Histone methylases as novel drug targets. Focus on EZH2 inhibition.

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ABSTRACT

Posttranslational modifications of histones (so-called epigenetic modifications) play a major role in transcriptional control and normal development, and are tightly regulated. Disruption of their control is a frequent event in disease. Particularly, the methylation of lysine 27 on histone H3 (H3K27), induced by the methylase Enhancer of Zeste homolog 2 (EZH2), emerges as a key control of gene expression, and a major regulator of cell physiology. The identification of driver mutations in EZH2 has already led to new prognostic and therapeutic advances, and new classes of potent and specific inhibitors for EZH2 show promising results in preclinical trials. This review examines roles of histone lysine methylases and demethylases in cells, and focuses on the recent knowledge and developments about EZH2.

Key-terms: epigenetic, histone methylation, EZH2, cancerology, tumors, apoptosis, cell death, inhibitor, stem cells, H3K27
Histone modifications and histone code

Epigenetic has been defined as inheritable changes in gene expression that occur without a change in DNA sequence. Key components of epigenetic processes are DNA methylation, histone modifications and variants, non-histone chromatin proteins, small interfering RNA (siRNA) and micro RNA (miRNA). They induce changes in gene expression in modifying accessibility of the eukaryotic transcription machinery to specific genes. In particular, the role of histones as active participants in gene regulation has only recently been appreciated.

Histones were discovered in 1884 by Albrecht Kossel. But until the early 1990s, these proteins, which are assembled into nucleosomes, forming beads around which the DNA is wrapped, were considered to be relatively inert scaffolding for packaging the genetic material. It is now known that histones play also a key-role in gene expression regulation, though post-translational modifications of histone (figure 1). In 2000, the concept of a ‘histone code’ emerged [1].

The histones' amino-terminal tails extend away from the central core, and are thus available for reversible acetylation, methylation, phosphorylation, ADP-ribosylation, and ubiquitination (figure 2). Histone modifications interact with DNA methylation to mark genes for silencing or transcription. By reading the combinatorial and/or sequential histone modifications that constitute the histone code (table 1), it was thought that it might be possible to predict which gene products will be transcribed and thus determine a cell’s RNA repertoire and ultimately its proteome, just as reading the DNA code allows us to predict the encoded protein sequence. However, some gene loci present both histone 3 lysine 4 trimethylation (H3K4me3), associated with transcriptional activation and histone 3 lysine 27 trimethylation (H3K27me3), and linked with repression. These bivalent domains are posited to be poised for either up- or downregulation and to provide an epigenetic blueprint for lineage determination [2], and are usually found in stem cells.

These post-translational modifications (PTM) undergone by histones have a profound effect on the remodeling of chromatin. Two distinct chromatin states can be distinguished: condensed “closed” heterochromatin, and de-condensed “open” euchromatin. The change from transcriptionally silenced heterochromatin to gene expression euchromatin is mediated by posttranslational modifications of histones and uses of distinct histone variants.
**Histone lysine methylation**

Histone methylation is an epigenetic mark actively studied in recent years. On about 11,000 articles referenced in Pubmed since 1964, more than half of them have been published during the last four years.

The most well-characterized histone methylation appears on lysine [3,4]. Histone lysine methylation occurs primarily on histone H3 at lysines 4, 9, 14, 18, 23, 27, 36 and 79 and on histone H4 at lysine 20 [4–6]. A number of these methylation events have been linked to transcriptional regulation, including those at H3 lysines 4, 36 and 79 (associated with active transcription) and those at H3 lysines 9 and 27 (associated with gene repression and heterochromatin formation) [3,7]. Unlike acetylation and phosphorylation, which in addition to recruit proteins to chromatin can also directly affect chromatin structure by altering the histone charge, lysine methylation does not alter the charge of the residue and is therefore thought to primarily modulate chromatin structure through the recruitment of distinct reader proteins that possess the ability to facilitate transcriptional activation or repression [3,4,6,8].

Lysine residues can be modified with up to three methyl groups (mono-, di- and trimethylation) on the epsilon amine of the side-chain (figure 3). Importantly, reader domains can distinguish between the different methyl states producing distinct functional outcomes [3,4,6,8]. These observations demonstrate the complexity and fine level of control that lysine methylation contributes to chromatin function and transcriptional regulation.

Among activation marks, trimethylation at lysine 4 of histone H3 (H3K4me3) is the prominent methyllysine species at active promoter regions [9–13]. This mark plays a major role in transcription initiation, notably in recruiting the general transcription factors, or in mediating interactions with RNA polymerase associated proteins [6]. H3K36 methylation, meanwhile, primarily exists with the lower methylation states (H3K36me1 and -me2) present near 5′ regions and higher methylation states (H3K36me2 and -me3) at the 3′ ends of genes [11,14]. The role of H3K36 methylation is also quite diverse and has been shown to be involved in numerous functions including transcription, mRNA splicing, DNA replication and DNA repair [15,16]. Its function that has been most well defined is its role in transcription elongation. Another modification found in gene bodies is methylation of H3K79, however, unlike H3K36 methylation, its role in actively transcribed genes is less clear. It may act as a protection from silencing [6].
At opposite, histone H3 lysine 9 methylation (H3K9) has been correlated with heterochromatin formation and transcriptional repression, making the methylation state of lysine 9 an interesting marker of transcriptional activity. H3K9me3 binds heterochromatin protein 1 (HP1) to constitutive heterochromatin [17]. HP1 is responsible for transcriptional repression and the actual formation and maintenance of heterochromatin. H3K9me2 is a characteristic mark of the inactivated X chromosome (Xi) [18,19]. H3K9 methylation is also involved in cell reprogramming, or cancer. H3K27 methylation is also another epigenetic repressive mark, which plays a major role in a plethora of cellular processes, such as stem cell renewal, cell fate, reprogramming, cancer, inflammation.

**Histone arginine methylation**

As lysine, arginine on histone can also be methylated. The addition of one or two methyl groups on arginine residues results in three different methylation states: monomethylated, asymmetrically dimethylated or symmetrically dimethylated arginine. The methyl groups are deposited by protein arginine methyltransferases (PRMTs). Histone arginine methylation associates with both active and repressed chromatin states depending on the residue involved and the status of methylation [20]. This process is involved in several cellular processes such as transcription, RNA processing, signal transduction and DNA repair. Besides, it is now clear that there is cross-talk between arginine and lysine methylation: this has been termed “arginine/lysine-methyl/methyl switch” [21,22]

**Histone methyltransferases and demethylases**

There are currently more than 60 predicted lysine methyltransferases and 30 predicted lysine demethylases in the human genome [23–25].

Histone methyltransferase (HMT) activity towards lysine (and arginine) residues is found in a family of enzymes with a conserved catalytic domain called SET (Suppressor of variegation, Enhancer of Zeste, Trithorax). The human genome encodes 49 SET domain-containing proteins and the histone lysine methyltransferase DOT1L, which does not contain a SET domain (table 2). The importance of HMTs for embryonic development has been demonstrated in numerous mouse knockout studies [26]. In addition, misregulation of HMTs has been linked to diseases or cancer aggressiveness. In particular, the Polycomb group
transcriptional repressor EZH2 (methylase of H3K27), is overexpressed in many different types of cancer [27], and has been proposed as a molecular marker of some cancer progression and metastasis [28–33].

In 2004, the first histone demethylase (HDM) has been discovered, and called LSD1 (lysine-specific demethylase 1). Since, more than 20 demethylases have been identified and characterized (table 3). They belong to either the LSD family or the JmjC family, demonstrating the reversibility of all methylation states at almost all major histone lysine methylation sites (table 3). The identification of these histone demethylases (HDMs) has completely changed our initial view of histone methylation as a permanent, heritable mark [34]. The presence of both histone methyltransferases and demethylases in the same complexes permits modifying of chromatin marks and subsequently switching of transcriptional states from silenced to activated status or vice-versa. Thus, a tight regulation of the expression, activity and recruitment of HMTs and HDMs is necessary. A deregulation of their activity or expression might modify the transcriptional balance, and lead to inappropriate gene expression programs that in turn could induce human disease (table 1 in supplementary material). In particular, the histone methylase EZH2 plays a major role in cell fate and cancer development, and appear now as a promising target for treat some diseases.

**Role of the lysine methyltransferase EZH2**

The methyltransferase Polycomb Group (PcG) protein Enhancer of zeste homolog 2 (EZH2), also called KMT6, is the catalytic subunit of the Polycomb Repressor Complex 2 (PRC2). Its C-terminal SET domain exhibits methyltransferase activity, leading to repress gene transcription by silencing target genes through methylation of histone H3 on lysine 27 (H3K27me3) [35]. In addition to methylation of H3K27, EZH2 has been shown to methylate cellular proteins and act as a co-activator of steroid hormone receptors [36]. This function is hypothesized to be independent of PRC2 and potentially induced by phosphorylation of EZH2 [36,37]. Besides its ability of methylate H3K27, EZH2 has recently been described to methylate lysine 120 of histone H2B which competes with ubiquitination on this site [38]. EZH2 is post-translationnaly regulate by O-linked N-acetylglucosamine (GlcNAc) transferase (OGT)-mediated O-GlcNAcylation at S75, which stabilizes EZH2 and hence facilitates the formation of H3K27me3 [39].
Unlike other SET domains, the methylase EZH2 is inactive on its own for histone substrates. To be functional, EZH2 need to form the PRC2 complex (figure 4) by interacting with other partners, including embryonic ectoderm development (EED), suppressor of zeste 12 homologue (SUZ12), and RBAP48/RBBP4 [40–43]. Collectively, these proteins regulate vital cellular processes, such as differentiation, cell identity, stem-cell plasticity, and proliferation [44–46]. As a result, aberrations in any PRC2 component can have powerful physiologic consequences on the cell.

**EZH2, stem cells and reprogramming**

EZH2 plays a central role in stem cells. Recent report showed that Ezh2 is important for establishing ES cell lines from blastocysts [47–49]. Additionally, Ezh2 is required for efficient somatic cell reprogramming by cell fusion and nuclear transfer [49,50]. Ezh2 is abundantly expressed in iPS cells (at a similar level as in ES cells), and Ezh2 knockdown severely impaired iPS cell generation. Proper differentiation of iPS cells and reprogramming require, thus, Ezh2 [51]. However, once pluripotency is established, Ezh2 knockdown leaves the pluripotent phenotype of iPS cells unaffected [52]. All this indicates that Ezh2 is critical for induction of pluripotency, but once pluripotency is established, Ezh2 is not required anymore. The mechanism of EZH2 in reprogramming is still poorly known, but it has been recently found that Ezh2 impacts on iPS cell generation at least in part through repression of the CDK inhibitor Ink4a/Arf, which represents a major roadblock for iPS cell generation [52]. Furthermore, c-Myc, one of the iPS cell inducing factors, was recently shown to directly regulate the Ezh2 expression and to be required for maintaining high Ezh2 expression in ES cells [53].

The role of EZH2 in reprogramming is, however, unclear. Indeed, in a recent paper[54], Fragola et al. generated iPS cells from MEF with a conditional Ezh2 knockout allele for the deletion of the catalytic Ezh2 SET domain [54]. Ezh2-deficient iPS cells, obtained using a cell-permeable TAT-Cre recombinase, exhibited a global loss of H3K27me3, and presented a typical iPS cell phenotype, including ES cell-like morphology, growth, and differentiation potential. This result on Ezh2-deficient iPS cells contrasts other papers which showed essential role of EZH2 in reprogramming [51,52]. It might be explain by used methodology, in that Ezh2 inactivation could have occurred after reprogramming.
**EZH2 and cell fate**

Enhancer of zeste homolog 2 also regulates expression of tissue-specific genes involved in cellular differentiation and developmental programs [35,55–58]. It is involved in differentiation of embryonic and adult stem cells into several cell lineages (myogenesis, adipogenesis, osteogenesis, neurogenesis, hematopoiesis, lymphopoiesis, epidermal differentiation and hepatogenesis) [59].

For instance, EZH2 was clearly shown to act as a negative regulator of skeletal muscle differentiation favoring the proliferation of myogenic precursors [60–62]. This function results from an EZH2-dependent direct repression of genes related to myogenic differentiation [60] through the H3K27me3 mark deposition on the promoters of myogenic genes [60,63]. Ezh2 is expressed early in the myotomal compartment of developing somites and in proliferation satellite cells and is down-regulated in terminally differentiated muscle cells [60]. In skeletal muscle progenitors, EZH2 is, thus, highly expressed and prevents an unscheduled differentiation by repressing muscle-specific gene expression. During the course of their differentiation, EZH2 is downregulated, favoring the expression of muscle specific genes, such as muscle creatine kinase (mCK), myogenin (MyoG), myh, or MyoD [64,65]. Furthermore, the key-role of EZH2 in control of self-renewal and safeguard of the transcriptional identity of skeletal muscle stem cells has been shown using mice with conditional ablation of Ezh2 in satellite cells. These mice have reduced muscle mass and fail to appropriately regenerate. These defects were associated with derepression of genes expressed in nonmuscle cell lineages [66]. Besides, in humans, abnormal expression of Ezh2 is observed in the muscular disorder Duchenne muscular dystrophy [67].

EZH2 was also found to be involved in commitment of mesenchymal stem cells towards osteoblast lineage [68]. Suppression of Ezh2 activity promotes differentiation of human mesenchymal stem cells into osteoblasts. The mechanism might be linked to Runx2 regulation since a striking decrease in Ezh2 mRNA levels has been found to be correlated to a increased Runx2 binding, suggesting that the transcription of Ezh2 is potentially negatively regulated by Runx2 [69]. At contrary, deletion of Ezh2 inhibits adipogenesis, by eliminating H3K27me3 on Wnt promoters and derepressing Wnt expression, which leads to activation of Wnt/b-catenin signaling [70]. These data show that Ezh2 facilitates adipogenesis whereas it suppresses osteogenesis.
**EZH2 and immune system**

EZH2 plays also a role in immune system, for both T and B cell development. Ezh2 is most abundant at sites of embryonic lymphopoiesis, such as fetal liver and thymus [71].

In B cell progenitors, Ezh2 expression is downregulated during differentiation. It is the highest in pro-B cells and very low in mature recirculating B cells (Su et al., 2002). Up-regulation of Ezh2 in proliferating human germinal center B cells (centroblasts) [72] and mitogen-stimulated lymphocytes [73] suggested an important role for this histone methylase in B cell division and activation. This is further supported by the association of EZH2 with Vav, one of the key regulators of the receptor-mediated signaling in lymphocytes [74]. But the major proof of a critical role for Ezh2 in early B cell development and rearrangement of the immunoglobulin heavy chain gene (Igh) has been established, in 2002, using Cre-mediated conditional mutagenesis. Ezh2 deficiency leads to diminished generation of pre-B cells and immature B cells in the bone marrow. Defective B cell development cannot be restored by the presence of the wild-type cells in the mixed bone marrow chimeras. The requirement for Ezh2 is development stage–specific: Ezh2 is a key regulator of histone H3 methylation in early B cell progenitors [75].

EZH2 is a master regulator of the germinal center (GC) B-cell phenotype [76]. It represses genes involved in proliferation checkpoints (e.g. CDKN1A) and in exit from the GC and terminal differentiation (e.g. IRF4 and PRDM1). This function is aberrantly reinforced by mutant EZH2Y146N lymphoma disease alleles [76]. EZH2 also established bivalent chromatin domains at key regulatory loci to transiently suppress GC B-cell differentiation. Beside, EZH2 cooperates with BCL2 to generate GC derived lymphomas [76].

A recent study also established a functional link between this histone methyltransferase EZH2 and transcriptional regulation of lineage-specifying genes in terminally differentiated CD4(+) T cells. EZH2 inactivation specifically enhanced T helper 1 (Th1) and Th2 cell differentiation and plasticity. Ezh2 directly binds Tbx21 and Gata3 genes, leading to substantial trimethylation at lysine 27 of histone 3 (H3K27me3) at these locus, thereby facilitating correct expression of these primordial genes in differentiating Th1 and Th2 cells. Additionally, Ezh2 deficiency leads to spontaneous generation of a small IFN-γ and Th2 cytokine-producing populations in non-polarizing cultures, and under these conditions, IFN-γ expression was largely dependent on increased expression of the transcription factor
Eomesodermin. Besides, in vivo, in a model of allergic asthma, Ezh2 loss results in exacerbated pathology with a progressive accumulation of memory phenotype Th2 cells [77].

**EZH2 and cancer**

Among EZH2 roles, its implication in cancer is the most studied: more than 70% of articles referenced in Pubmed for “Ezh2” term, are related to cancer. Alterations in **EZH2** were first discovered in breast and prostate cancer, where amplification and overexpression first implied it may function as an oncogene [28,31]. Since, increasing evidence demonstrates that EZH2 is not only aberrantly expressed in several types of human cancers, but often behaves as a molecular biomarker of poor prognosis [27,28,31,78–84]. The role of EZH2 in cancer development was initially validated both *in vitro* and *in vivo*, with **EZH2** overexpression proving sufficient to drive proliferation in cancer cells and transform primary fibroblasts [27,85].

Overexpression of EZH2 has now been found in a number of human cancers, such as prostate cancers, gastric cancers, breast cancer, renal cancer, colorectal cancer, non small cell lung cancer, squamous cell carcinomas, urothelial carcinomas in addition to synovial sarcomas, chondrosarcoma, lymphomas and melanomas [31,86–91]. EZH2 expression is correlated with aggressiveness, metastasis, and poor prognosis in most of these cancers. Elevated expression of EZH2 has, also, been identified as a marker for breast cancer initiating cells, possibly reflecting its role in maintaining “stemness” [31,92].

In addition, several mutations, located the most often in SET domain leading to increased trimethylation efficiency, have been associated to cancers (table 4) [93–98]. Recurrent mutations of EZH2 have been found in germinal center B-cell like diffuse large B-cell lymphoma, follicular lymphoma, and melanoma [99]. The mutated residues alter the substrate specificity of EZH2 and facilitate the conversion from a dimethylated to a trimethylated state, thus resulting in significantly elevated global H3K27me3 levels [93,98]. The most frequent identified mutation appears on Y641 (mutations Y/F, Y/N, Y/H, Y/C) [98,100,101]. Another mutation has been identify (A677G and A687V) though these mutants are less prevalent in [93,102].
Together this data suggests a causative role for elevated catalytic activity of EZH2 in the development of cancer. The functional consequence of increased EZH2 (either by overexpression or mutations) in cancer tissues includes the silencing of genes that promote differentiation and restrain proliferation.

Nonetheless, a high expression of EZH2 and trimethylation of histone H3 at lysine 27 were sometimes associated with improvements in survival. Thus, increased EZH2 expression is correlated to better overall survival in diffuse large B-cell lymphoma and lung cancer [103,104]. In the same way, a recent report showed that EZH2 serves as a tumor suppressor in myelodysplastic syndromes, which was evidenced by EZH2 deletions, missense and frameshift mutations [105]. Besides, enhanced trimethylation of H3K27me3 has been correlated with longer overall survival and better prognosis in non-small cell lung cancer, breast, ovarian and pancreatic cancers [106,107].

Mechanistically, EZH2 is usually believed to function predominately as a transcriptional repressor that silences an array of target genes, including more than 200 tumor suppressors [88,108]. EZH2 is identified as a downstream mediator of the retinoblastoma protein (pRB) pathway-E2F pathway which controls multiple key cell-cycle regulators during cell proliferation in normal and cancer cells [27]. Additionally, EZH2 represses the p16, p19 and p15 directly or indirectly which activates the cyclin D-CDK4/6 complex and promotes progression through G1 phase and cell proliferation [109,110]. Furthermore, enforced expression of EZH2 increases cancer cell proliferation, epithelial-mesenchymal transition, metastatic spreading and other oncogenic properties, whereas its depletion inhibited cell proliferation, migration and invasion and induced cell apoptosis and senescence both in vitro and in vivo [87,111,112]. Besides, EZH2 could cause a rise in cell migration and invasion in cancer cells by regulating E-cadherin and MMP [113]. Increasing evidence also suggests that aberrant overexpression of EZH2 could contribute to acquired chemotherapeutic resistance in multiple cancers [114–116].

In addition to its role as a transcriptional repressor, several studies have shown that EZH2 may also function in target gene activation [36,117,118]. Recently, Xu et al reported that EZH2 plays an important role in castration-resistant prostate cancer, and its oncogenic function does not depend on silencing but rather on transcriptional induction of its target
Many of these genes were downregulated upon EZH2 knockdown, suggesting that the role of EZH2 as an activator was independent of the PRC2 complex. This function is hypothesized to be induced by phosphorylation of EZH2 [36,37].

**Antagonistic relationship between PRC2 and SWI/SNF**

Accumulating evidence has suggested that SWI/SNF (SWitch/Sucrose NonFermentable) chromatin-remodeling complex oppose epigenetic silencing by PcG proteins, and functions as a tumor suppressor in some cancers. This SWI/SNF complex is a multi-subunit chromatin remodeling complex that uses the energy of ATP hydrolysis to reposition nucleosomes, thereby regulating access to the DNA and modulating transcription and DNA replication/repair [119].

The activity of SWI/SNF complex can be counteracted by PcG proteins [120,121]. This antagonistic relationship between SWI/SNF components and PcG proteins were first uncovered via genetic studies in *Drosophila*. In 1988, mutations in core components of the SWI/SNF complex were found to suppress defects in body segment identity conferred by mutations in PcG proteins [122]. Latter, in 90’s year, it was discovered that the SWI/SNF complex promotes *Hox* gene activation during embryogenesis, while PcG proteins maintain their repression [123,124]. SWI/SNF is also capable of displacing PcG proteins from the INK4a/ARF locus [125].

Furthermore, there seems to be a balanced function between SWI/SNF and PcG. Accumulating evidence raises the possibility that the antagonistic relationship between these two complexes plays important roles in preventing tumor formation in mammals. Intriguingly, while PcG proteins are frequently overexpressed in cancers, specific inactivating mutations of SWI/SNF complex have been identified in several human cancers [126]. The most compelling case has been that of *SMARCB1* (SNF5), which was discovered to be homozygously inactivated in nearly all rhabdoid tumors (a rare pediatric malignancy) [127]. Interestingly, *SMARCB1*-heterozygous mice develop sarcomas that closely resemble human rhabdoid tumors [128]. Tumorigenesis can be completely suppressed by tissue-specific codeletion of *EZH2*, suggesting an antagonistic interaction between PRC2 and SWI/SNF [129].
EZH2 inhibitors

As described above, most findings have established that EZH2 functions as an important oncogenic biomarker for cancer initiation and progression, thus leading to the hypothesis that blocking EZH2 expression/activity and its downstream signaling cascade may represent a promising strategy for novel anticancer treatment. That’s why several groups have developed small-molecule inhibitors of EZH2 [130]. Over the past few years, several potent inhibitors of EZH2, with various selectivities, have been discovered and demonstrated promising preclinical results (figure 5, table 5).

DZNep as an indirect inhibitor

The first EZH2 inhibitor which was described is a cyclopentanyl analog of 3-deazaadenosine, called 3-Deazaneplanocin A (DZNep). It is a cyclopentanyl analog of 3-deazaadenosine that potently inhibits the activity of S-adenosylhomocysteine hydrolase (SAH), resulting in cellular accumulation of (SAH) which in turn represses the S-adenosyl-L-methionine-dependent histone lysine methyltransferase activities [143] (figure 5). Initially studied for its antiviral proprieties, recent findings indicate that DZNep is a chromatin-remodeling compound that induces degradation of cellular PRC2 proteins including EZH2 and concomitant removal of H3K27me3 mark [79,132]. Disruption of EZH2 by DZNep leads to the reactivation of the epigenetically silenced targets. This induces apoptosis, inhibits cell invasion and enhances chemotherapeutic sensitivity in tumoral cells, but not in normal and untransformed cells at tumor-inhibiting doses [79]. As DZNep has minimal toxicity in vivo [144], it may be a promising drug candidate for anti-cancer treatment. That’s why, it has been widely examined as a possible epigenetic therapeutic agent for the treatment of various cancers, including lung cancer [145], gastric cancer [146], myeloma [133], acute myeloid leukemia [132], lymphoma [147], but also chondrosarcoma [91]. DZNep-induced inhibition of EZH2 dramatically diminished the number and self-renewal capacity of cancer cells with tumor-initiating properties and significantly decreased tumor xenograft growth and improved survival [134,148]. DZNep selectively induced apoptosis in cancer cells but not in normal cells by preferential reactivation of genes repressed by PRC2 including the apoptosis effector FBOX32 [79]. EZH2
depletion induced not only cell cycle arrest and apoptosis, but also cell senescence. EZH2 decrease triggered simultaneous remarkable gains of two senescence-associated regulators p16 and p21. These data suggest that DZNep exerts its anticancer roles partially through inducing cell apoptosis and senescence and inhibiting cell proliferation [149]. Interestingly, DZNep also reduces tumoral cell migration and invasion, in part through upregulating E-cadherin [150].

These findings suggested DZNep may be a promising therapeutic agent for cancer treatment through multiple mechanisms. Besides its antitumoral role, DZNep has been reported to modulate allogeneic T cell responses and may represent a novel therapeutic approach for treatment of graft versus host disease [151]. DZNep also promotes erythroid differentiation of K562 cells, presumably through a mechanism that is not directly related to EZH2 inhibition [152], suggesting that this inhibitor may also be exploited for therapeutic applications for hematological diseases, including anemia.

**SAM-competitive inhibitor**

Because DZNep is not totally specific to EZH2, significant efforts have been made over the past few years to obtain compounds that are potent and highly selective for EZH2 (table 6) [99,138,140,141,153]. To identify inhibitors of EZH2 methyltransferase activity, high-throughput biochemical screening experiments have been performed. Although the structure of the EZH2 active site has not yet been determined, the conserved SET domain architecture predicts two essential binding pockets: one for the SAM methyl donor and another for the Lys27 substrate. Because more than 50 SET domain proteins have been identified in humans thus far, the selectivity of the inhibitors is crucial for minimizing off-target effects [154]. From the end of 2012, several SAM-competitive inhibitors were announced with promising preclinical results [153] (figure 5, table 6).

The compound EPZ005687 has a $K_i$ value of 24 nmol/L and is over 500-fold more selective for EZH2 versus 15 other PMTs and 50-fold more selective for EZH2 versus the closely related enzyme EZH1 [138]. Interestingly, EPZ005687 can also inhibit H3K27 methylation induced by the EZH2 mutants Y646 and A682, and it has been shown to selectively kill lymphoma cells that are heterozygous for one of these EZH2 mutations, with minimal effect on the proliferation of wild-type cells [138]. Another EZH2 inhibitor developed by Epizyme Inc. is EPZ-6438 (also called E7438). It shares similar in vitro properties (i.e. mechanism of action,
specificity, and cellular activity) as EPZ005687, but it demonstrates significantly improved pharmacokinetic properties, including good oral bioavailability in animals. Interestingly, oral dosing of EPZ-6438 leads to potent in vivo target inhibition and antitumor activity in a SMARCB1-deleted malignant rhabdoid tumor xenograft model (21). The ability of EPZ-6438 to reduce global H3K27Me3 levels was further demonstrated in several other human lymphoma cell lines, including lines expressing either wild-type or mutant EZH2. This compound is currently under study in a phase 1/2 trial as a single agent in subjects with advanced solid tumors or with B cell lymphomas. The primary goal of the phase 1 trial is to establish the safety and define the maximal tolerated dose of the drug.

EI1, another inhibitor of EZH2, was developed by Novartis [140] and shows very good selectivity with a low $K_i$ value (approximately 13 nmol/L). Loss of the H3K27 methylation function and activation of PRC2 target genes have been observed in EI1-treated cells. EI1 is equally active against both wild type and the Y646 mutant form of EZH2, and the inhibition of the EZH2 Y646 mutant in B-cell lymphomas decreases the H3K27 methylation level genome-wide and activates PRC2 target genes, leading to decreased proliferation, cell cycle arrest, and apoptosis [140].

Another EZH2 inhibitor is GSK126 (developed by GlaxoSmithKline), which has a $K_i$ of 0.5–3 nmol/L [99]. The selectivity of GSK126 for EZH2 is more than 1000-fold higher than its selectivity for 20 other human methyltransferases containing SET or non-SET domains, and it is over 150-fold more selective for EZH2 than for EZH1. McCabe et al. showed that the compound GSK126 decreased global methylation at H3K27 and reactivated silenced PRC2 target genes in EZH2-mutant diffuse large B-cell lymphoma (DLBCL) cell lines [99]. Furthermore, this compound effectively inhibited the proliferation of the EZH2-mutant DLBCL cells, and suppressed tumor growth in a mouse xenograft model.

UNC1999, an analogue of GSK126, is the first orally bioavailable inhibitor that has high \textit{in vitro} potency against wild type and mutant EZH2 over a broad range of epigenetic and non-epigenetic targets. As GSK126, UNC1999 potently reduced H3K27me3 levels in cells ($IC_{50}<50$ nmol/L) and selectively killed DLBCL cell lines harboring the Y646N mutation [141]. However, UNC1999 shows less selectivity for EZH1 than the inhibitors mentioned above.

\textbf{Stabilized $\alpha$-helix of EZH2 (SAH-EZH2)}
Most recently, Kim et al. developed a peptide called stabilized α-helix of EZH2 (SAH-EZH2), which inhibits EZH2 inhibition by a different mechanism from previous inhibitors [142]. SAH-EZH2 selectively disrupts the contact between EZH2 and EED, another subunit in the PRC2 complex, whereas the other EZH2 inhibitors target the HMT catalytic domain (figure 5). As in the case of GSK126, SAH-EZH2 decreases the H3K27 trimethylation level, resulting in growth arrest of PRC2-dependent MLL-AF9 leukemia cells (table 6).

**Future perspective**

Due to frequent activation of EZH2 in cancers, these new targeted therapies hold exciting promise in the clinic. Indeed, as discusses above, several reports have shown that genetic silencing and pharmacologic inhibition of EZH2 induced cell apoptosis, inhibited cell invasion and tumor angiogenesis, ultimately suppressed cancer growth and progression [155,156]. More importantly, given the advantages of specific chemical compounds including convenient to use and reversible nature of epigenetic modifications behind carcinogenesis, administration of small molecules targeting EZH2 seems to be a plausible and appealing way as a novel anti-cancer strategy [157]. However, the down-regulation of the EZH2 causes the hepatocytes to become more susceptible to lipid accumulation and inflammation. Significantly, from a translational point of view, because EZH2 inhibitors are potential and promising drugs useful in the treatment of various types of cancer, the patients who will be eventually treated with them should be monitored for the induction of non-alcoholic fatty liver disease (NAFLD) as a potential side effect [158].

**Executive summary**

- Histone modifications and histone code: Post-translational modifications of histone play a major role in transcriptional control and normal development, and are tightly regulated (histone code).
- Role of the lysine methyltransferase EZH2:
  * H3K27 methylation is a major epigenetic mark, related to gene silencing, and its control by HMTs (EZH2) and HDMs (JMJD3 and UTX) is a major regulator of cell physiology (reprogrammation, cell differentiation, immune system, cancers...).
  * EZH2 is overexpressed or mutated in numerous types of cancers.
- EZH2 inhibitors: EZH2 inhibitors are promising anticancer drugs

**Defined key-terms**

1) A current search of the PubMed database for the term ‘epigenetic’ returns more than 33 000 papers, with about half of them published during the past 4 years, marking an explosion of research efforts on this topic. Striking is the diversity of biological processes that are described in these articles, including fundamental aspects of development, cell fate or reprogramming in diverse organisms, as well as basic mechanisms of transcriptional control or DNA damage repair. Thus, epigenetic, through the modulation of genetic information, plays roles in fundamental life processes, such as cell proliferation, cell development, cell fate, or decision between cell survival and cell death.

2) **EZH2** (Enhancer of Zeste Drosophila Homolog 2) was initially cloned in 1996. This genes located on human chromosome 21 encodes a histone methyltransferase and constitutes the catalytic component of the polycomb repressive complex-2 (PRC2). EZH2 specifically methylates the histone H3 at lysine-27 (H3K27). It plays a major role in a plethora of biological processes, including development, cell fate or reprogramming, as well as regulation of immune system or cancers.

**Acknowledgements**

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**References**

1. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 403(6765), 41–45 (2000). ** This article is one of the first paper about the concept “histone code”.


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85. Croonquist PA, Van Ness B. The polycomb group protein enhancer of zeste homolog 2 (EZH2) is an oncogene that influences myeloma cell growth and the mutant ras phenotype. Oncogene. 24(41), 6269–6280 (2005).


25


The fundamental DNA packing unit is known as a nucleosome. Each nucleosome is about 11nm in diameter. The DNA double helix wraps around a central core of eight histone protein molecules (an octamer containing 2 H2A, 2 H2B, 2 H3 and 2 H4) to form a single nucleosome. The N-terminal “tail” of these histones can undergo post-translational modifications (acetylation, methylation or phosphorylation).
Figure 2: Schematic representation of major histone modifications

Histone modifications mainly occur on the N-terminal tails of histones but also on the C-terminal tails and globular domains. The major modifications shown include acetylation (A), methylation (M), phosphorylation (P) and ubiquitination (U).
Figure 3: Methyl group transfer reaction on lysine

The lysine amino group of the substrate histone polypeptide engages in a SN2 reaction with the activated co-factor S-adenosyl-L-methionine (SAM), resulting in the formation of an N-methylated lysine and S-adenosyl-L-homocysteine (SAH).
Figure 4: Schematic representation of PRC2 complex.

(A) Domain organizations of each subunit in the human PRC2 complex.
Domain “1”, binding region for PHF1 in human cells; domain “2”, binding region for SUZ12; CXC, cysteine-rich domain; SANT, domain that allows chromatin remodeling protein to interact with histones; SET, catalytic domain of EZH2; VEFS, VRN2-EMF2-FIS2-SUZ12 domain; WD, WD-40 domain; WDB, WD-40 binding domain; Zn, Zn-finger region.

(B) The subunits of human PRC2 complexes, their interactions, and schematic function of PRC2 are shown.
Figure 5: Modes of inhibition of PRC2.

Three types of inhibitors are indicated: DZNep as an SAH hydrolase inhibitor, SAM competitive inhibitors, and SAH-EZH2 peptides as disrupters of the contact between EZH2 and EED.
### Table 1: The histone code.

<table>
<thead>
<tr>
<th>Methylation</th>
<th>Mono-methylation</th>
<th>di-methylation</th>
<th>tri-methylation</th>
<th>Acetylation</th>
<th>Ubiquitination</th>
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<td>repression</td>
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</table>

1 For each post-translational modification, the known functional association on gene transcription is shown. By reading the combinatorial and/or sequential histone modifications that constitute the histone code, it may be possible to predict which gene products will be transcribed. However, this code is controversial, since some gene loci present marks both associated with transcriptional activation and linked with repression. These bivalent domains are posited to be poised for either up- or downregulation and to provide an epigenetic blueprint for lineage determination, and are usually found in stem cells.
Table 2: Histone target substrates and domain structure of histone lysine methyltransferases. ²

<table>
<thead>
<tr>
<th>Synonyms</th>
<th>Protein structure</th>
<th>Histone substrates</th>
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<tr>
<td><strong>With SET domain</strong></td>
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</table>

² For each protein, the official name as well as the most commonly used synonyms, the histone target substrates and domain structure are provided.

SET: Suppressor of variegation, Enhancer of Zeste, Trithorax domain; pre/post-SET: cysteinerich motifs found adjacent to a subset of SET domains; PHD: plant homeodomain zinc finger; ANK: ankyrin repeats; AT hook: A/T DNA binding motif; C2H2 Znf: C2H2-type zinc finger; HMG: high mobility group; SANT: SWI3, ADA2, N-CoR and TFIIB DNA-binding domain; CxxC: CxxC zinc finger; MBD: methyl CpG binding domain.
<table>
<thead>
<tr>
<th>Domain</th>
<th>Histone Modification</th>
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<tr>
<td>SETD7, SET7/9</td>
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<td>SUV4-20H1, KMT5B</td>
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<td>SUV4-20H2, KMT5C</td>
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<tr>
<td>SMYD5</td>
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<td>SETD6</td>
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<td>PRDM6, MEL1, PFM3</td>
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<td>Without SET domain</td>
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**Legend:**
- SET
- bromo
- TUDOR
- C2H2 Znf
- CxxC
- pre-SET
- chromo
- ANK
- HMG
- MBD
- pre-SET
- PHD
- AT hook
- SANT
- DOT
Table 3: Histone target substrates and domain structure of histone lysine demethyltransferases. 3

<table>
<thead>
<tr>
<th>Synonyms</th>
<th>Protein structure</th>
<th>Histone substrates</th>
<th>Other substrates</th>
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</thead>
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<td>LSD1, KDM1A, AOF2, BHC110</td>
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<td>H3K4me1, H3K4me2</td>
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<tr>
<td><strong>JMJC demethylases</strong></td>
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<td>JMJD5, KDM8</td>
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<td>H3K36me2</td>
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<td>H3K9me1, H3K9me2</td>
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</tbody>
</table>

3 For each protein, the official name as well as the most commonly used synonyms, the histone target specificities and domain structure are provided. Structural domains are annotated.

ARID: AT-rich interacting domain; amine oxidase: amine oxidase domain; C5HC2: C5HC2 zinc-finger domain; CXXC: CXXC zinc-finger domain; DNMT1: DNA methyltransferase 1; FBOX: F-box domain; FBXL: F-box and Leu-rich repeat protein; HIF1AN: hypoxia-inducible factor 1A inhibitor; HR: hairless domain; HSPBAP1: heat shock protein-associated protein 1; JARID: Jumonji domain-ARID-containing protein; JMJC: Jumonji C domain; LRR: Leu-rich repeat domain; LSD: Lys-specific demethylase; MINA: MYC induced nuclear antigen; NFkB: nuclear factor kB; NO66: nucleolar protein 66; PHD: plant homeodomain; SWIRM: Swi3p Rsc8p and Moira domain; TPR: tetratricopeptide domain; TUDOR: Tudor domain; UTX: ubiquitously transcribed X chromosome tetratricopeptide repeat protein; UTY: ubiquitously transcribed Y chromosome tetratricopeptide repeat protein.
| Gene/Group                        | H4K20me1 | H3K9me2 | ARID5B | H3K9me1, H3K9me2 | H3K27me2, H3K27me3 | H3K27me2, H3K27me3 | H3K9me2, H3K9me3 | H3K36me2, H3K36me3 | H1.4K26me2, H1.4K26me3 | H3K9me2, H3K9me3 | H3K36me2, H3K36me3 | H1.4K26me2, H1.4K26me3 | H3K9me2, H3K9me3 | H3K36me2, H3K36me3 | H1.4K26me2, H1.4K26me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 | H3K4me2, H3K4me3 |
|----------------------------------|----------|---------|--------|------------------|-------------------|-------------------|------------------|-------------------|-------------------|------------------|-------------------|-------------------|------------------|-------------------|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| PHF2, JHDM1E                     |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| HR                               |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| KDM3B                            |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD1A, JHDM2A, TSGA, KDM3A      |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD1C                           |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD3, KDM6B                     |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| UTX, KDM6A                       |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| UTY                              |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD2A, JHDM3A, KDM4A            |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD2C, JHDM3C, GASC1, KDM4C     |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD2B, JHDM3B, KDM4B            |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JMJD2D, JHDM3D, KDM4D            |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JARID1B, PLU1, KDM5B             |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JARID1C, SMCX, KDM5C             |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JARID1D, SMCY, KDM5D             |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JARID1A, RBP2, KDM5A             |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| JARID2                            |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| MINA                             |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
| NO66                             |          |         |        |                  |                   |                   |                  |                   |                   |                  |                   |                   |                   |                   |                   |                  |                   |                  |                   |                   |                  |                   |                   |                   |
Table 4: Association between EZH2 mutations and disease.⁴

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<th>Mutation</th>
<th>Phenotype</th>
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<tbody>
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<td>CS47fs</td>
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<td></td>
<td>CS71Y</td>
<td>Myelofibrosis</td>
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<td>CS76W</td>
<td>Myelodysplastic syndrome, myeloproliferative neoplasms</td>
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<td></td>
<td>P577L</td>
<td>Early T-cell precursor acute lymphoblastic leukaemia</td>
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<td></td>
<td>R583X</td>
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<td>V626M</td>
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<tr>
<td></td>
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<td>V662fs</td>
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<td>Werner syndrome, neuroblastoma</td>
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<tr>
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<td>A682V</td>
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<td>R684C</td>
<td>Werner syndrome, Myelofibrosis</td>
</tr>
<tr>
<td></td>
<td>R684H</td>
<td>Early T-cell precursor acute lymphoblastic leukaemia</td>
</tr>
<tr>
<td></td>
<td>K685fs</td>
<td>Chronic myelomonocytic leukemia</td>
</tr>
<tr>
<td></td>
<td>R690H</td>
<td>Refractory Cytopenia with Multilineage Dysplasia, Chronic myelomonocytic leukemia</td>
</tr>
<tr>
<td></td>
<td>R690C</td>
<td>Myelodysplastic syndrome</td>
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</table>

⁴ EZH2 mutations identified in association with disease are annotated below with the disease-associated with each mutation, the nature of the mutation, and the structural domain involved. The sequence is numbered in accordance with EZH2 isoform A and the numbering for some mutations has been transposed from the original references so that all mutations can be referred to relative to the same sequence. (Abbreviations: fs, frameshift; X, nonsense).
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Condition</th>
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<tbody>
<tr>
<td>A692V</td>
<td>Diffuse large B-cell lymphoma</td>
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<tr>
<td>N693T</td>
<td>Acute Myelomonocytic Leukemia</td>
<td></td>
</tr>
<tr>
<td>N693Y</td>
<td>Early T-cell precursor acute lymphoblastic leukaemia, Myelofibrosis</td>
<td></td>
</tr>
<tr>
<td>H694Y</td>
<td>Werner syndrome</td>
<td></td>
</tr>
<tr>
<td>H694R</td>
<td>Chronic myelomonocytic leukemia</td>
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<tr>
<td>S695L</td>
<td>Werner syndrome, Early T-cell precursor acute lymphoblastic leukaemia</td>
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<tr>
<td>I727fs</td>
<td>Myelodysplastic/myeloproliferative neoplasm, unclassifiable</td>
<td></td>
</tr>
<tr>
<td>F728fs</td>
<td>Early T-cell precursor acute lymphoblastic leukaemia</td>
<td></td>
</tr>
<tr>
<td>Y731X</td>
<td>Chronic myelomonocytic leukemia</td>
<td></td>
</tr>
<tr>
<td>Y733fs</td>
<td>Myelodysplastic syndrome</td>
<td></td>
</tr>
<tr>
<td>Y733X</td>
<td>Werner syndrome</td>
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</tr>
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<td>Y741C</td>
<td>Werner syndrome</td>
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</tr>
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<td>V742ins</td>
<td>Acute myeloid leukemia</td>
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<td>V742D</td>
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<tr>
<td>I744fs</td>
<td>Acute myeloid leukemia</td>
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<tr>
<td>E745K</td>
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<td>E745fs</td>
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Table 5: Chemical structures and biochemical data for small-molecule inhibitors of EZH2

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound</th>
<th>Mechanism and potency</th>
<th>Selectivity toward EZH2</th>
<th>Highest clinical status</th>
<th>Références</th>
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</thead>
<tbody>
<tr>
<td><img src="image" alt="DZNep" /></td>
<td>DZNep</td>
<td>SAH hydrolase inhibitor</td>
<td>non selective</td>
<td>preclinical</td>
<td>[79,131–134]</td>
</tr>
<tr>
<td><img src="image" alt="GSK126" /></td>
<td>GSK126</td>
<td>SAM-competitive inhibitor of PRC2, $K_i = 0.5-3$ nM</td>
<td>&gt;1000-fold over 20 other HMTs; over EZH1</td>
<td>preclinical</td>
<td>[99,135]</td>
</tr>
<tr>
<td><img src="image" alt="GSK343" /></td>
<td>GSK343</td>
<td>SAM-competitive inhibitor of PRC2, $K_i = 0.5-3$ nM</td>
<td>IC50 = 4nM and is over 1000-fold selective for other HMTs except EZH1 (60-fold selectivity)</td>
<td>preclinical</td>
<td>[136,137]</td>
</tr>
<tr>
<td><img src="image" alt="EPZ005687" /></td>
<td>EPZ005687</td>
<td>SAM-competitive inhibitor of PRC2, $K_i = 24$ nM</td>
<td>&gt;500-fold over 15 other HMTs; about 50-fold over EZH1</td>
<td>preclinical</td>
<td>[138]</td>
</tr>
<tr>
<td><img src="image" alt="EPZ-6438" /></td>
<td>EPZ-6438</td>
<td>SAM-competitive inhibitor of PRC2, $K_i = 0.5-3$ nM IC50 = 11 nM</td>
<td>35-fold selectivity versus EZH1; &gt;4,500-fold selectivity relative to 14 other HMTs</td>
<td>phase I/II</td>
<td>[139]</td>
</tr>
</tbody>
</table>
| Peptide: FSSNRKILXRTQILNQEWKQRRIPQV | E11 | SAM-competitive inhibitor of PRC2
IC50=15 nM; Ki=13 nM | preclinical | [140] |
| Peptide: stabilized a-helix of EZH2 peptide (SAH-EZH2) | UNC1999 | SAM-competitive inhibitor of PRC2
IC50=2-15 nM; Ki=13 nM | over 1000-fold selective for other HMTs except EZH1 (22-fold selectivity). | preclinical | [141] |
| Hydrocarbon-stapled peptide that mimics the a-helical EED-dinding domain of EZH2, disrupting the EZH2–EED complex | not selective for EZH1 | preclinical | [142] |
## Supplementary material

1. Table 1: List of histone lysine methyltransferases and demethylases linked to disease.  

<table>
<thead>
<tr>
<th>Gene</th>
<th>histone substrate</th>
<th>Disease</th>
<th>Genetic/epigenetic aberration</th>
<th>Aberrant expression</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>MLL</td>
<td>H3K4</td>
<td>Leukemia (AML, ALL, MLL)</td>
<td>&gt; 50 different MLL fusions</td>
<td></td>
<td>[1–7]</td>
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<tr>
<td></td>
<td></td>
<td>Acute myeloid leukemia (AML)</td>
<td>MLL-PTD</td>
<td></td>
<td>[8–10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wiedemann-Steiner syndrome</td>
<td>intragenic mutations</td>
<td></td>
<td>[11–13]</td>
</tr>
<tr>
<td>MLL2</td>
<td>H3K4</td>
<td>Hepatocellular carcinoma (HCC)</td>
<td>Hepatitis B virus integration into MLL2 : HBx-MLL2 fusion</td>
<td></td>
<td>[14]</td>
</tr>
<tr>
<td></td>
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<td>Acute myeloid leukemia (AML) (mice)</td>
<td>NUP98-JARID1A</td>
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<td>[15]</td>
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<tr>
<td></td>
<td></td>
<td>Kabuki syndrome 1 (KABUK1)</td>
<td>intragenic mutations</td>
<td></td>
<td>[16,17]</td>
</tr>
<tr>
<td>MLL3</td>
<td>H3K4</td>
<td>Leukemia</td>
<td></td>
<td></td>
<td>[18,19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colorectal cancer</td>
<td>Intragenic mutations</td>
<td></td>
<td>[19–22]</td>
</tr>
<tr>
<td>DOT1L</td>
<td>H3K79</td>
<td>Leukemia (AML, ALL)</td>
<td>MLL-AF10 fusion, MLL-AF4 fusion</td>
<td></td>
<td>[23,24]</td>
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<tr>
<td></td>
<td></td>
<td>T cell acute lymphoblastic leukemia (T-ALL)</td>
<td>CALM-AF10 fusion, SET-NUP214 fusion</td>
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<td>[25,26]</td>
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<td></td>
<td></td>
<td>Osteoarthritis</td>
<td>intragenic mutations</td>
<td></td>
<td>[27,28]</td>
</tr>
<tr>
<td>EZH2</td>
<td>H3K27</td>
<td>Bladder carcinoma</td>
<td>overexpression</td>
<td></td>
<td>[29–36]</td>
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<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
<td>overexpression</td>
<td></td>
<td>[29,30,37,38]</td>
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<td>Colorectal cancer</td>
<td>overexpression</td>
<td></td>
<td>[29,39–45]</td>
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<td>overexpression</td>
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<td>[46–53]</td>
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<td>overexpression</td>
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<td>[54–59]</td>
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<td>Lymphoma</td>
<td>Intragenic mutations</td>
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<td>Myeloproliferative neoplasms</td>
<td>Intragenic mutations</td>
<td>downregulation</td>
<td>[64]</td>
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<tr>
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<td>Rhabdoid tumors</td>
<td>Intragenic mutations; Mutations of opposing chromatin modifying complex SWI-SNF</td>
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<td>[83–91]</td>
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<td>Melanoma</td>
<td>overexpression</td>
<td></td>
<td>[38,96–99]</td>
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<td>overexpression</td>
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<td>[100–121]</td>
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<td>chondrosarcoma</td>
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<td>[122]</td>
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<td></td>
<td>lung cancer</td>
<td>downregulation</td>
<td></td>
<td>[123]</td>
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<td></td>
<td></td>
<td>Various other cancers</td>
<td>overexpression</td>
<td></td>
<td>[29,38]</td>
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<tr>
<td></td>
<td></td>
<td>Weaver syndrome</td>
<td>Intragenic mutations</td>
<td></td>
<td>[124–126]</td>
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</table>

1 However, in many cases the molecular mechanisms of disease development are not well understood yet, and it remains to be shown whether misregulation of these HMTs contributes to disease initiation or progression.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Data</th>
<th>Function/Condition</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td><strong>NSD1</strong></td>
<td>H3K36</td>
<td>Acute myeloid leukemia (AML) t(5;11)(q32;p15,5); tranlocation: NUP98-NSD1 fusion</td>
<td>[127–138]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myelodysplastic syndrome t(5,11)(q35;p15,5)</td>
<td>[131,139]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beckwith-Wiedemann syndrome intragenic mutations</td>
<td>[140]</td>
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<td></td>
<td></td>
<td>Sotos syndrome intragenic mutations; 5q35 microdeletions</td>
<td>[140–173]</td>
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<tr>
<td><strong>NSD2</strong></td>
<td>H3K36</td>
<td>glioblastoma multiform (GBM) overexpression</td>
<td>[174]</td>
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<tr>
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<td></td>
<td>hepatocellular carcinoma (HCC) overexpression</td>
<td>[175]</td>
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<tr>
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<td></td>
<td>leukemia overexpression</td>
<td>[176]</td>
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<td>multiple myeloma (MM) t(4;14)(p16;q32): altered expression of FGFR3, NSD2</td>
<td>[177,178]</td>
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<td></td>
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<td>Various other cancers Wolf-Hirschhorn syndrome</td>
<td>[179]</td>
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<tr>
<td><strong>NSD3</strong></td>
<td></td>
<td>acute myeloid leukemia (AML) t(8;11)(p11,2;p15); translocation: NUP98-NSD3 fusion</td>
<td>[181]</td>
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<td><strong>EHMT1</strong></td>
<td>H3K9</td>
<td>9q subtelomeric deletion syndrome / Kleefstra syndrome haploinsufficiency of EHMT1; microdeletion of 9q34.3: intragenic mutation downregulation</td>
<td>[175,182–185]</td>
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<td></td>
<td>breast cancer intragenic mutations</td>
<td>[186]</td>
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<td>medulloblastoma downregulation</td>
<td>[187]</td>
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<td><strong>SETDB1</strong></td>
<td>H3K9</td>
<td>Huntington’s disease (HD) overexpression</td>
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<td><strong>SETDB2</strong></td>
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<td>asthma mutation</td>
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<td>H3K4</td>
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<td>[193,194]</td>
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<td>[195]</td>
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<td>hepatocellular carcinoma (HCC) overexpression</td>
<td>[196]</td>
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<td><strong>SMYD4</strong></td>
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<td>medulloblastoma downregulation</td>
<td>[187]</td>
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<td><strong>PRDM1</strong></td>
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<td>lymphoma mutations in PRDM1 gene, epigenetic silencing</td>
<td>[198–207]</td>
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<td>lupus erythematosus overexpression</td>
<td>[208–210]</td>
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<td>[211–213]</td>
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<td><strong>PRDM2</strong></td>
<td>H3K9</td>
<td>breast cancer mutations in PRDM2 gene; promoter DNA methylation</td>
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<td>Event Type</td>
<td>Mutation/Expression</td>
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<td>gastric cancer</td>
<td>mutations in PRDM2 genes, promoter DNA methylation</td>
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5 **References**

1. Rapin N, Porse BT. Oncogenic fusion proteins expressed in immature hematopoietic cells fail to recapitulate the transcriptional changes observed in human AML. *Oncogenesis*. 3, e106 (2014).


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