HHS Public Access

Author manuscript

Nat Med. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Nat Med. 2016 February ; 22(2): 128–134. doi:10.1038/nm.4036.

Targeting EZH2 in cancer

Kimberly H. Kim[1,2,3,4] and **Charles W. M. Roberts**[1,2,3,4,*,5]

[1]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[2]Division of Hematology/Oncology, Boston Children's Hospital, MA, USA

[3]Department of Pediatrics, Harvard Medical School, Boston MA, USA

[4]Broad Institute of Harvard and Massachusetts Institute of Technology, Boston, MA, USA

Dynamic regulation of covalent histone modifications at enhancers and promoters has a key role in the modulation of gene expression and consequently fate specification [1–6]. Recent findings emerging from human cancer genome sequencing efforts have revealed that many genes that encode chromatin regulators that modify histones are frequently mutated across a wide variety of cancers[7–11]. Converging lines of investigation have particularly highlighted links between enhancer of zeste homologue 2 (EZH2) and cancer.

EZH2 is the enzymatic subunit of Polycomb repressive complex 2 (PRC2), a complex that methylates lysine 27 of histone H3 to promote transcriptional silencing [12,13]. Distinct cancer-associated perturbations of this regulatory axis have emerged and include both gain-of-function and loss-of-function mutations in *EZH2*, overexpression of *EZH2*, mutations in the H3K27 demethylase *UTX*, and frequent mutations in the SWI/SNF chromatin remodeling complex that partially antagonizes Polycomb function. Recurrent missense mutations of the H3K27 site itself have also been identified. Interest in elucidating the roles of EZH2 in cancer has been further enhanced by the development of small molecules that effectively inhibit the enzymatic activity of EZH2 and the translation of these molecules into early phase trials with preliminary evidence of clinical responses.

Here we begin by reviewing the spectrum of EZH2 and H3K27 perturbations found in cancer and synthesize a perspective that unites the function of EZH2 as a master regulator of transcription. We then review the development, translation, and early clinical findings from therapeutic targeting of EZH2.

Mutations Resulting in Gain of Function of EZH2 in Cancer

EZH2 is the enzymatically active core subunit of the PRC2 complex, which also includes EED, SUZ12, and RbAp46/48. PRC2 methylates of the lysine residue at position 27 of

*Correspondence: Charles.Roberts@stjude.org.

[5]Current affiliation: Comprehensive Cancer Center and Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, USA

Conflict of Interest Statement: As a recipient of a Dana-Farber Cancer Institute-Novartis Institutes for Biomedical Research Drug Discovery Program research grant, C.W.M.R. received research funding and consulting fees from the Novartis Institutes for Biomedical Research.

histone 3 (H3K27) [14,15], which facilitates chromatin compaction and gene silencing (Figure 1). Several lines of evidence have implicated EZH2 in the development and progression of a variety of cancers. An early indication came from the observation that EZH2 overexpression is associated with worse progression of prostate cancer [16]. Similar findings have emerged in other human cancers including breast cancer, bladder cancer, endometrial cancer, and melanoma as high levels of EZH2 were shown to correlate with aggressiveness and advanced disease in each of these cancer types [16–19] (Table 1). EZH2 has been shown to be essential for proliferation of cancer cell lines and independently ectopic EZH2 expression to confer a proliferative advantage upon non-cancerous cells [17]. Forced expression of EZH2 leads to the development of myeloproliferative disorder in mice [20]. In an immortalized human epithelial cell line, expression of causes neoplastic transformation of breast epithelial cells, a phenotype that is dependent upon its methyltransferase domain [21].

Additional findings to support an oncogenic role for EZH2 have more recently emerged. Studies have shown that recurrent heterozygous point mutations at tyrosine 641 (Y641) within the C-terminal catalytic SET domain of EZH2 occur in 22% of germinal center B-cell (GCB) diffuse large cell B-cell lymphomas (DLBCL) and in 7% to 12% of follicular lymphomas (FL) [22,23] (Table 1). The Y641 mutation was initially thought to be a loss-of-function mutation. However, via *in vitro* biochemical enzymatic assays this mutation was subsequently shown to confer gain-of-function of enzyme activity resulting in augmented conversion of H3K27 to the trimethylated form [24]. Y641 mutants (Y641F, Y641N, Y641S, Y641C, and Y641H) have reduced methylation activity of unmethylated H3K27 but enhanced activity for the dimethylated version of H3k27. The mutant thus cooperates together with wild-type EZH2 to shift the steady state of H3K27 to favor trimethylation and thus represses expression of Polycomb targets [25]. EZH2 point mutations at the A677 and A687 residues have also been identified in non-Hodgkin lymphomas (NHL), where they similarly result in hypertrimethylation of H3K27 [26,27].

Additional support for a gain-of-function role for mutant EZH2 in cancer comes from the identification of cancer-associated loss-of-function mutations in other chromatin regulators that normally antagonize EZH2 activity. UTX (ubiquitously transcribed tetratricopeptide repeat gene on X chromosome) is a histone demethylase that functions in part by antagonizing EZH2 activity by removing methyl groups from di- and trimethylated H3K27. Inactivating mutations affecting *UTX* occur in several types of human cancer, including multiple myeloma, medulloblastoma, esophageal cancer, bladder cancer, pancreatic cancer, and renal cancers [8,28–30]. These mutations include homozygous (in females) or hemizygous (in males) large deletions, nonsense mutations, small frame-shifting insertion/deletions, and consensus splice site mutations that lead to a premature termination codon [31]. Almost all mutations are predicted to result in loss of the JmjC domain of UTX, which is essential for its demethylase activity, and have been shown to cause increased levels of H3K27 trimethylation [31,32]. Consequently, the loss-of-function mutations in UTX may be analogous to those caused by gain-of-function mutations in EZH2.

Genetic studies in many different organisms have also revealed an evolutionarily conserved antagonistic relationship between Polycomb proteins and SWI/SNF complexes, which utilizes the energy of ATP hydrolysis to remodel chromatin [33–36]. SWI/SNF complexes are

comprised of 12 to 15 subunits which have collectively been found to be mutated in 20% of all human cancers [37,38]. Unopposed EZH2 activity is also a driver of cancers driven by loss of the SWI/SNF core subunit SNF5/SMARCB1 as originally shown in malignant rhabdoid tumor, a highly aggressive type of pediatric cancer [39,40]. Recent studies have extended this antagonistic relationship to cancers linked to inactivation of other SWI/SNF subunits as EZH2 inhibition is synthetic lethal in ovarian cancer xenografts mutant for the SWI/SNF subunit *ARID1A* and sensitizes lung cancer xenografts mutant for the SWI/SNF ATPase core subunit *BRG1*/*SMARCA4*-to chemotherapy in mice [41,42]. Most recently, a broad role for EZH2 in progression of cancers that have mutations in the SWI/SNF tumor suppressor subunits *ARID1A*, *PBRM1*, and *SMARCA4* has been demonstrated in both cell lines and *in vivo* models [43]. Notably, a non-catalytic role for EZH2 was identified in this context, indicating that dependency upon EZH2 for cancer progression can be derived from both catalytic and non-catalytic functions of EZH2.

Mechanism of EZH2 Oncogenic Activity

The extensive evidence linking EZH2 activity to cancer has prompted interest in the underlying mechanism. EZH2/PRC2 is known to be recruited to specific loci during development to silence genes associated with alternative fates [44–46]. Analogous to its role in normal stem cells where EZH2 suppresses differentiation by repressing lineage-specifying factors [45–47], it is expressed at higher levels in cancer stem cell (CSC) populations isolated from human breast cancer xenografts and primary breast tumor cells compared to noncancer cell lines and is essential for the maintenance of these populations [48]. In at least some CSC models, EZH2 suppresses expression of genes associated with lineage specification leading to the hypothesis that EZH2 facilitates transformation by blocking differentiation [47,49]. However, it is important to note that EZH2 has also been shown to be essential to facilitate some differentiation programs of several tissue types [50,51]. Ultimately, the central function of EZH2/PRC2 during development may not be to either promote stemness or differentiation per se, but rather to suppress transcriptional programs that underlie alternate fates. Thus, the consequences of perturbing EZH2 on fate control are likely to be highly cell-type specific.

Given its role as a transcriptional regulator, substantial efforts have been dedicated to the identification of downstream targets or pathways that contribute to transformation driven by EZH2. EZH2 has been shown to be downstream of the cell cycle regulatory retinoblastoma-E2F pathway and was in turn required for the expression of proliferative genes and for E2F-driven proliferation [17]. The Ink4a/Arf tumor suppressor locus is thought to be another key target that can be silenced by Polycomb activity, and EZH2-mediated silencing of *E-cadherin* and *FOXC1* and DNA damage repair pathways have also been shown to contribute to oncogenesis [48,52–54]. An unresolved question that carries potential therapeutic relevance is whether any EZH2 targets are universally essential for EZH2-mediated transformation independent of cell lineage or whether EZH2-induced oncogenic transcriptional changes are context-specific depending upon cancer type and cell-of-origin.

Several studies have also identified a PRC2-independent function of EZH2 in transcriptional activation rather than repression [55–58] (Figure 1). In a model of castration-resistant prostate cancer, the oncogenic function of EZH2 was shown to be independent of its role as a

Polycomb transcriptional repressor, but instead due to a PRC2-independent coactivator role of EZH2 for transcription factors including androgen receptor [59]. An activating role of EZH2 was also demonstrated in breast cancer cells via activation of NF-κB targets and *NOTCH1* [57,58]. EZH2 has also been implicated in transcriptional activation of gene expression in breast cancer, where it has been shown to induce the expression of genes that are regulated by estrogen receptor (ER) and Wnt signaling transcription factors by physically bridging between the ER and components of Wnt signaling (β–catenin) [55]. In addition to its known roles in histone modification and transcriptional regulation, it has been shown that EZH2 can methylate non-histone substrates. EZH2 binds and methylates STAT3, which promotes tumorigenicity of CSC in glioblastoma and in a prostate cancer model, EZH2 has been shown methylate the androgen receptor (AR), modulating AR recruitment to the sites bound by both AR and EZH2 [59,60]. EZH2 has also been implicated in the methylation of non-histone substrates, this mechanism may facilitate recognition by the ubiquitination machinery driving degradation of the methylated proteins [61,62]. Collectively, these results suggest that in addition to its known role as an H3K27 histone methyltransferase and transcriptional suppressor, EZH2 may also have PRC2-independent roles as a transcriptional coactivator and directly modulate the activity of transcription factors and other proteins. However, the contribution of these noncanonical functions to the overall cellular role of EZH2 and their link to any role of EZH2 in oncogenic transformation remain unclear.

EZH2 as a therapeutic target

Given the evidence for EZH2 enzymatic gain-of-function being a cancer driver, development of EZH2-specific inhibitors has been an active area of investigation and multiple biotech and pharmaceutical companies have been developing such compounds. Promising preclinical results have been obtained and human phase I trials are now underway, with early results suggesting potential clinical activity (Table 2).

Enzymatic inhibition of EZH2

The first EZH2 inhibitor that was widely used for experimental work was 3-deazaneplanocin A (DZNep). It is a cyclopentanyl analog of 3-deazaadenosine that potently interferes with *S*-adenosyl-L-homocysteine hydrolase (SAH), a component of the methionine cycle, which causes cellular SAH levels to increase, repressing the activity of *S*-adenosyl-L-methionine-dependent histone lysine methyltransferase activity [63]. Therefore, the effect of DZNep on inhibition of histone methylation is not specific to EZH2. Treatment with DZnep induces significant antitumor activity in various cancer types corresponding with inhibition of PRC2, and removal of H3K27me3 marks [64]. In spite of potentially promising results *in vitro* and *in vivo,* DZNep has a very short plasma half-life, confers non-specific inhibition of histone methylation, and is toxic in animal models [65].

In order to improve anti-tumor activity and reduce toxicity, significant efforts have been directed toward developing compounds that are potent and selective inhibitors of EZH2. High-throughput biochemical screens have yielded several potent inhibitors based on the conserved SET domain architecture, which enabled prediction of two essential binding

pockets for the S-adenosyl-L-methionine (SAM) methyl donor and the H3K27 substrate. In 2012, several groups announced independent development of SAM-competitive inhibiting compounds derived from high throughput screens. EPZ005687 binds to wild-type and Y641-mutant EZH2 and displays greater than 500-fold selectivity for EZH2 compared with 15 other human protein methyltransferases and 50-fold selectivity over EZH1 [66]. EPZ005687 shows dose-dependent inhibition of H3K27me3 in EZH2–wild-type and, Y641- and A677-mutant lymphoma cells as well as in cell lines of other cancer types, including breast and prostate cancer. The simultaneously developed small-molecule EZH2 inhibitor, GSK126, inhibits both wild-type and mutant EZH2 and has greater than 1,000-fold selectivity for EZH2 compared with other methyltransferases and 150-fold compared to EZH1 [67,68]. GSK126 markedly inhibits the growth of lymphomas carrying activating *EZH2* mutations *in vivo* [68]. A third independent SAM-competitive inhibitor, EI1, inhibits both wild-type and mutant EZH2 with an IC_{50} 15 nM, shows >10,000 fold selectivity for EZH2 over other methyltransferases and 90-fold selectivity over EZH1 [69]. EI1 inhibits H3K27me2/3 levels without affecting EZH2 protein levels in *EZH2*-mutant DLBCL cells and a *SMARCB1*-mutant rhabdoid tumor cell line and inhibits cell growth and causes cell cycle arrest and apoptosis in cells carrying *EZH2* mutations. These effects were accompanied by downregulation of a proliferation gene expression signature and increased expression of PRC2 targets. In pursuit of compounds more suitable for long-term animal studies by not requiring frequent injection, UNC1999 was synthesized as the first orally bioavailable inhibitor that was highly selective for wild-type EZH2 and the EZH2 Y641 mutant. UNC1999 is also relatively active against EZH1 with only 10-fold less potency than for EZH2, therefore offering the potential to target both EZH2 and EZH1 [70]. EPZ-6438 was subsequently developed and is more potent compared to EPZ005687 with improved pharmacokinetic properties including good oral bioavailability [71]. Treatment of mice carrying *SMARCB1*-mutant malignant rhabdoid tumors with EPZ-6438 resulted in decreased levels of H3K27me3 and dose-dependent tumor regression [72]. In June 2013, a phase I/II clinical trial of EPZ-6438 in patients with advanced solid tumors or with B-cell lymphomas was launched (NCT01897571). Preliminary reports from the study have been presented at scientific meetings (http://www.epizyme.com/wp-content/uploads/2014/11/Ribrag-ENA-FINAL.pdf and http://www.epizyme.com/wp-content/uploads/2015/07/ICML-Slides-Presented-062015-v2.pdf). The data show potentially encouraging activity of the drug: partial or complete responses in nine of fifteen NHL patients, including a partial response in the one patient who had an EZH2 mutation (EZH2 Y646H), and one complete response and partial responses in malignant rhabdoid tumor patients were observed. Two other clinical trials (NCT02395601 and NCT02082977) are actively enrolling patients and further investigation in *EZH2*-mutant B-cell NHL and *SMARCB1*-deficient tumors is currently being pursued.

Inhibitors that Disrupt PRC2 Stability

The discovery of non-enzymatic functions for EZH2 and the implication of these in SWI/SNF-mutant cancers raises the possibility that the enzymatic inhibitors currently in clinical trials may not fully suppress the transformation promoting activity of EZH2. EZH2 can also be inhibited by disrupting its interaction with other PRC2 subunits with a peptide known as

stabilized alpha-helix of EZH2 (SAH-EZH2) that is derived from the domain of EZH2 that interacts with EED [73]. SAH-EZH2 disrupts the EZH2–EED complex, reduces EZH2 protein levels, and selectively inhibits H3K27 trimethylation in a dose-dependent manner. This peptide is efficacious against EZH2-dependent MLL–AF9 leukemia and *EZH2*-mutant lymphoma cells but has no effect on non-transformed and *EZH2*–wild-type controls. Notably, whereas the anti-proliferative effect of the SAH-EZH2 correlated with the reduction in H3K27me3 levels, the effect seemed to correlate even more strongly with reduction of EZH2 protein levels, consistent with the findings of dependence upon non-enzymatic roles of EZH2 in SWI/SNF-mutant cancers [43].

Therapy Resistance and Combination Therapies

Therapy resistance typically enables cancer cells to escape the effects of any single agent and mechanisms of resistance to EZH2 inhibitors are beginning to emerge. In a cell line model, two novel secondary mutations of EZH2 (Y111L and Y661D) were identified in resistant cells following prolonged exposure to EZH2 inhibitors and were found to cooperate to confer resistance [74]. Separately, it had earlier been reported that loss of PRC2 subunits can amplify Ras-driven transcription in PNS tumors, high-grade gliomas, and melanomas and co-occurrence of a Ras pathway mutation with mutations in SWI/SNF correlated with resistance to EZH2 inhibition [75,76] (Figure 2). Consequently, there is interest in identifying therapies that have the potential to cooperate with EZH2 inhibitors. In preclinical models of EZH2-mutant NHL, combining EPZ-6438 with conventional NHL-directed chemotherapy was synergistic in preventing tumor growth [77]. The combination of EPZ-6438 and a glucocorticoid receptor agonist (GRag) also enhanced inhibition of proliferation both in cells harboring *EZH2* mutations and in germinal center NHL. In prostate cancer models, combination of the chemotherapeutic agent etoposide with GSK126 significantly increased death of murine and human prostate cancer cell lines [78]. Finally, in the preclinical study of non-small cell lung cancers, EZH2 inhibition was found to have differing effects in subsets of these cancers as defined by their differing mutations. In tumors that carried either BRG1/SMARCA4 loss-of-function mutations or EGFR gain-of-function mutations, EZH2 inhibition sensitized the malignancies to TopoII inhibitors. In contrast, in tumors that lacked these mutations, EZH2 inhibition conversely promoted resistance to TopoII inhibitors [42]. Collectively, while development of EZH2 inhibition as a therapeutic is only in early stages, evidence of resistance mechanisms is beginning to emerge as are potential approaches for combination therapy, both areas of ongoing study.

Loss-of-function EZH2 mutations in cancer

In the case of EZH2, although overexpression and gain-of-function mutations suggest oncogenic activity, there is also evidence suggesting that EZH2 acts as a tumor suppressor in some cancer types [79–81] (Table 3). Recurrent inactivating deletion, frameshift, nonsense, and missense mutations in *EZH2* occur in a subset of myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), and MDS/MPN overlap disorders [80,81]. Whereas the truncating mutations are dispersed throughout the gene, the missense mutations preferentially occur in highly conserved residues in the domain required for interaction with SUZ12 of PRC2 and the CXC-SET domain required for EZH2 catalytic activity. These

mutations can be found in both monoallelic and biallelic states and individuals whose myeloid disorders had homozygous mutations were shown to have reduced survival compared to those with heterozygous mutations [80]. Mice lacking *Ezh2* had promoted initiation and propagation of *Runx1*-mutant MDS, consistent with a tumor suppressive role of EZH2 in MDS [79]. Loss-of-function mutations and deletions of *EZH2* also occur in human T cell acute lymphoblastic leukemia (T-ALL) [82,83] (Table 3). Notably, EZH2 was not the only PRC2 subunit affected, as missense mutations were identified in both *EZH2* (11 of 68 cases) and *SUZ12* (3 of 68 cases) [82]. The frequency of PRC2 mutations was even higher in a pediatric subtype of T-ALL, early T cell precursor (ETP) ALL where deletions and sequence mutations of *EED*, *EZH2*, and *SUZ12* are found in 42% of ETP ALL, and are also found in 12% of non-ETP pediatric T-ALL [84]. Mutation of genes encoding PRC2 subunits other than EZH2 also occurs in other cancers [85–87] (Table 3). In endometrial stromal tumors, fusion of *SUZ12* (also known as *JJAZ1)* with *JAZF1* was identified [86,87]. Loss-of-function somatic alterations of *EED* or *SUZ12* occur in 70–90% of malignant peripheral nerve sheath tumors (MPNSTs) where they correspond with complete loss of H3K27me3 and activation of multiple developmentally suppressed pathways [85,88]. *EED* mutations that affect EED protein stability, its interaction with EZH2, or its binding to H3K27me3 also occur in a subset of patients with MDS and related diseases and haploinsufficiency of EED leads to a myeloproliferative disorder in mice [89–91].

The canonical substrate of PRC2, lysine residue 27 of histone H3 and its variants, has itself been found to harbor specific recurrent missense mutations in highly restricted cancer types, including 31% of pediatric glioblastoma multiforme (GBM), 78% of diffuse intrinsic pontine gliomas (DIPG), and 50% of pediatric high-grade gliomas (pHGG) [92–94]. *In vitro*, the H3K27M mutation has been shown to block PRC2 activity as it has an increased affinity for the protein, causing it to act as a sink for EZH2 binding and thus inhibit methylation at other H3 sites [94]. Expression of H3.3 K27M also increases H3K27ac, a modification mutually exclusive to H3K27me3 that correlates with transcriptional activation rather than repression [95]. Therefore, histone H3 mutations may contribute to tumorigenesis through aberrant activation of key regulatory loci by reducing H3K27 trimethylation and facilitating acetylation. Correspondingly, the high frequency of H3K27 mutations in these pediatric brain cancers but their rarity in other cancers suggests a highly context-dependent cancer-promoting role, perhaps reflecting perturbation of a role for EZH2/PRC2 in regulation of neural stem cell fate. However, PRC2 subunit gene mutations have not been detected in pediatric GBM or DIPG, so it remains to be determined whether the H3K27M mutations are similar in mechanism to EZH2/PRC2 loss-of-function mutations or whether the these mutations exert effects via additional non-Polycomb mechanisms. Collectively, the discoveries that EZH2/PRC2 loss-of-function can drive oncogensis in certain contexts suggest that some caution is warranted in the clinical application of EZH2 inhibitors.

Conclusions, questions, and future directions

The parallel development and testing of independent EZH2 inhibitors, three of which have now moved to clinical trials, should soon yield a trove of data on the toxicity as well as potential efficacy of this approach to enzymatic inhibition in cancers that carry a variety of EZH2 activation mechanisms. As only data from patients with *EZH2*–wild-type tumors has

been reported thus far, it will be of great interest to determine efficacy of these inhibitors in cancers that carry activating *EZH2* mutations. Given the emerging non-PRC2–based functions of EZH2, it will also be of interest to understand the extent to which *EZH2* mutations promote cancer via PRC2-dependent versus independent effects. Similarly, it will also be important to understand the extent to which the cancer-promoting effects of EZH2 depend upon its enzymatic activity compared to non-enzymatic structural contributions to PRC2 integrity as this carries substantial implications for drug development.

Given its clear gain-of-function contributions to cancer and the capability of directly inhibiting its enzymatic function, EZH2 constitutes a compelling target for anti-cancer therapy, albeit with potential caveats from its developmental and tumor suppressor roles. The story of EZH2, from discovery as a regulator of body patterning in fruit flies to transcriptional regulator of chromatin structure to driver of cancer should soon have a new chapter when clinical trials reveal whether targeting EZH2 can bring a substantial therapeutic advance.

Acknowledgments

K.H.K. was supported by an award from National Cancer Center. C.W.M.R. was supported by R01CA172152 and R01CA113794. The Garrett B. Smith Foundation, the Cure AT/RT Now foundation, The Avalanna Fund, and Miles for Mary provided additional support.

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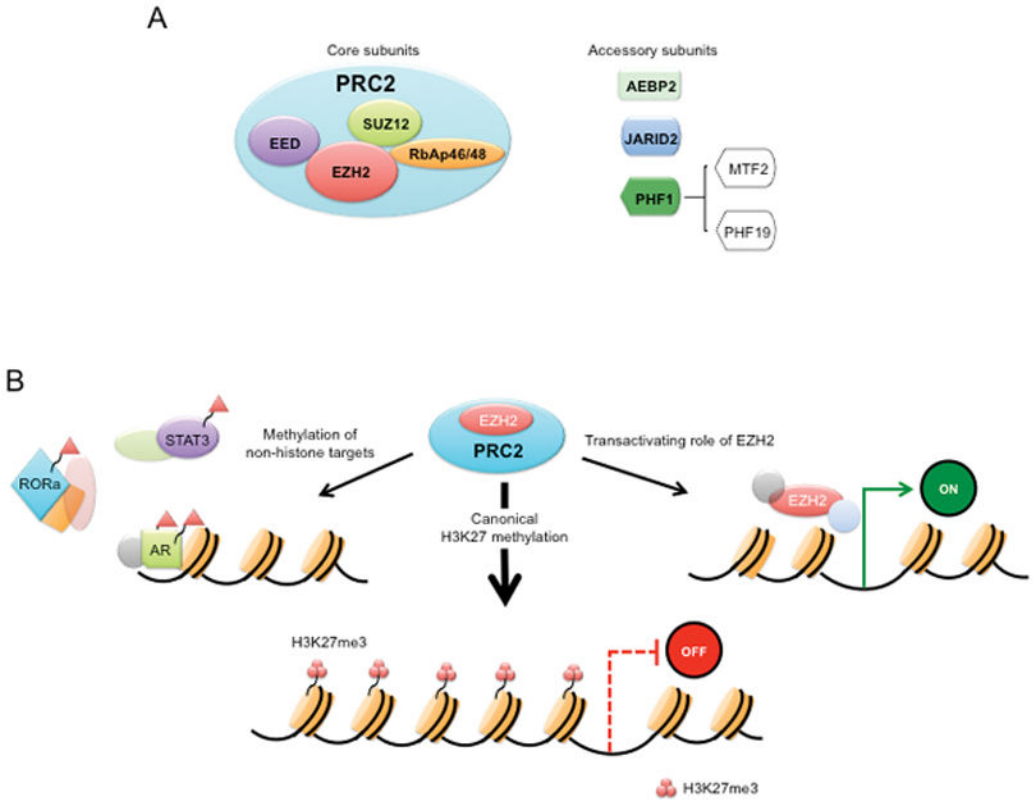

Figure 1. The PRC2 complex and its function in transcriptional regulation

a. The mammalian PRC2 complex consists of four core subunits: the catalytic subunit enhancer of zeste 2 (EZH2), embryonic ectoderm development (EED), suppressor of zeste 12 (SUZ12), and retinoblastoma (Rb)-associated protein 46/48 (RbAp46/48) and additional proteins, including AEBP2, PCL, and JARID2 have also at times been found to be associating with PRC2 complex to modulate the activity of PRC2 in different context. **b.** EZH2 regulates transcriptional activity: 1) EZH2 is also capable of methylating a number of non-histone protein substrates, 2) PRC2 methylates Histone 3 on lysine 27, which contributes to transcriptional silencing, 3) EZH2 also has a PRC2-independent role in transcriptional activation.

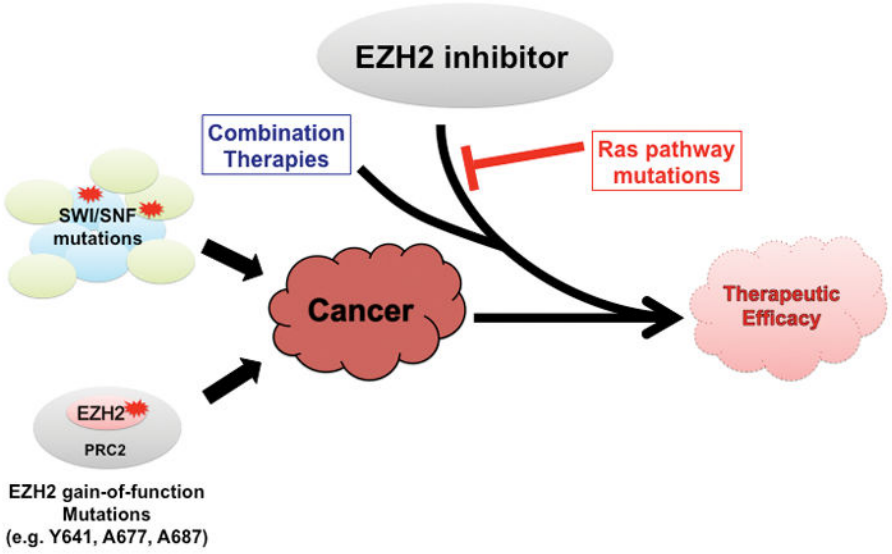

Figure 2. EZH2 as a therapeutic target in cancer

a. The roles of EZH2 mediated transcriptional silencing are context specific. For cell types in which its hyperactivity drives oncogenesis, contributions from silencing of lineage specification genes, the tumor suppressor Rb and DNA repair genes have been identified. **b.** Cancers harboring SWI/SNF mutations and gain-of-function EZH2 mutations confer dependency on EZH2 inhibition. Early pre-clinical evidence suggests potential benefit of combination therapy with an EZH2 inhibitor. At least in the case of cancers driven by SWI/SNF mutation, Ras pathway mutations can confer resistance to EZH2 inhibition.

Table 1

EZH2 overexpression and gain-of-function mutations identified in different cancer types and affected target genes

EZH2 status	Associated cancer type	Target genes	Reference
EZH2 overexpression	Breast	CK5/6. P-cadherin, RAD51 paralogs, RUNX3, CDKN1C, FOXC1, RAF1-B-catenin signaling, DNA damage repair RKIP, CIITA, KLF2,	16–18,20,48,52, 96–100
	Prostate	DAB2IP, p16, CDK4, MSMB, ADRB2, E-Cadherin, Ras, NF-kappaB, SLIT2, TIMP-2/3, EMT regulator,	16,18,53,59, 101–105
	Endometrial	p16, E-cadherin, sFRP1, DKK3, B-Catenin	18,107,108
	Melanoma	p21/CDKN1A, DCK, AMD1, WDR19,	17,18,109,110
	Bladder	unknown	17,111,112
	Glioblastoma	BMPR1B	17,113
	Liver	unknown	114
	Lung	Dkk-1	115
	Natural killer/T-cell lymphoma	Cyclin D1	116
	Ovarian	VASH1	117
EZH2 mutation (gain-of-function)	Non-Hodgkin's Lymphoma (FL, GCB-subtype DLBCL), melanoma	Cdkn2a, Cdkn1a/p21, BLIMP1/PRDM1	22,24–26,118

Table 2

EZH2 inhibitors and their status in clinical development

Compound	Mechanism	Potency (Ki, nM)	Selectivity	Status	Reference
DZNep	SAH hydrolase inhibitor of methyltransferases	Unknown	Unknown	Preclinical	119–122
EI1	SAM-competitive inhibitor of PRC2	13−/+3 nM	>10,000-fold over other HMTs, ~90-fold over EZH1	Preclinical	69
EPZ005687	SAM-competitive inhibitor of PRC2	24−/+7 nM	>500-fold over other HMTs, ~50-fold over EZH1	Preclinical	66
GSK343	SAM-competitive inhibitor of PRC2	1.2−/+0.2 nM	1,000-fold over other HMTs, 60-fold over EZH1	Preclinical	67
GSK126	SAM-competitive inhibitor of PRC2	0.5−/+3 nM	>1,000-fold over 20 other HMTs, 150-fold over EZH1	Phase I	68
UNC1999	SAM-competitive inhibitor of PRC2	4.6−/+0.8 nM	10,000-fold over other HMTs, 10-fold over EZH1	Preclinical	70
EPZ-6438	SAM-competitive inhibitor of PRC2	2.5−/+0.5 nM	>4,500-fold over 14 other HMTs, 35-fold over EZH1	Phase I/II	71,72
Stabilized a-helix of EZH2 peptide (SAH-EZH2)	Hydrocarbon-stapled peptides that mimics the a-helical EED binding domain of EZH2 (aa 40–68) to disrupt the protein interation between EZH2 and EED	Unknown	More selective for EZH2 over EZH1	Preclinical	73

Table 3

EZH2/PRC2 loss-of-function mutations identified in different cancer types and affected target genes

EZH2/PRC2 status	Associate cancer type	Target genes	Reference
EZH2 mutation (loss-of-function)	MDS, MPN, T-ALL	NOTCH/JAK-STAT, NOTCH1	[79–83]
SUZ12 fusion	Endometrial stromal tumors	unknown	[86,87]
SUZ12/EED mutation	T-ALL, ETP ALL, MDS/MPN/MPD, MPNSTs	NOTCH1, JAK-STAT, FOXN4, IGF2, PAX2, TLX1	[82–85,89,90]