)	Ewing Sarcoma	I	2018	NCT03600649
LSD1	INCB059872	Ewing Sarcoma	I	2018	NCT03514407
LSD1	INCB059872	AML/MDS, SCLC, myelofibrosis, Ewing sarcoma and poorly differentiated neuroendocrine tumours	I/II	2016	NCT02712905
LSD1	CC-90011	Non-Hodgkin Lymphoma, advanced solid tumours	I	2016	NCT02875223
DNA Methyltransferase Inhibitors					
DNMT	Guadecitabine (SGI-110)	Paraganglioma, gastrointestinal stromal tumours, phaeochromocytoma, hereditary leiomyomatosis and renal cell carcinoma	II	2017	NCT03165721
DNMT	Guadecitabine (SGI-110)	Higher risk MDS	II	2014	NCT02131597
DNMT	Guadecitabine (SGI-110)	AML	III	2017	NCT02920008
DNMT	5-aza-4'-Thio-2'-Deoxycytidine (Aza-TdC)	Advanced solid tumours	I	2017	NCT03366116
DNMT	4'-Thio-2'-Deoxycytidine (TdCyd)	Advanced solid tumours	I	2015	NCT02423057
DNMT	5-Fluoro-2-Deoxycytidine (FdCyd) + THU	Lung, breast, bladder and head and neck cancer	II	2009	NCT00978250

Combinations with immune checkpoint inhibitors					
EZH2 + CTLA-4	CPI-1205 + ipilimumab	Advanced solid tumours	I/II	2017	NCT03525795
EZH2 + PD-L1	EPZ-6438 (Tazemetostat) + atezolizumab	Relapsed or refractory DLBCL	I	2014	NCT02220842
LSD1 + PD-1	INCB059872 + nivolumab	SCLC	I/II	2016	NCT02712905
DNMT + PD-1	THU-Decitabine + nivolumab	NSCLC	II	2016	NCT02664181
DNMT + PD-1	Azacitidine + pembrolizumab	NSCLC	II	2015	NCT02546986
DNMT + PD-1	Decitabine + anti-PD-1	Relapsed or refractory malignancies	I/II	2016	NCT02961101
DNMT + CTLA-4	Decitabine + ipilimumab	Relapsed or refractory MDS or AML	I	2017	NCT02890329
DNMT + PD-L1	Guadecitabine (SGI-110)+ atezolizumab	MDS, AML, CMML	I/II	2016	NCT02935361
DNMT + PD-1	Azacitidine + pembrolizumab	Metastatic melanoma	II	2017	NCT02816021
DNMT + CTLA-4	Guadecitabine (SGI-110) + ipilimumab	Metastatic melanoma	I	2015	NCT02608437
DNMT + PD-1	Guadecitabine (SGI-110) + nivolumab	Refractory metastatic colorectal cancer	I/II	2019	NCT03576963
DNMT + PD-1	Guadecitabine (SGI-110) + pembrolizumab	Recurrent ovarian, primary peritoneal or fallopian tube cancer	II	2016	NCT02901899
DNMT + PD-L1	Guadecitabine (SGI-110) + durvalumab	Advanced renal cancer	Ib/II	2017	NCT03308396
DNMT + PD-L1	Guadecitabine (SGI-110) + durvalumab	Hepatocellular carcinoma, pancreatic adenocarcinoma and cholangiocarcinoma	Ib	2018	NCT03257761
DNMT + PD-L1	Guadecitabine (SGI-110) + atezolizumab	Advanced urothelial carcinoma	I/II	2017	NCT03179943
DNMT + PD-1	Guadecitabine (SGI-110) + pembrolizumab	NSCLC, CRPC	I	2017	NCT02998567
DNMT + PD-L1 or CTLA-4	Azacitidine + durvalumab or tremelimumab	Head and neck cancer	I/II	2017	NCT03019003
DNMT, HDAC + PD-1	Azacitidine, Entinostat + nivolumab	NSCLC	II	2013	NCT01928576
DNMT, HDAC + PD-1	Guadecitabine pembrolizumab, and mocetinostat	Advanced NSCLC	I	2017	NCT03220477
DNMT, IDO-1 + PD-1	Azacitidine, epacadostat pembrolizumab,	Advanced solid tumours	I/II	2017	NCT02959437
LSD1, IDO-1 + PD-1	INCB059872, epacadostat pembrolizumab	Advanced solid tumours	I/II	2017	NCT02959437
Other combinations					
PRMT5 + DNMT	GSK3326595 + Azacitidine	Myelodysplastic syndrome (MDS), CMML, AML	I/II	2018	NCT03614728
LSD1 + DNMT	INCB059872 + ATRA or azacitidine	AML	I/II	2016	NCT02712905
LSD1 + DNMT	GSK2879552 ± Azacitidine	Myelodysplastic syndrome	II	2017	NCT02929498
EZH2	EPZ-6438 (Tazemetostat) + R-CHOP	DLBCL	I/II	2016	NCT02889523
DNMT + androgen receptor	Decitabine + enzalutamide	Castration resistant prostate cancer	I/II	2019	NCT03709550
EZH2 + androgen receptor	CPI-1205 + enzalutamide	Castration resistant prostate cancer	I/II	2017	NCT03480646
DNMT	Azacitidine + chemotherapy	AML (newly diagnosed)	II	2017	NCT03164057
DNMT + NEDD8 activating enzyme	Azacitidine + pevonedistat	Relapsed refractory MDS or MDS/MPN	II	2017	NCT03238248
DNMT + Poly ADP Ribose Polymerase	Decitabine + talazoparib	AML	I/II	2016	NCT02878785

BCL: B cell lymphoma; DLBCL: Diffuse large B cell lymphoma; AML: Acute myeloid leukaemia; ALL: Acute lymphoblastic leukaemia; CMML: Chronic myelomonocytic leukaemia; MDS: Myelodysplastic syndrome; MLL: Mixed lineage leukaemia; CRPC: Castration resistant prostate cancer; SCLC: Small cell lung cancer; NSCLC: Non-small cell lung cancer; THU: Tetrahydrouridine; IDO: indoleamine 2,3-dioxygenase; ATRA: All-trans retinoic acid; R-CHOP: Rituximab, Cyclophosphamide, Vincristine, Doxorubicin, Prednisolone. Trial information from https://www.clinicaltrials.gov/

References

1. Dawson, M.A. & Kouzarides, T. Cancer epigenetics: from mechanism to therapy. *Cell* **150**, 12-27 (2012).
2. Jin, B., Li, Y. & Robertson, K.D. DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes Cancer* **2**, 607-617 (2011).
3. Luo, C., Hajkova, P. & Ecker, J.R. Dynamic DNA methylation: In the right place at the right time. *Science* **361**, 1336-1340 (2018).
4. Stojkovic, V. & Fujimori, D.G. Mutations in RNA methylating enzymes in disease. *Curr Opin Chem Biol* **41**, 20-27 (2017).
5. Xie, P., Zang, L.Q., Li, X.K. & Shu, Q. An epigenetic view of developmental diseases: new targets, new therapies. *World J Pediatr* **12**, 291-297 (2016).
6. Kohli, R.M. & Zhang, Y. TET enzymes, TDG and the dynamics of DNA demethylation. *Nature* **502**, 472-479 (2013).
7. Xiao, C.L.*, et al.* N(6)-Methyladenine DNA Modification in the Human Genome. *Mol Cell* **71**, 306-318 e307 (2018).
8. Xie, Q.*, et al.* N(6)-methyladenine DNA Modification in Glioblastoma. *Cell* **175**, 1228-1243 e1220 (2018).
9. Barbieri, I.*, et al.* Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control. *Nature* **552**, 126-131 (2017).
10. Berulava, T., Rahmann, S., Rademacher, K., Klein-Hitpass, L. & Horsthemke, B. N6-adenosine methylation in MiRNAs. *PLoS One* **10**, e0118438 (2015).
11. Pendleton, K.E.*, et al.* The U6 snRNA m(6)A Methyltransferase METTL16 Regulates SAM Synthetase Intron Retention. *Cell* **169**, 824-835 e814 (2017).
12. Squires, J.E.*, et al.* Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. *Nucleic Acids Res* **40**, 5023-5033 (2012).
13. Xuan, J.J.*, et al.* RMBase v2.0: deciphering the map of RNA modifications from epitranscriptome sequencing data. *Nucleic Acids Res* **46**, D327-D334 (2018).
14. Jia, G.*, et al.* N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol* **7**, 885-887 (2011).
15. Li, Z.*, et al.* FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N(6)-Methyladenosine RNA Demethylase. *Cancer Cell* **31**, 127-141 (2017).
16. Clarke, S. Protein methylation. *Curr Opin Cell Biol* **5**, 977-983 (1993).
17. Xhemalce, B., Dawson, M.A. & Bannister, A.J. *Histone modifications. Epigenetic regulation and epigenomics : advances in molecular biology and medicine*, (Wiley-Blackwell, Weinheim, 2012).
18. Deguchi, T. & Barchas, J. Inhibition of transmethylations of biogenic amines by S-adenosylhomocysteine. Enhancement of transmethylation by adenosylhomocysteinase. *J Biol Chem* **246**, 3175-3181 (1971).
19. Wang, Y., Sun, Z. & Szyf, M. S-adenosyl-methionine (SAM) alters the transcriptome and methylome and specifically blocks growth and invasiveness of liver cancer cells. *Oncotarget* **8**, 111866-111881 (2017).
20. Tessarz, P.*, et al.* Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. *Nature* **505**, 564-568 (2014).

21. Goll, M.G.*, et al.* Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science* **311**, 395-398 (2006).
22. Bannister, A.J., Schneider, R. & Kouzarides, T. Histone methylation: dynamic or static? *Cell* **109**, 801-806 (2002).
23. Schubeler, D. Function and information content of DNA methylation. *Nature* **517**, 321-326 (2015).
24. Maunakea, A.K., Chepelev, I., Cui, K. & Zhao, K. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res* **23**, 1256-1269 (2013).
25. Papin, C.*, et al.* Combinatorial DNA methylation codes at repetitive elements. *Genome Res* **27**, 934-946 (2017).
26. Baba, Y.*, et al.* Epigenomic diversity of colorectal cancer indicated by LINE-1 methylation in a database of 869 tumors. *Mol Cancer* **9**, 125 (2010).
27. Howard, G., Eiges, R., Gaudet, F., Jaenisch, R. & Eden, A. Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. *Oncogene* **27**, 404-408 (2008).
28. Bannister, A.J. & Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res* **21**, 381-395 (2011).
29. Kolasinska-Zwierz, P.*, et al.* Differential chromatin marking of introns and expressed exons by H3K36me3. *Nat Genet* **41**, 376-381 (2009).
30. Kim, W., Choi, M. & Kim, J.E. The histone methyltransferase Dot1/DOT1L as a critical regulator of the cell cycle. *Cell Cycle* **13**, 726-738 (2014).
31. Wood, K., Tellier, M. & Murphy, S. DOT1L and H3K79 Methylation in Transcription and Genomic Stability. *Biomolecules* **8**, 11 (2018).
32. Garrett-Bakelman, F.E. & Melnick, A.M. Mutant IDH: a targetable driver of leukemic phenotypes linking metabolism, epigenetics and transcriptional regulation. *Epigenomics* **8**, 945-957 (2016).
33. Bernstein, B.E.*, et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326 (2006).
34. Jorgensen, S., Schotta, G. & Sorensen, C.S. Histone H4 lysine 20 methylation: key player in epigenetic regulation of genomic integrity. *Nucleic Acids Res* **41**, 2797-2806 (2013).
35. Henikoff, S. & Shilatifard, A. Histone modification: cause or cog? *Trends Genet* **27**, 389-396 (2011).
36. Biggar, K.K. & Li, S.S. Non-histone protein methylation as a regulator of cellular signalling and function. *Nat Rev Mol Cell Biol* **16**, 5-17 (2015).
37. Popis, M.C., Blanco, S. & Frye, M. Posttranscriptional methylation of transfer and ribosomal RNA in stress response pathways, cell differentiation, and cancer. *Curr Opin Oncol* **28**, 65-71 (2016).
38. Dominissini, D.*, et al.* Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* **485**, 201-206 (2012).
39. Roundtree, I.A., Evans, M.E., Pan, T. & He, C. Dynamic RNA Modifications in Gene Expression Regulation. *Cell* **169**, 1187-1200 (2017).
40. Patil, D.P.*, et al.* m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature* **537**, 369-373 (2016).
41. Bartke, T.*, et al.* Nucleosome-interacting proteins regulated by DNA and histone methylation. *Cell* **143**, 470-484 (2010).
42. Foster, B.M.*, et al.* Critical Role of the UBL Domain in Stimulating the E3 Ubiquitin Ligase Activity of UHRF1 toward Chromatin. *Mol Cell* **72**, 739-752 e739 (2018).

43. Bell, A.C. & Felsenfeld, G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature* **405**, 482-485 (2000).
44. Hashimoto, H.*, et al.* Structural Basis for the Versatile and Methylation-Dependent Binding of CTCF to DNA. *Mol Cell* **66**, 711-720 e713 (2017).
45. Wilson, V.L. & Jones, P.A. DNA methylation decreases in aging but not in immortal cells. *Science* **220**, 1055-1057 (1983).
46. Unnikrishnan, A.*, et al.* Revisiting the genomic hypomethylation hypothesis of aging. *Ann N Y Acad Sci* **1418**, 69-79 (2018).
47. De Cecco, M.*, et al.* Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. *Aging (Albany NY)* **5**, 867-883 (2013).
48. Belgnaoui, S.M., Gosden, R.G., Semmes, O.J. & Haoudi, A. Human LINE-1 retrotransposon induces DNA damage and apoptosis in cancer cells. *Cancer Cell Int* **6**, 13 (2006).
49. Gasior, S.L., Wakeman, T.P., Xu, B. & Deininger, P.L. The human LINE-1 retrotransposon creates DNA double-strand breaks. *J Mol Biol* **357**, 1383-1393 (2006).
50. Belancio, V.P., Deininger, P.L. & Roy-Engel, A.M. LINE dancing in the human genome: transposable elements and disease. *Genome Med* **1**, 97 (2009).
51. Tan, L.*, et al.* Naked Mole Rat Cells Have a Stable Epigenome that Resists iPSC Reprogramming. *Stem Cell Reports* **9**, 1721-1734 (2017).
52. Beerman, I.*, et al.* Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell* **12**, 413-425 (2013).
53. Hannum, G.*, et al.* Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* **49**, 359-367 (2013).
54. Maegawa, S.*, et al.* Caloric restriction delays age-related methylation drift. *Nat Commun* **8**, 539 (2017).
55. Rakyan, V.K.*, et al.* Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res* **20**, 434-439 (2010).
56. Teschendorff, A.E.*, et al.* Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res* **20**, 440-446 (2010).
57. Esteller, M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. *Oncogene* **21**, 5427-5440 (2002).
58. Field, A.E.*, et al.* DNA Methylation Clocks in Aging: Categories, Causes, and Consequences. *Mol Cell* **71**, 882-895 (2018).
59. Horvath, S. & Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet* **19**, 371-384 (2018).
60. Marioni, R.E.*, et al.* DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol* **16**, 25 (2015).
61. Steensma, D.P.*, et al.* Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **126**, 9-16 (2015).
62. Chen, B.H.*, et al.* DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging (Albany NY)* **8**, 1844-1865 (2016).
63. Sen, P., Shah, P.P., Nativio, R. & Berger, S.L. Epigenetic Mechanisms of Longevity and Aging. *Cell* **166**, 822-839 (2016).
64. McCauley, B.S. & Dang, W. Histone methylation and aging: lessons learned from model systems. *Biochim Biophys Acta* **1839**, 1454-1462 (2014).
65. Liu, L.*, et al.* Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. *Cell Rep* **4**, 189-204 (2013).
66. Sun, D.*, et al.* Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. *Cell Stem Cell* **14**, 673-688 (2014).

67. Buschbeck, M.*, et al.* The histone variant macroH2A is an epigenetic regulator of key developmental genes. *Nat Struct Mol Biol* **16**, 1074-1079 (2009).
68. Herbig, U., Ferreira, M., Condel, L., Carey, D. & Sedivy, J.M. Cellular senescence in aging primates. *Science* **311**, 1257 (2006).
69. Kreiling, J.A.*, et al.* Age-associated increase in heterochromatic marks in murine and primate tissues. *Aging Cell* **10**, 292-304 (2011).
70. Dang, W.*, et al.* Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature* **459**, 802-807 (2009).
71. O'Sullivan, R.J., Kubicek, S., Schreiber, S.L. & Karlseder, J. Reduced histone biosynthesis and chromatin changes arising from a damage signal at telomeres. *Nat Struct Mol Biol* **17**, 1218-1225 (2010).
72. Min, K.W.*, et al.* Profiling of m6A RNA modifications identified an age-associated regulation of AGO2 mRNA stability. *Aging Cell* **17**, e12753 (2018).
73. Belancio, V.P., Roy-Engel, A.M., Pochampally, R.R. & Deininger, P. Somatic expression of LINE-1 elements in human tissues. *Nucleic Acids Res* **38**, 3909-3922 (2010).
74. Berman, B.P.*, et al.* Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. *Nat Genet* **44**, 40-46 (2011).
75. Hansen, K.D.*, et al.* Increased methylation variation in epigenetic domains across cancer types. *Nat Genet* **43**, 768-775 (2011).
76. Genovese, G.*, et al.* Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* **371**, 2477-2487 (2014).
77. Jaiswal, S.*, et al.* Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* **371**, 2488-2498 (2014).
78. Xie, M.*, et al.* Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* **20**, 1472-1478 (2014).
79. Buscarlet, M.*, et al.* Lineage restriction analyses in CHIP indicate myeloid bias for TET2 and multipotent stem cell origin for DNMT3A. *Blood* **132**, 277-280 (2018).
80. Challen, G.A.*, et al.* Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* **44**, 23-31 (2011).
81. Jeong, M.*, et al.* Loss of Dnmt3a Immortalizes Hematopoietic Stem Cells In Vivo. *Cell Rep* **23**, 1-10 (2018).
82. Ko, M.*, et al.* Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc Natl Acad Sci U S A* **108**, 14566-14571 (2011).
83. Li, Z.*, et al.* Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood* **118**, 4509-4518 (2011).
84. Moran-Crusio, K.*, et al.* Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **20**, 11-24 (2011).
85. Quivoron, C.*, et al.* TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell* **20**, 25-38 (2011).
86. Coombs, C.C.*, et al.* Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell* **21**, 374-382 e374 (2017).
87. Fuster, J.J.*, et al.* Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* **355**, 842-847 (2017).
88. Jaiswal, S.*, et al.* Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med* **377**, 111-121 (2017).

89. You, J.S. & Jones, P.A. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* **22**, 9-20 (2012).
90. Elkashef, S.M.*, et al.* IDH Mutation, Competitive Inhibition of FTO, and RNA Methylation. *Cancer Cell* **31**, 619-620 (2017).
91. Flavahan, W.A.*, et al.* Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature* **529**, 110-114 (2016).
92. Xu, W.*, et al.* Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell* **19**, 17-30 (2011).
93. Dang, L.*, et al.* Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **465**, 966 (2010).
94. Cairns, R.A. & Mak, T.W. Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discov* **3**, 730-741 (2013).
95. Figueroa, M.E.*, et al.* Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* **18**, 553-567 (2010).
96. Glass, J.L.*, et al.* Epigenetic Identity in AML Depends on Disruption of Nonpromoter Regulatory Elements and Is Affected by Antagonistic Effects of Mutations in Epigenetic Modifiers. *Cancer Discov* **7**, 868-883 (2017).
97. Gaidzik, V.I.*, et al.* TET2 mutations in acute myeloid leukemia (AML): results from a comprehensive genetic and clinical analysis of the AML study group. *J Clin Oncol* **30**, 1350-1357 (2012).
98. Su, R.*, et al.* R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. *Cell* **172**, 90-105 e123 (2018).
99. Piunti, A. & Shilatifard, A. Epigenetic balance of gene expression by Polycomb and COMPASS families. *Science* **352**, aad9780 (2016).
100. Mohammad, F. & Helin, K. Oncohistones: drivers of pediatric cancers. *Genes Dev* **31**, 2313-2324 (2017).
101. Schwartzentruber, J.*, et al.* Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* **482**, 226-231 (2012).
102. Sturm, D.*, et al.* Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* **22**, 425-437 (2012).
103. Wu, G.*, et al.* Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* **44**, 251-253 (2012).
104. Wan, Y.C.E., Liu, J. & Chan, K.M. Histone H3 Mutations in Cancer. *Curr Pharmacol Rep* **4**, 292-300 (2018).
105. Nikbakht, H.*, et al.* Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma. *Nat Commun* **7**, 11185 (2016).
106. Mackay, A.*, et al.* Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. *Cancer Cell* **32**, 520-537 e525 (2017).
107. Lewis, P.W.*, et al.* Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science* **340**, 857-861 (2013).
108. Bender, S.*, et al.* Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell* **24**, 660-672 (2013).
109. Chan, K.M.*, et al.* The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev* **27**, 985-990 (2013).
110. Justin, N.*, et al.* Structural basis of oncogenic histone H3K27M inhibition of human polycomb repressive complex 2. *Nat Commun* **7**, 11316 (2016).
111. Herz, H.M.*, et al.* Histone H3 lysine-to-methionine mutants as a paradigm to study chromatin signaling. *Science* **345**, 1065-1070 (2014).

112. Wang, X.*, et al.* Molecular analysis of PRC2 recruitment to DNA in chromatin and its inhibition by RNA. *Nat Struct Mol Biol* **24**, 1028-1038 (2017).
113. Stafford, J.M.*, et al.* Multiple modes of PRC2 inhibition elicit global chromatin alterations in H3K27M pediatric glioma. *Sci Adv* **4**, eaau5935 (2018).
114. Castel, D.*, et al.* Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol* **130**, 815-827 (2015).
115. Grasso, C.S.*, et al.* Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med* **21**, 555-559 (2015).
116. Hashizume, R.*, et al.* Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. *Nat Med* **20**, 1394-1396 (2014).
117. Kruidenier, L.*, et al.* A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature* **488**, 404-408 (2012).
118. Mohammad, F.*, et al.* EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nat Med* **23**, 483-492 (2017).
119. Piunti, A.*, et al.* Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med* **23**, 493-500 (2017).
120. Dawson, M.A. The cancer epigenome: Concepts, challenges, and therapeutic opportunities. *Science* **355**, 1147-1152 (2017).
121. Pleyer, L. & Greil, R. Digging deep into "dirty" drugs - modulation of the methylation machinery. *Drug Metab Rev* **47**, 252-279 (2015).
122. Dombret, H.*, et al.* International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood* **126**, 291-299 (2015).
123. Fenaux, P.*, et al.* Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol* **28**, 562-569 (2010).
124. Fenaux, P.*, et al.* Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* **10**, 223-232 (2009).
125. Silverman, L.R.*, et al.* Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. *J Clin Oncol* **20**, 2429-2440 (2002).
126. Jones, P.A., Issa, J.P. & Baylin, S. Targeting the cancer epigenome for therapy. *Nat Rev Genet* **17**, 630-641 (2016).
127. Oki, Y., Jelinek, J., Shen, L., Kantarjian, H.M. & Issa, J.P. Induction of hypomethylation and molecular response after decitabine therapy in patients with chronic myelomonocytic leukemia. *Blood* **111**, 2382-2384 (2008).
128. Tsai, H.C.*, et al.* Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. *Cancer Cell* **21**, 430-446 (2012).
129. Agrawal, K., Das, V., Vyas, P. & Hajduch, M. Nucleosidic DNA demethylating epigenetic drugs - A comprehensive review from discovery to clinic. *Pharmacol Ther* **188**, 45-79 (2018).
130. Pappalardi, M.B.*, et al.* Abstract 2994: Discovery of selective, noncovalent small molecule inhibitors of DNMT1 as an alternative to traditional DNA hypomethylating agents. *Cancer Research* **78**, 2994-2994 (2018).
131. Arrowsmith, C.H., Bountra, C., Fish, P.V., Lee, K. & Schapira, M. Epigenetic protein families: a new frontier for drug discovery. *Nat Rev Drug Discov* **11**, 384-400 (2012).
132. McCabe, M.T., Mohammad, H.P., Barbash, O. & Kruger, R.G. Targeting Histone Methylation in Cancer. *Cancer J* **23**, 292-301 (2017).

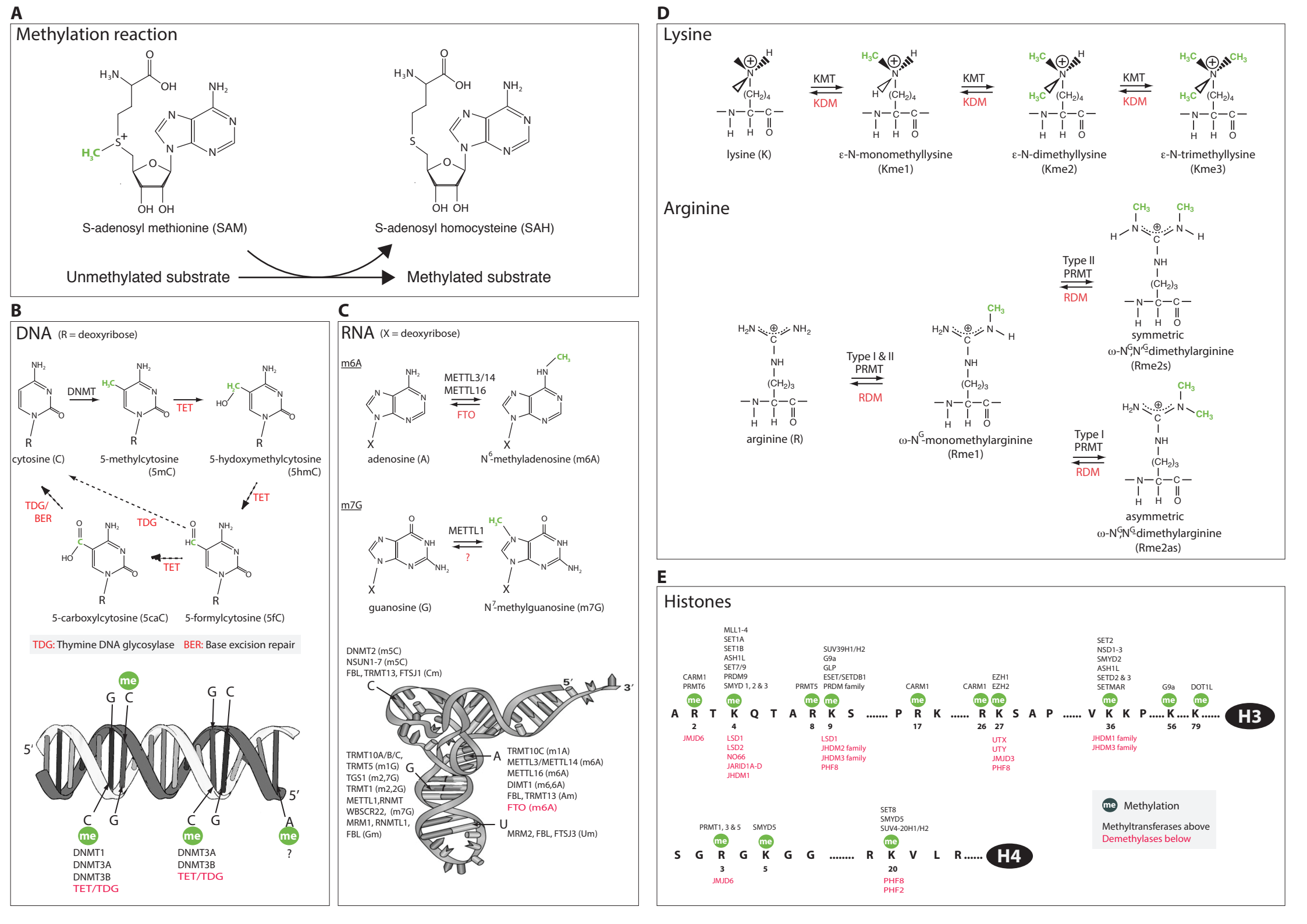
133. Margueron, R. & Reinberg, D. The Polycomb complex PRC2 and its mark in life. *Nature* **469**, 343-349 (2011).
134. Wassef, M. & Margueron, R. The Multiple Facets of PRC2 Alterations in Cancers. *J Mol Biol* **429**, 1978-1993 (2017).
135. Beguelin, W.*, et al.* EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. *Cancer Cell* **23**, 677-692 (2013).
136. Morin, R.D.*, et al.* Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* **42**, 181-185 (2010).
137. Sneeringer, C.J.*, et al.* Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. *Proc Natl Acad Sci U S A* **107**, 20980-20985 (2010).
138. Bodor, C.*, et al.* EZH2 mutations are frequent and represent an early event in follicular lymphoma. *Blood* **122**, 3165-3168 (2013).
139. LaFave, L.M.*, et al.* Loss of BAP1 function leads to EZH2-dependent transformation. *Nat Med* **21**, 1344-1349 (2015).
140. Kim, K.H.*, et al.* SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat Med* **21**, 1491-1496 (2015).
141. Bitler, B.G.*, et al.* Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med* **21**, 231-238 (2015).
142. Wilson, B.G.*, et al.* Epigenetic antagonism between polycomb and SWI/SNF complexes during oncogenic transformation. *Cancer Cell* **18**, 316-328 (2010).
143. Stanton, B.Z.*, et al.* Smarca4 ATPase mutations disrupt direct eviction of PRC1 from chromatin. *Nat Genet* **49**, 282-288 (2017).
144. Kadoch, C.*, et al.* Dynamics of BAF-Polycomb complex opposition on heterochromatin in normal and oncogenic states. *Nat Genet* **49**, 213-222 (2017).
145. Bitler, B.G., Aird, K.M. & Zhang, R. Epigenetic synthetic lethality in ovarian clear cell carcinoma: EZH2 and ARID1A mutations. *Mol Cell Oncol* **3**, e1032476 (2016).
146. Campagne, A.*, et al.* BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation. *Nat Commun* **10**, 348 (2019).
147. Schoumacher, M.*, et al.* Uveal melanoma cells are resistant to EZH2 inhibition regardless of BAP1 status. *Nat Med* **22**, 577-578 (2016).
148. Ernst, T.*, et al.* Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet* **42**, 722-726 (2010).
149. Puda, A.*, et al.* Frequent deletions of JARID2 in leukemic transformation of chronic myeloid malignancies. *Am J Hematol* **87**, 245-250 (2012).
150. Score, J.*, et al.* Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. *Blood* **119**, 1208-1213 (2012).
151. Ueda, T.*, et al.* EED mutants impair polycomb repressive complex 2 in myelodysplastic syndrome and related neoplasms. *Leukemia* **26**, 2557-2560 (2012).
152. Nikoloski, G., van der Reijden, B.A. & Jansen, J.H. Mutations in epigenetic regulators in myelodysplastic syndromes. *Int J Hematol* **95**, 8-16 (2012).
153. Lee, W.*, et al.* PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. *Nat Genet* **46**, 1227-1232 (2014).
154. Ntziachristos, P.*, et al.* Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med* **18**, 298-301 (2012).
155. Zhang, J.*, et al.* The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* **481**, 157-163 (2012).

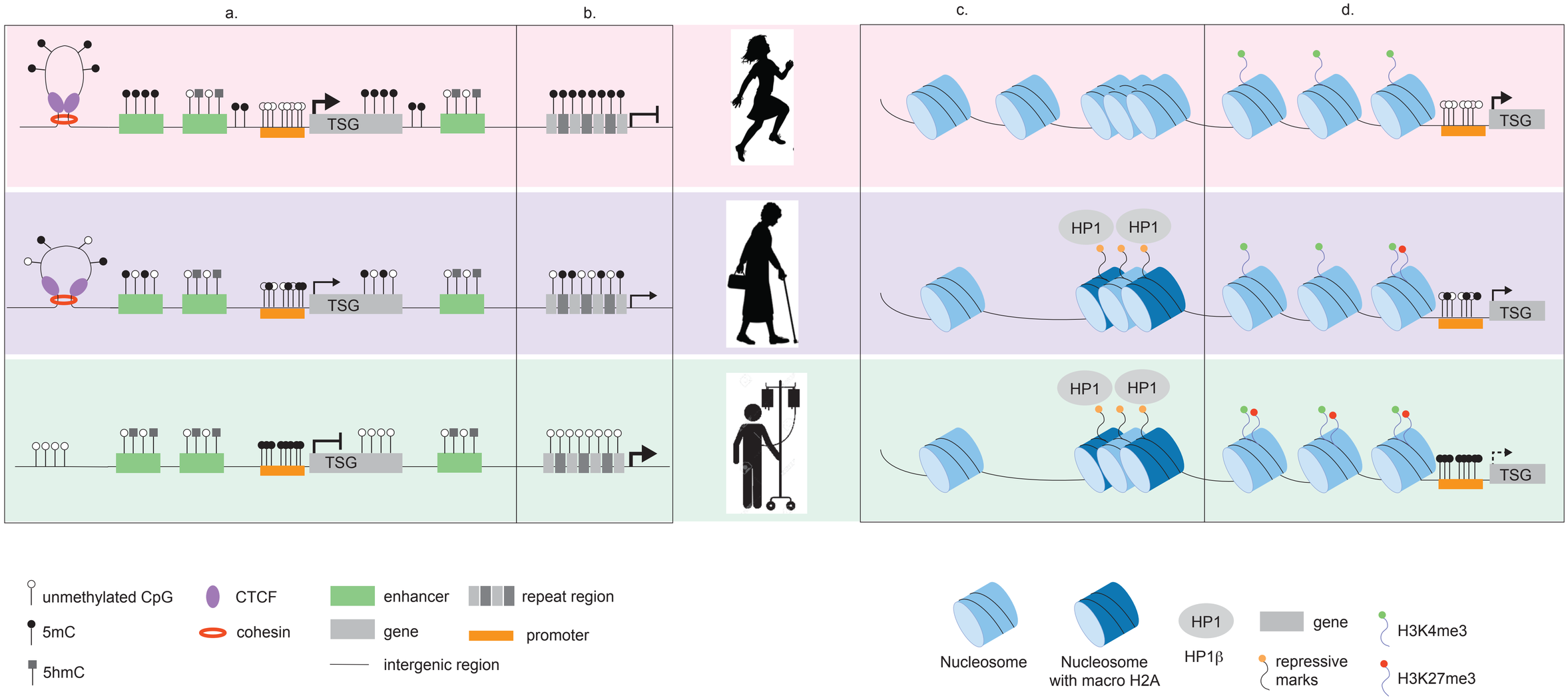
156. Comet, I., Riising, E.M., Leblanc, B. & Helin, K. Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. *Nat Rev Cancer* **16**, 803-810 (2016).
157. Chen, L.*, et al.* CRISPR-Cas9 screen reveals a MYCN-amplified neuroblastoma dependency on EZH2. *J Clin Invest* **128**, 446-462 (2018).
158. Tsubota, S.*, et al.* PRC2-Mediated Transcriptomic Alterations at the Embryonic Stage Govern Tumorigenesis and Clinical Outcome in MYCN-Driven Neuroblastoma. *Cancer Res* **77**, 5259-5271 (2017).
159. Iliopoulos, D.*, et al.* Loss of miR-200 inhibition of Suz12 leads to polycomb-mediated repression required for the formation and maintenance of cancer stem cells. *Mol Cell* **39**, 761-772 (2010).
160. Gardner, E.E.*, et al.* Chemosensitive Relapse in Small Cell Lung Cancer Proceeds through an EZH2-SLFN11 Axis. *Cancer Cell* **31**, 286-299 (2017).
161. Hubaux, R.*, et al.* EZH2 promotes E2F-driven SCLC tumorigenesis through modulation of apoptosis and cell-cycle regulation. *J Thorac Oncol* **8**, 1102-1106 (2013).
162. Kleer, C.G.*, et al.* EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A* **100**, 11606-11611 (2003).
163. Varambally, S.*, et al.* The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419**, 624-629 (2002).
164. N. Chi, S.*, et al. A phase I study of the EZH2 inhibitor tazemetostat in pediatric subjects with relapsed or refractory INI1-negative tumors or synovial sarcoma*, (2016).
165. Zauderer, M.G.*, et al.* Phase 2, multicenter study of the EZH2 inhibitor tazemetostat as monotherapy in adults with relapsed or refractory (R/R) malignant mesothelioma (MM) with BAP1 inactivation. *Journal of Clinical Oncology* **36**, 8515-8515 (2018).
166. Positive Results for Tazemetostat in Follicular Lymphoma. *Cancer Discovery* **8**, OF3-OF3 (2018).
167. McCabe, M.T.*, et al.* EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* **492**, 108-112 (2012).
168. Knutson, S.K.*, et al.* A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat Chem Biol* **8**, 890-896 (2012).
169. Souroullas, G.P.*, et al.* An oncogenic Ezh2 mutation induces tumors through global redistribution of histone 3 lysine 27 trimethylation. *Nat Med* **22**, 632-640 (2016).
170. Stein, E.M.*, et al.* The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. *Blood* **131**, 2661-2669 (2018).
171. Gulla, A.*, et al.* Protein arginine methyltransferase 5 has prognostic relevance and is a druggable target in multiple myeloma. *Leukemia* **32**, 996-1002 (2018).
172. Blanc, R.S. & Richard, S. Arginine Methylation: The Coming of Age. *Mol Cell* **65**, 8-24 (2017).
173. Hu, D.*, et al.* Interplay between arginine methylation and ubiquitylation regulates KLF4-mediated genome stability and carcinogenesis. *Nat Commun* **6**, 8419 (2015).
174. Tarighat, S.S.*, et al.* The dual epigenetic role of PRMT5 in acute myeloid leukemia: gene activation and repression via histone arginine methylation. *Leukemia* **30**, 789-799 (2016).
175. Yan, F.*, et al.* Genetic validation of the protein arginine methyltransferase PRMT5 as a candidate therapeutic target in glioblastoma. *Cancer Res* **74**, 1752-1765 (2014).
176. Koh, C.M.*, et al.* MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. *Nature* **523**, 96-100 (2015).
177. Li, Y.*, et al.* PRMT5 is required for lymphomagenesis triggered by multiple oncogenic drivers. *Cancer Discov* **5**, 288-303 (2015).
178. Mack, S.C.*, et al.* Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. *Nature* **506**, 445-450 (2014).

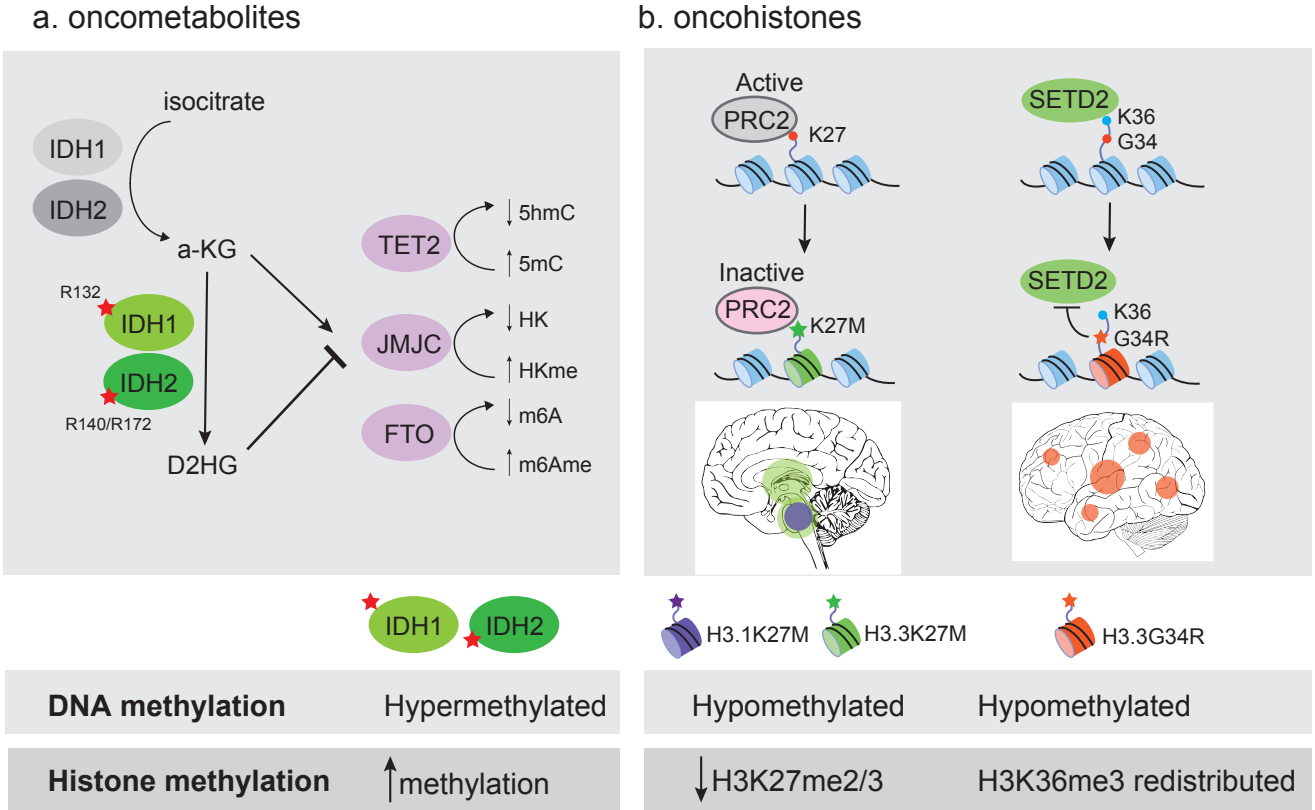
179. Chan-Penebre, E.*, et al.* A selective inhibitor of PRMT5 with in vivo and in vitro potency in MCL models. *Nat Chem Biol* **11**, 432-437 (2015).
180. Klose, R.J., Kallin, E.M. & Zhang, Y. JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet* **7**, 715-727 (2006).
181. Somervaille, T.*, et al.* Safety, Phamacokinetics (PK), Pharmacodynamics (PD) and Preliminary Activity in Acute Leukemia of Ory-1001, a First-in-Class Inhibitor of Lysine-Specific Histone Demethylase 1A (LSD1/KDM1A): Initial Results from a First-in-Human Phase 1 Study. *Blood* **128**, 4060-4060 (2016).
182. Hamamoto, R., Saloura, V. & Nakamura, Y. Critical roles of non-histone protein lysine methylation in human tumorigenesis. *Nature Reviews Cancer* **15**, 110 (2015).
183. Biggar, K.K. & Li, S.S.C. Non-histone protein methylation as a regulator of cellular signalling and function. *Nature Reviews Molecular Cell Biology* **16**, 5 (2014).
184. Fong, C.Y.*, et al.* BET inhibitor resistance emerges from leukaemia stem cells. *Nature* **525**, 538-542 (2015).
185. Rathert, P.*, et al.* Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. *Nature* **525**, 543-547 (2015).
186. Shu, S.*, et al.* Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer. *Nature* **529**, 413-417 (2016).
187. Tyler, D.S.*, et al.* Click chemistry enables preclinical evaluation of targeted epigenetic therapies. *Science* **356**, 1397-1401 (2017).
188. Holohan, C., Van Schaeybroeck, S., Longley, D.B. & Johnston, P.G. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer* **13**, 714-726 (2013).
189. Marazzi, I., Greenbaum, B.D., Low, D.H.P. & Guccione, E. Chromatin dependencies in cancer and inflammation. *Nat Rev Mol Cell Biol* **19**, 245-261 (2018).
190. Sigalotti, L., Fratta, E., Coral, S. & Maio, M. Epigenetic drugs as immunomodulators for combination therapies in solid tumors. *Pharmacol Ther* **142**, 339-350 (2014).
191. Chiappinelli, K.B.*, et al.* Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. *Cell* **162**, 974-986 (2015).
192. Roulois, D.*, et al.* DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts. *Cell* **162**, 961-973 (2015).
193. Sheng, W.*, et al.* LSD1 Ablation Stimulates Anti-tumor Immunity and Enables Checkpoint Blockade. *Cell* **174**, 549-563 e519 (2018).
194. Topper, M.J.*, et al.* Epigenetic Therapy Ties MYC Depletion to Reversing Immune Evasion and Treating Lung Cancer. *Cell* **171**, 1284-1300 e1221 (2017).
195. Stone, M.L.*, et al.* Epigenetic therapy activates type I interferon signaling in murine ovarian cancer to reduce immunosuppression and tumor burden. *Proc Natl Acad Sci U S A* **114**, E10981-E10990 (2017).
196. Arenas-Ramirez, N., Sahin, D. & Boyman, O. Epigenetic mechanisms of tumor resistance to immunotherapy. *Cell Mol Life Sci* **75**, 4163-4176 (2018).
197. Han, D.*, et al.* Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. *Nature* **566**, 270-274 (2019).
198. Brach, D.*, et al.* EZH2 Inhibition by Tazemetostat Results in Altered Dependency on B-cell Activation Signaling in DLBCL. *Mol Cancer Ther* **16**, 2586-2597 (2017).
199. Beguelin, W.*, et al.* EZH2 and BCL6 Cooperate to Assemble CBX8-BCOR Complex to Repress Bivalent Promoters, Mediate Germinal Center Formation and Lymphomagenesis. *Cancer Cell* **30**, 197-213 (2016).
200. Matei, D.*, et al.* Epigenetic resensitization to platinum in ovarian cancer. *Cancer Res* **72**, 2197-2205 (2012).

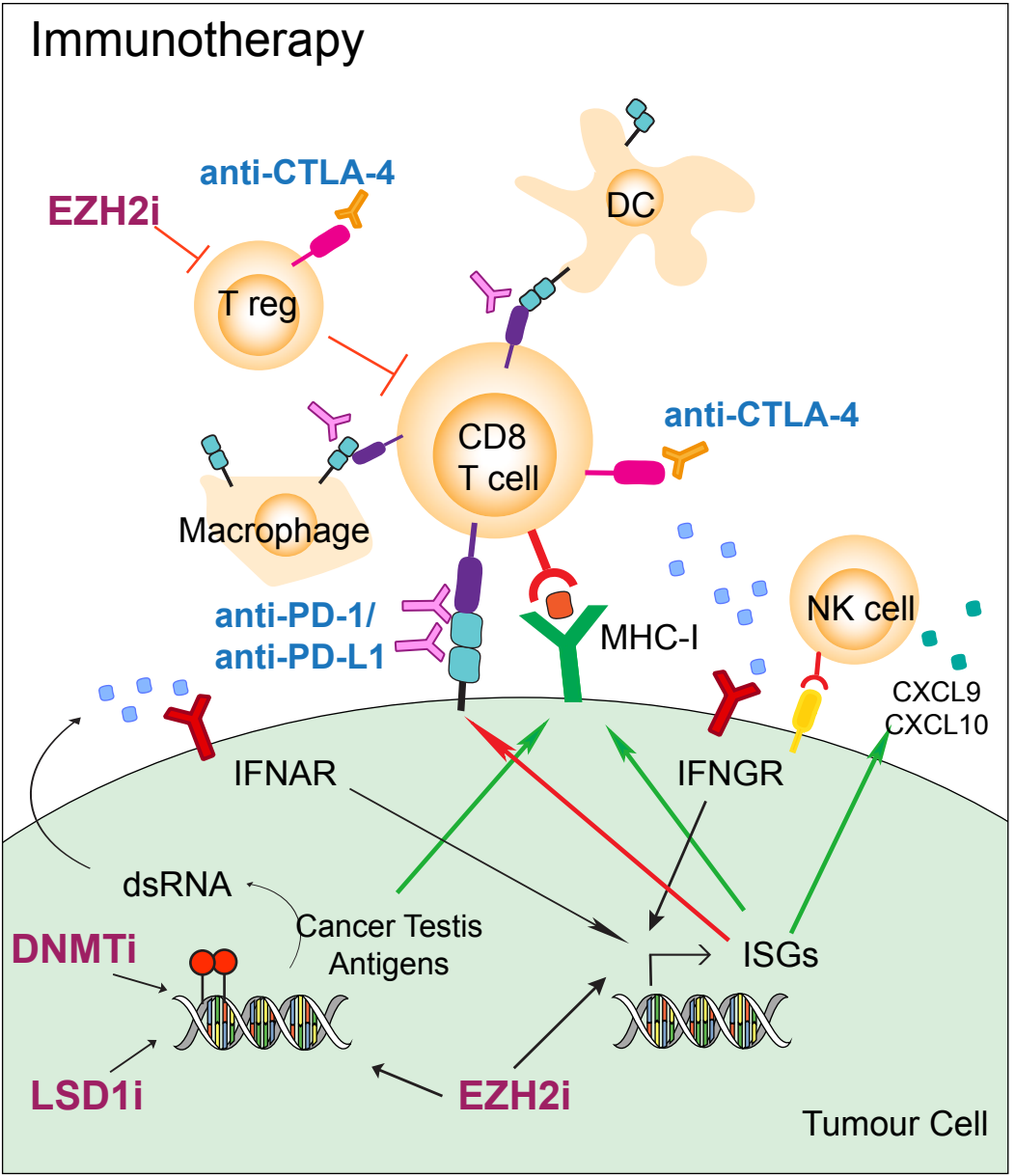
201. Nguyen, A.T. & Zhang, Y. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev* **25**, 1345-1358 (2011).
202. Huyen, Y.*, et al.* Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* **432**, 406-411 (2004).
203. Lin, Y.H.*, et al.* Global reduction of the epigenetic H3K79 methylation mark and increased chromosomal instability in CALM-AF10-positive leukemias. *Blood* **114**, 651-658 (2009).
204. Wakeman, T.P., Wang, Q., Feng, J. & Wang, X.F. Bat3 facilitates H3K79 dimethylation by DOT1L and promotes DNA damage-induced 53BP1 foci at G1/G2 cell-cycle phases. *EMBO J* **31**, 2169-2181 (2012).
205. Oksenych, V.*, et al.* Histone methyltransferase DOT1L drives recovery of gene expression after a genotoxic attack. *PLoS Genet* **9**, e1003611 (2013).
206. Prebet, T.*, et al.* Prolonged administration of azacitidine with or without entinostat for myelodysplastic syndrome and acute myeloid leukemia with myelodysplasia-related changes: results of the US Leukemia Intergroup trial E1905. *J Clin Oncol* **32**, 1242-1248 (2014).
207. Kalin, J.H.*, et al.* Targeting the CoREST complex with dual histone deacetylase and demethylase inhibitors. *Nat Commun* **9**, 53 (2018).
208. Liu, X.S.*, et al.* Editing DNA Methylation in the Mammalian Genome. *Cell* **167**, 233-247 e217 (2016).
209. Liszczak, G.P.*, et al.* Genomic targeting of epigenetic probes using a chemically tailored Cas9 system. *Proceedings of the National Academy of Sciences* **114**, 681-686 (2017).
210. Luskin, M.R.*, et al.* A clinical measure of DNA methylation predicts outcome in de novo acute myeloid leukemia. *JCI Insight* **1**(2016).
211. Koch, A.*, et al.* Analysis of DNA methylation in cancer: location revisited. *Nat Rev Clin Oncol* **15**, 459-466 (2018).
212. Bienkowski, M.*, et al.* Clinical Neuropathology practice guide 5-2015: MGMT methylation pyrosequencing in glioblastoma: unresolved issues and open questions. *Clin Neuropathol* **34**, 250-257 (2015).
213. Shen, S.Y.*, et al.* Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature* **563**, 579-583 (2018).
214. Dawson, S.J.*, et al.* Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med* **368**, 1199-1209 (2013).
215. Agarwal, R.*, et al.* Dynamic molecular monitoring reveals that SWI-SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. *Nat Med* (2018).
216. Rodriguez-Terrones, D. & Torres-Padilla, M.E. Nimble and Ready to Mingle: Transposon Outbursts of Early Development. *Trends Genet* **34**, 806-820 (2018).
217. Faulkner, G.J.*, et al.* The regulated retrotransposon transcriptome of mammalian cells. *Nature Genetics* **41**, 563 (2009).
218. Tchasovnikarova, I.A.*, et al.* GENE SILENCING. Epigenetic silencing by the HUSH complex mediates position-effect variegation in human cells. *Science* **348**, 1481-1485 (2015).
219. Liu, N.*, et al.* Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. *Nature* **553**, 228-232 (2018).
220. Stadler, M.B.*, et al.* DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* **480**, 490-495 (2011).
221. Ohm, J.E.*, et al.* A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. *Nat Genet* **39**, 237-242 (2007).
222. Schlesinger, Y.*, et al.* Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. *Nat Genet* **39**, 232-236 (2007).

223. Widschwendter, M.*, et al.* Epigenetic stem cell signature in cancer. *Nat Genet* **39**, 157-158 (2007).
224. Qiu, L.*, et al.* Mechanism of cancer: Oncohistones in action. *J Genet Genomics* **45**, 227-236 (2018).
225. Fang, J.*, et al.* Cancer-driving H3G34V/R/D mutations block H3K36 methylation and H3K36me3-MutSalpha interaction. *Proc Natl Acad Sci U S A* **115**, 9598-9603 (2018).
226. Canadas, I.*, et al.* Tumor innate immunity primed by specific interferon-stimulated endogenous retroviruses. *Nat Med* **24**, 1143-1150 (2018).
227. Zingg, D.*, et al.* The Histone Methyltransferase Ezh2 Controls Mechanisms of Adaptive Resistance to Tumor Immunotherapy. *Cell Rep* **20**, 854-867 (2017).
228. Abou El Hassan, M.*, et al.* Cancer Cells Hijack PRC2 to Modify Multiple Cytokine Pathways. *PLoS One* **10**, e0126466 (2015).
229. Peng, D.*, et al.* Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature* **527**, 249-253 (2015).
230. Wang, D.*, et al.* Targeting EZH2 Reprograms Intratumoral Regulatory T Cells to Enhance Cancer Immunity. *Cell Rep* **23**, 3262-3274 (2018).
231. Goswami, S.*, et al.* Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. *J Clin Invest* **128**, 3813-3818 (2018).

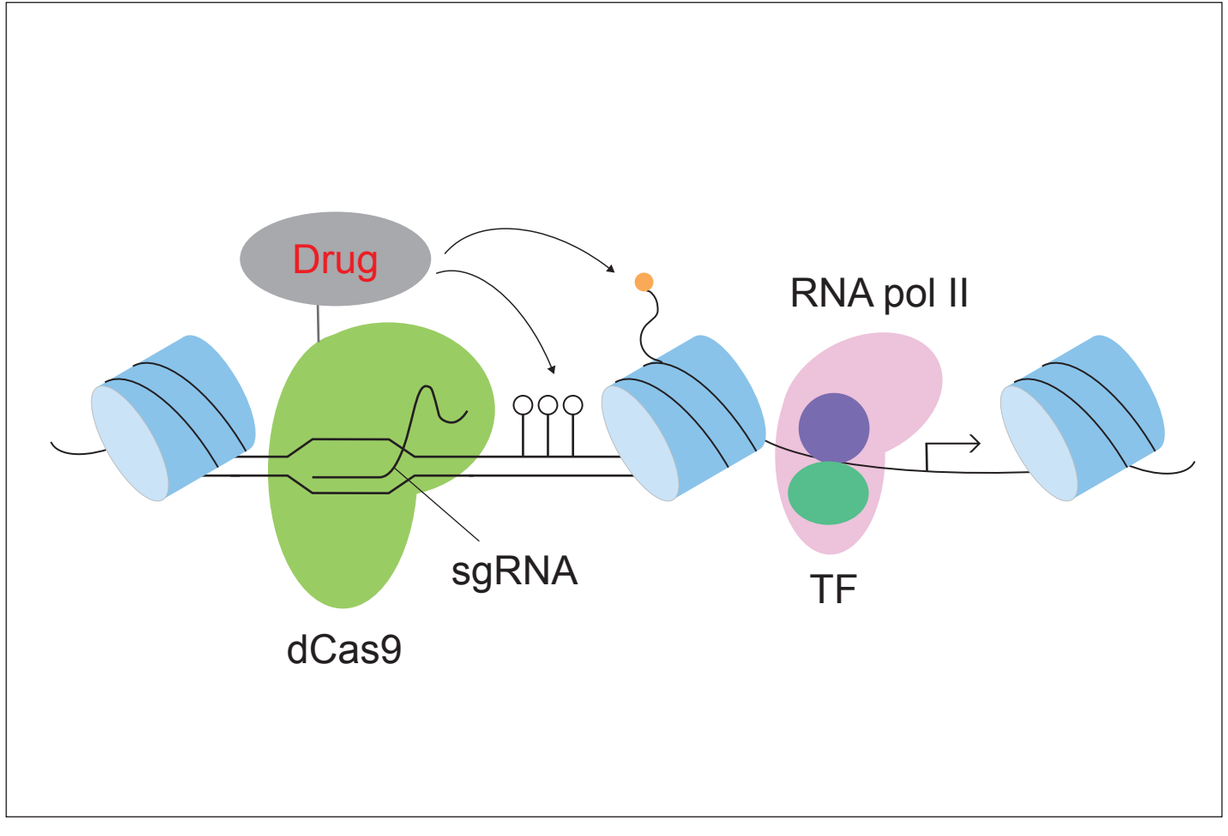

A
Methylation reaction
S-adenosyl methionine (SAM)
S-adenosyl homocysteine (SAH)
Unmethylated substrate
Methylated substrate
B
DNA (R = deoxyribose)
DNMT
TET
cytosine (C)
5-methylcytosine (5mC)
5-hydoxymethylcytosine (5hmC)
TDG/ BER
TDG
TET
TET
5-carboxylcytosine (5caC)
5-formylcytosine (5fC)
TDG: Thymine DNA glycosylase
BER: Base excision repair
me
DNMT1
DNMT3A
DNMT3B
TET/TDG
DNMT3A
DNMT3B
TET/TDG
?
C
RNA (X = deoxyribose)
m6A
METTL3/14
METTL16
FTO
adenosine (A)
N6-methyladenosine (m6A)
m7G
METTL1
?
guanosine (G)
N7-methylguanosine (m7G)
DNMT2 (m5C)
NSUN1-7 (m5C)
FBL, TRMT13, FTSJ1 (Cm)
TRMT10A/B/C,
TRMT5 (m1G)
TGS1 (m2,7G)
TRMT1 (m2,2G)
METTL1,RNMT
WBSCR22, (m7G)
MRM1, RNMTL1,
FBL (Gm)
TRMT10C (m1A)
METTL3/METTL14 (m6A)
METTL16 (m6A)
DIMT1 (m6,6A)
FBL, TRMT13 (Am)
FTO (m6A)
MRM2, FBL, FTSJ3 (Um)
D
Lysine
KMT
KDM
lysine (K)
ε-N-monomethyllysine (Kme1)
ε-N-dimethyllysine (Kme2)
ε-N-trimethyllysine (Kme3)
Arginine
Type I & II PRMT
RDM
arginine (R)
ω-NG-monomethylarginine (Rme1)
Type II PRMT
RDM
symmetric ω-NG,NG-dimethylarginine (Rme2s)
Type I PRMT
RDM
asymmetric ω-NG,NG-dimethylarginine (Rme2as)
E
Histones
MLL1-4
SET1A
SET1B
ASH1L
SET7/9
PRDM9
SMYD 1, 2 & 3
CARM1
PRMT6
SUV39H1/H2
G9a
GLP
ESET/SETDB1
PRDM family
PRMT5
CARM1
CARM1
EZH1
EZH2
SET2
NSD1-3
SMYD2
ASH1L
SETD2 & 3
SETMAR
G9a
DOT1L
A R T K Q T A R K S ..... P R K ..... R K S A P ..... V K K P ..... K ..... K
2 4 8 9 17 26 27 36 56 79
H3
JMJD6
LSD1
LSD2
NO66
JARID1A-D
JHDM1
LSD1
JHDM2 family
JHDM3 family
PHF8
UTX
UTY
JMJD3
PHF8
JHDM1 family
JHDM3 family
Methylation
Methyltransferases above
Demethylases below
PRMT1, 3 & 5
SMYD5
SET8
SMYD5
SUV4-20H1/H2
S G R G K G G ..... R K V L R.....
3 5 20
H4
JMJD6
PHF8
PHF2


a.
b.
c.
d.
TSG
HP1
HP1
unmethylated CpG
5mC
5hmC
CTCF
cohesin
enhancer
gene
intergenic region
repeat region
promoter
Nucleosome
Nucleosome with macro H2A
HP1β
gene
repressive marks
H3K4me3
H3K27me3


a. oncometabolites
isocitrate
IDH1
IDH2
a-KG
R132
IDH1
IDH2
R140/R172
D2HG
TET2
5hmC
5mC
JMJC
HK
HKme
FTO
m6A
m6Ame
b. oncohistones
Active
PRC2
K27
Inactive
PRC2
K27M
SETD2
K36
G34
SETD2
K36
G34R
IDH1
IDH2
H3.1K27M
H3.3K27M
H3.3G34R
DNA methylation
Hypermethylated
Hypomethylated
Hypomethylated
Histone methylation
methylation
H3K27me2/3
H3K36me3 redistributed


Immunotherapy
anti-CTLA-4
EZH2i
DC
T reg
CD8
T cell
anti-CTLA-4
Macrophage
anti-PD-1/
anti-PD-L1
MHC-I
NK cell
CXCL9
CXCL10
IFNAR
IFNGR
dsRNA
Cancer Testis
Antigens
DNMTi
ISGs
LSD1i
EZH2i
Tumour Cell

a. Targeted drug delivery



b. Liquid biopsies