



# The roles of EZH2 in cancer and its inhibitors

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## Abstract

The enhancer of zeste homolog 2 (EZH2) is encoded by the Enhancer of zeste 2 polycomb repressive complex 2 subunit gene. EZH2 is involved in the cell cycle, DNA damage repair, cell differentiation, autophagy, apoptosis, and immunological modulation. The main function of EZH2 is to catalyze the methylation of H3 histone of H3K27Me<sub>3</sub>, which inhibits the transcription of target genes, such as tumor suppressor genes. EZH2 also forms complexes with transcription factors or directly binds to the promoters of target genes, leading to regulate gene transcriptions. EZH2 has been as a prominent target for cancer therapy and a growing number of potential targeting medicines have been developed. This review summarized the mechanisms that EZH2 regulates gene transcription and the interactions between EZH2 and important intracellular signaling molecules (Wnt, Notch, MEK, Akt) and as well the clinical applications of EZH2-targeted drugs.

**Keywords** EZH2 · PRC2 · Histone methylation · Cancer treatment · Signal pathways

## Function and structure of EZH2

Polycomb group (PcG) protein complexes inhibit transcription initiation of target gene by methylating histones on chromatin. PcG protein forms a stable multi-protein complex with some protein factors and acts collectively to keep their target genes in a transcriptional repression state. PRC1 (Polycomb repressive complex 1) and PRC2 (Polycomb repressive complex 2) are the two primary core protein complexes in mammalian cells [1]. Embryonic Ectoderm Development (EED), Zeste 12 inhibitor (Suppressor of Zeste 12, SUZ12), and EZH1 or EZH2 are the components of PRC2. The catalytic subunit of PRC2, Enhancer of zeste homolog 2 (EZH2), plays a crucial role in the H3 methylation pathway [2].

The EZH2 gene is located on chromosome 7q35 and comprises 20 exons that can code for 746 amino acids. There are EED interaction Domain (EID), Domain 1, Domain 2, Cysteine-rich domain (CXC Domain), and Enhancer domain of zeste and trithorax (SET Domain) in the EZH2 protein [3]. The SET domain can offer the active site of methyltransferase. Due to the solo EZH2 subunit lacks histone

methyltransferase activity, it must be combined with the EED/ESC and SUZ12 domains to function [4].

Histone methylation modifications can specifically activate or inhibit gene transcriptional activity, which is linked to many human diseases. Histone methyltransferase EZH2 of the PRC2 complex promotes trimethylation of H3 histone 27 lysine, changing the structure of chromatin and limiting gene transcription.

EZH2 has the ability to directly methylate a variety of target molecules, including GATA4, STAT3,  $\beta$ -catenin, and the lysines at positions 510, 514, and 515 of PRC2 [5–10]. EZH2 may also bind to particular molecules directly, creating a ternary complex with Rel A and Rel B in the NF- $\kappa$ B component, EZH2 interacts to TCF,  $\beta$ -catenin, and ER to activate the c-Myc and cyclin D1 genes, which are located downstream [11, 12]. Another mode of action of EZH2 is to bind to the promoter region of target genes and affect gene transcription, commonly found in c-Myc and Notch1 [13, 14] (Fig. 1).

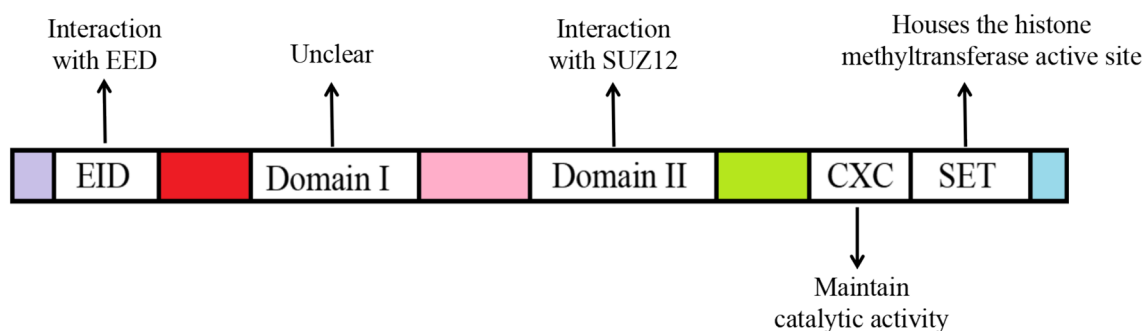
## EZH2-related diseases and signal pathways

### EZH2/Wnt/ $\beta$ -Catenin signaling pathway

By binding to some long non-coding RNAs, such as NEAT1, EZH2 could control the enrichment of H3K27Me<sub>3</sub> in

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**Fig. 1** Domain composition and function of EZH2. Hierarchical analysis expression of EZH2 protein included five individual conservative structure area: EID structure area, Domain I, Domain II, CXC structure area, and SET Domain

various gene promoters and activate or inhibit the Wnt/ $\beta$ -catenin signaling pathway [15–17]. Additionally, EZH2 could assemble into a complex with  $\beta$ -catenin directly, control the expression of downstream FTO genes, and then increase the expression of c-Myc to promote the growth of tumor cells [18] (Fig. 2).

### MEK/ERK/EZH2 signal pathway

Elk-1 can bind to the EZH2 promoter after being phosphorylated, which increases EZH2 expression. The expression of EZH2 is decreased when the MEK-ERK-Elk-1 signaling pathway is compromised [19]. E2F4 can bind to EZH2 and activate MAPK signaling in AML (acute myeloid leukemia) patients. Additionally, EZH2 can block the MAPK signaling pathway's ability to inhibit E2F4 [20]. Patients with diffuse large B-cell lymphoma develop resistance to EZH2 inhibitors as a result of MAPK signaling reduction of TNFSF10 and BAD expression through a FOXO3-dependent mechanism [21]. In non-small cell lung cancer cells with the KRASG12C mutation, MEK1 controls EZH2 expression [22]. EZH2 inhibits MEK-ERK1/2 signaling in vascular smooth muscle cells (VSMC), prevents aortic dissection (AD), inhibits ATG5 and ATG7 via H3K27Me3, inhibits autophagic cell death (ACD), and regulates the MEK-ERK1/2 signaling pathway [23]. CXXC4 is directly controlled by EZH2; it could inhibit MAPK signaling by severing the MEK1/2-ERK 1/2 connection [24] (Fig. 3).

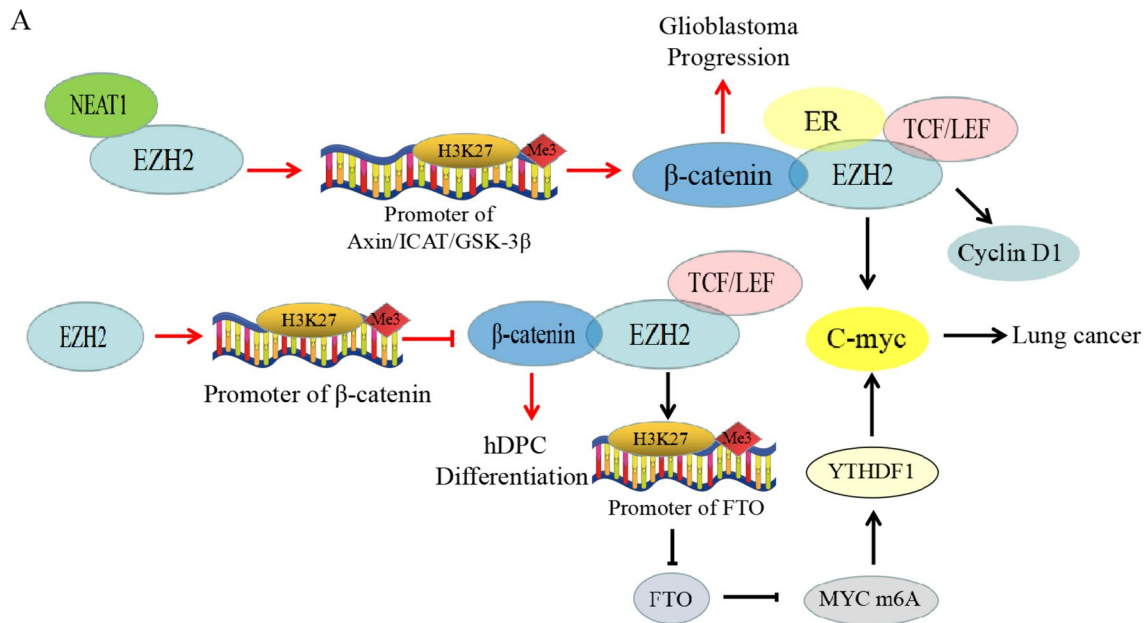
### EZH2/Notch signal pathway

Breast cancer and melanoma tumor cells proliferate more readily when EZH2 levels are elevated, which is related to the interaction of the histone methyltransferase NSD3 with EZH2 and RNA polymerase II to enhance H3K36me2/3 [25, 26]. PVT1, a long-chain non-coding RNA, can interact with EZH2 in non-small cell lung cancer (NSCLC) to

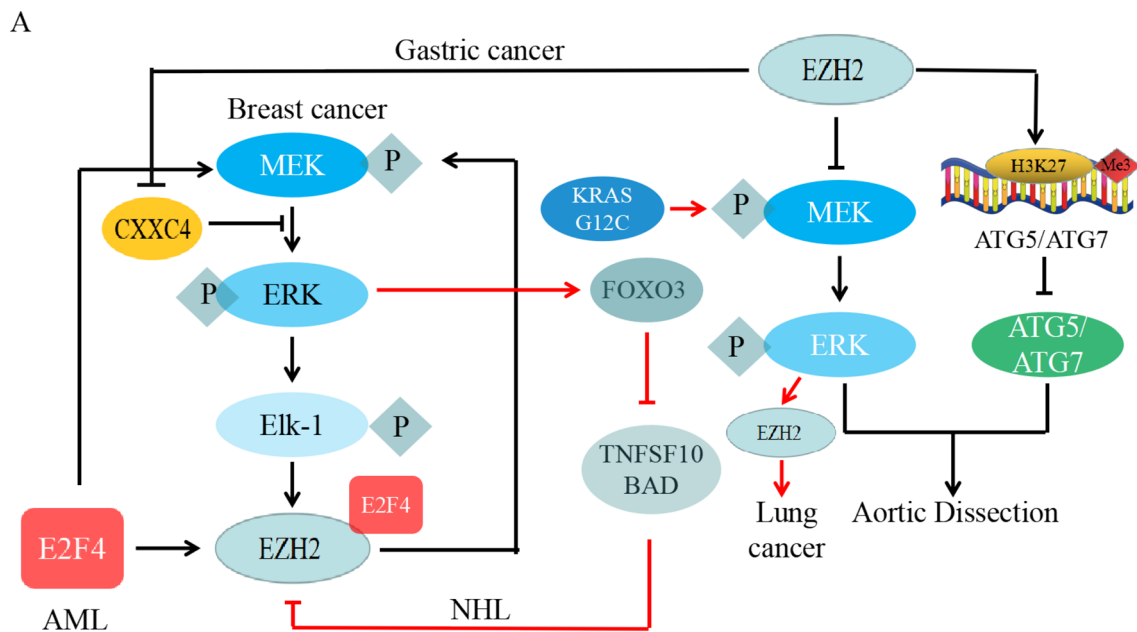
mediate miR-497 promoter methylation and prevent miR-497 and YAP1 upregulation [27]. Notch1, Notch2, and Jagged 1 are inhibited by miR-34a, and HOTAIR, a long-chain non-coding RNA, could block miR-34a through H3K27me3 by EZH2 [28, 29]. Mutation or translocation of Notch1 or Notch2 in breast cancer can lead to the upregulation of PRC2/EZH2, promote the binding of EZH2 to the HES-1 transcription factor in the PTEN promoter region, and inhibit PTEN expression [30]. EZH2 inhibits Notch function by blocking the Notch ligand DLL4 and under the induction of TNF and promoting the methylation of the Notch1 promoter region [31, 32] (Fig. 4).

### EZH2/PI3K/Akt signal pathway

When JNK/STAT3 is activated, it results in the upregulation of IKBEK and miR-21, increases the phosphorylation of STAT3 and Akt, decreases the expression of Spry2, phosphorylates the 21st serine of EZH2 through p-Akt, inhibits the expression of H3K27Me3, PI3K, Id3, and increases the expression of Id2, Prdm1, and Eomes [33–35], and EZH2 can also interact with STAT3 and lead to increased STAT3 activity [36]. A significant amount of E2F1 is released by Akt, which then hyperphosphorylates Rb, activates cyclins and cyclin-dependent kinases (CDKs), and binds to the EZH2 promoter to increase EZH2 transcription [37]. When IGF1 binds to its receptor IGF1R, and VCAM-1 binds to VLA-4, it will activate the PI3K/Akt signaling pathway, promote the phosphorylation of EZH2, and lead to the increase of HIF-1, IGF1, and Bcl2, resulting in drug resistance [38]. By attaching to the EZH2 promoter, the transcription factor E2F7 increases H3K27Me3, decreases PTEN expression, and activates the Akt/mTOR signaling pathway [39, 40]. By activating TNFSF13B, EZH2 increases Akt phosphorylation, but when the level of the demethylase KDM2B is lowered, EZH2 inhibits the phosphorylation of PI3K and Akt [41, 42]. EZH2 has the ability to control the amount of VEGF-A through the PI3K/Akt pathway [43]. EZH2



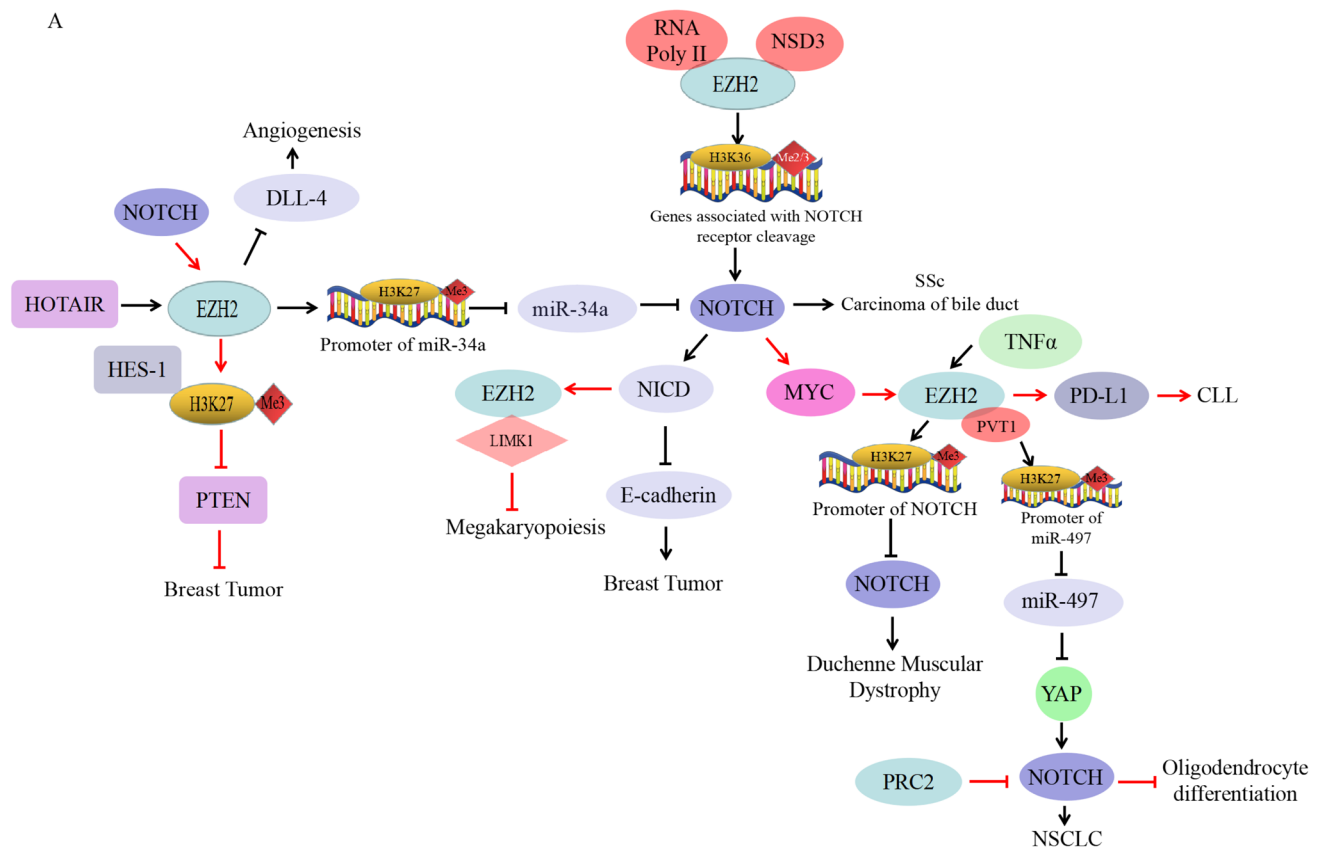
**Fig. 2** EZH2/Wnt/β-Catenin signal transduction and related diseases. Interrelationships between EZH2 and Wnt/β-Catenin and the resulting illnesses



**Fig. 3** MEK/ERK/EZH2 signal transduction and related diseases. Interrelationships between EZH2 and MEK/ERK and the resulting illnesses

control on PI3K can be accomplished by PIK3IP; however, EZH2 regulation requires ARID1A. Although ARID1A and EZH2 have a mutual inhibitory effect, EZH2 has the ability to inhibit PIK3IP1 and activate the PI3K/Akt pathway, while ARID1A has the ability to stimulate PIK3IP1 [44]. Cell Cycle-Related Kinase (CCRK) in immortalized human hepatocytes increases the amount of EZH2, promotes

H3K27me3, and speeds tumor growth. At the same time, CCRK’s phosphorylation of Akt and EZH2 may stimulate CCRK transcription, forming a self-reinforcing regulatory circuit [45]. PTEN transcription will be significantly increased by MYC gene activation, while EZH2 phosphorylation will be decreased. Additionally, EZH2 can direct MYC to the IGF1R promoter region, where it can then activate



**Fig. 4** EZH2/Notch signal transduction and related diseases. Interrelationships between EZH2 and Notch and the resulting illnesses

IGF1R and Akt to aid in the development of tumors [46, 47]. The oncoprotein binding domain of Yin Yang 1 (YY1) could attract EZH2 in breast cancer, causing PTEN and PTENP1 to be down-regulated. The combination of YY1 and EZH2 inhibited the transcription of PTEN and PTENP1, resulting in increased phosphorylation of S473 and T308 of Akt and increased Akt activation [48]. FRA1/C-Jun can be activated by the ERK/AKT signaling pathway, which causes AP-1 to occupy the EZH2 promoter and cause primary EZH2 expression. Finally, trimethylates H3K27 to repress the ITG2 promoter and inhibit transcription [49] (Fig. 5).

Long non-coding RNA can also play a role in the EZH2 and Akt regulatory mechanism. Urothelial cancer associated 1 (UCA1) interacted directly with EZH2, increasing EZH2 expression and activating the Akt/GSK-3/cyclin D1 pathway. EZH2 may also interact directly with the cyclin D1 promoter, promoting cyclin D1 expression. Furthermore, Akt phosphorylation might indirectly affect EZH2 expression, generating a positive feedback loop with EZH2. Long-chain non-coding RNA PART1 (Prostate Androgen Regulated Transcript 1) inhibited downstream PI3K / Akt by boosting the production of PLZF (Promyelocytic Leukemia Zinc Finger) and subsequently recruiting EZH2 [50]. lncRNA-SNHG1 could bind to EZH2 and activated

the PI3K/Akt/mTOR and Wnt/ $\beta$ -Catenin signaling pathways [51]. LINC01559 and CASC11 can bind EZH2 to the PTEN promoter, increase PTEN promoter methylation, inhibit PTEN, and activate PI3K/Akt [52, 53]. LINC00665 and lncRNA-AFAP1-AS1 can interact with EZH2 to activate the Akt signaling pathway, resulting in drug resistance in patients [54, 55] (Fig. 5).

### EZH2 other related signal pathway

CHK1 is involved in the regulation of cell apoptosis. Inhibiting CHK1 has been shown to greatly increase the production of Caspase3 and Caspase9, as well as promote cell apoptosis [56]. By interacting with the promoter of CHK1, EZH2 could enhance the expression of CHK1, blocking the downstream apoptotic pathway. High expression of EZH2 and CHK1 has been linked to poor prognosis and treatment resistance in ovarian cancer patients [57], implying that EZH2 is also linked to other apoptotic pathways. EZH2 could enhance cell death and increase intestinal epithelial cell permeability by inactivating JAK2/STAT signaling through H3K27Me3 in inflammatory bowel illness [58]. Not only does EZH2 play a crucial part in cell death, but it is also a significant target in other disorders. The epithelial–mesenchymal transition



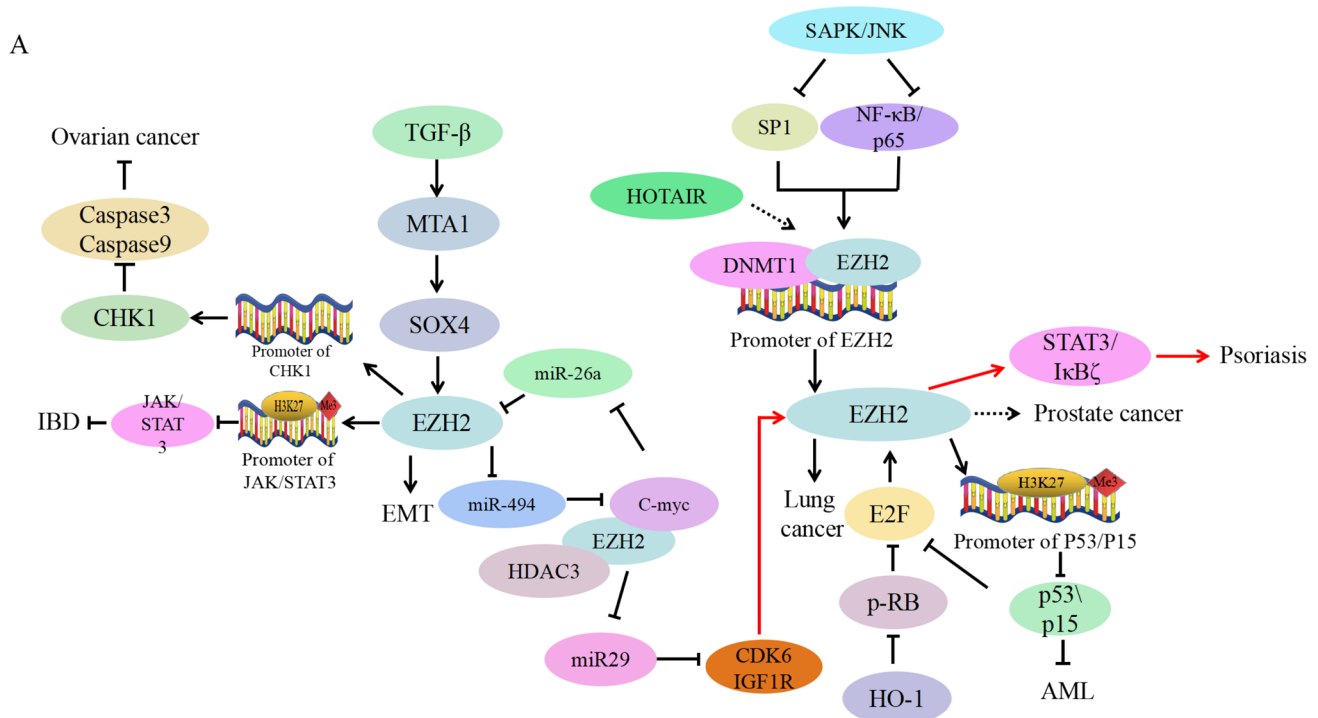


MDS cells results in the overexpression of EZH2, which suppresses p53 and p15 and finally leads to AML. p53 can counteract the action of pRB-E2F in normal circumstances. The balance between p53 and pRB-E2F is essential for normal cell growth, but HO-1 disrupts this balance by over-activating the pRB-E2F pathway, which increases the inhibition of p53 and p15 from EZH2, allowing tumor cells to evade cell cycle-level checks and making chemotherapy drugs difficult to eliminate tumor cells [60]. Excessive stimulation of the CDK4/6-EZH2 pathway in psoriasis causes STAT3, IB, and the inflammatory response in the body to be triggered. Inhibitors targeting EZH2 could be a new sort of psoriasis therapy method for this pathway [61]. MYC boosted EZH2 expression and accelerated tumor growth in lymphomas via blocking miR-26a [62]. By suppressing miR-494, EZH2 can also boost MYC expression in a positive feedback loop. MYC can also form a complex with HDAC3 and EZH2 to block miR-29 expression, resulting in increased expression of downstream CDK6 and IGF1R and lymphoma [63]. Furthermore, through its interaction with DNA methyltransferase 1 (DNA methyltransferases, DNMT1), EZH2 can not only regulate histone methylation but also participate in DNA methylation and affect the transcription of downstream targets [64]. Upstream of EZH2-DNMT1, there are regulatory mechanisms. In non-small cell lung cancer, activation of SAPK/JNK inhibits the interaction between EZH2 and DNMT1 by suppressing SP1 and NF-B/p65 [65, 66].

lncRNA HOTAIR can also regulate EZH2-DNMT1 activity, enhance EZH2 expression, and cancer cell production [67] (Fig. 6).

## Targeting EZH2 has become an important way to treat cancer

Melanoma, breast cancer, prostate cancer, lung and liver cancer, psoriasis, hematological malignancies, and other disorders have all been linked to EZH2 mutations and expression imbalances. At this time, EZH2 inhibitors can be split into two categories: those that inhibit EZH2 methyltransferase activity and those that damage the structure of PRC2. There are two types of inhibitors of EZH2 methyltransferase activity. S-adenosine-L-homocysteine (SAH) hydrolase inhibitors, for example, inhibit EZH2 indirectly by increasing SAH levels. The second is an S-adenosylmethionine (SAM) competitive inhibitor that inhibits EZH2 by occupying the SAM-binding site in the EZH2 binding pocket [68]. The use of EZH2 inhibitors in combination with other medications has also been shown to have a positive therapeutic impact [69]. Because EZH2 possesses both cancer suppressor and cancer promoter qualities, it is thought that drugs may be created to target EZH2's cancer promoter domain without compromising its tumor suppressor activity (Table 2).



**Fig. 6** EZH2 and other signal transduction and related diseases. Interrelationships between EZH2 and other molecule and the resulting illnesses

## EZH2/EZH1 inhibitors

Tazemetostat was recently approved by the FDA for metastatic or advanced epithelioid sarcoma (ES) that is not suitable for surgical resection. Tazemetostat is a selective EZH2 inhibitor, in cell-free test, and  $K_i$  and  $IC_{50}$  are 2.5 nM and 11 nM, respectively, which are 35 times more selective than EZH1 [70]. In diffuse large B-cell lymphoma cells, EL1 can significantly inhibit the methyltransferase activity of EZH2, reduce H3K27Me3, and activate the expression of p16 to inhibit the proliferation of diffuse large B-cell lymphoma cells (DLBCL). EL1 can also inhibit the Y641F mutation of EZH2, the  $IC_{50}$  is 13 nM, and for WT EZH2,  $IC_{50}$  is 15 nM [71]. GSK126 is a potent and highly selective EZH2 methyltransferase inhibitor with  $IC_{50}$  is 9.9 nM in cell-free experiments. In vitro experiments, GSK126 could significantly inhibit EZH2 activity in DLBCL cells and induce tumor suppressor gene transcription. GSK126 also has inhibitory effect on EZH2 mutants [72]. CPI-169 is a potent and selective EZH2 inhibitor, the  $IC_{50}$  is 0.24 nM, 0.51 nM, and 6.1 nM for EZH2 WT, EZH2 Y641N, and EZH1. In non-Hodgkin's lymphoma (NHL) cells, CPI-169 could significantly inhibit the activity of EZH2, reduce the trimethylation level of H3K27, and the safety of CPI-169 is reliable [73]. EPZ005687 could bind to the SAM pocket of the EZH2 SET domain. It is a SAM-competitive inhibitor of EZH2. The  $K_i$  is 24 nM. EPZ005687 could act on a variety of different lymphoma cells to reduce H3K27 methylation and prolong the G1 phase and shorten the S and G2/M phases. Interestingly, EPZ005687 has stronger inhibitory activity against EZH2 containing Tyr641 or Ala677 mutants, while WT EZH2 has a weaker effect [74]. EPZ011989 is an effective, selective, and orally active EZH2 inhibitor, the  $K_i < 3$  nM. EPZ011989 has a significant tumor suppressor effect on mouse model of human B-cell lymphoma and EPZ011989 could reduce intracellular H3K27 methylation in human lymphoma cells with Y641F mutation [75]. ZLD10A is a new type of EZH2 inhibitor, which could inhibit the methyltransferase activity of EZH2 with high selectivity. The NHL cells treated with ZLD10A showed decreased H3K27 methylation and increased tumor cell apoptosis and also had a good inhibitory effect on EZH2 with Y641F and A677G mutants. ZLD10A's  $IC_{50}$  for WT EZH2, Y641F, and A677G are 18.6 nM, 27.1 nM, and 0.9 nM [76]. GSK503 is a highly effective EZH2 inhibitor with potential anti-tumor potential. The  $K_i$  is about 3~27 nM. It has a significant inhibitory effect on the proliferation of DLBCL which carried GCB (Germinal Center B cells) cells and could also affect the lymphatic transformation [77]. JQEZ5 is an inhibitor of EZH2 lysine methyltransferase.  $IC_{50}$  is 11.1 nM, which inhibited the colony formation of human primary CD34<sup>+</sup> chronic myeloid leukemia (CML) stem/progenitor cells [78].

GSK926 and GSK343 with the same maternal structure have EZH2 inhibitory activity and could significantly inhibit the trimethylation of H3K27, and the  $IC_{50}$  are  $324 \pm 174$  nM and  $174 \pm 84$  nM. GSK343 could effectively inhibit proliferation of breast cancer cells and prostate cancer cells [79]. PF-06726304 is a selective EZH2 inhibitor,  $K_i$  values are 0.7 nM and 3 nM for WT EZH2 and EZH2 (Y641N), and  $IC_{50}$  for H3K27me3 inhibition is 15 nM. In the subcutaneous Karpas-422 xenograft model, PF-06726304 could significantly inhibit tumor growth [80]. EZH2-IN-3 is an inhibitor of EZH2 and EZH1. The  $IC_{50}$  for EZH2 (WT) is  $0.032 \pm 0.019$  nM. When the level of H3K27Me3 in the cell decreases, the effect of EZH2-IN-3 will change significantly. And it has a selective effect on the growth of diffuse large B-cell lymphoma cells [81]. Liracetostat (CPI-1205) is an inhibitor of selective histone lysine methyltransferase EZH2 with oral biological activity, and the  $IC_{50}$  is 2 nM and 52 nM for EZH2 and EZH1. It has potential anti-tumor activity. In multiple myeloma and plasmacytoma cell models, CPI-1205 could cause tumor cell apoptosis [82]. EBI-2511 is a potent and orally active EZH2 inhibitor,  $IC_{50}$  is 4 nM for EZH2 (A667G) and an  $IC_{50}$  value of 55 nM in the WSU-DLCL2 cell line. This compound is a scaffold based on Tazemetostat. In the mouse model of generalized xenotransplantation, the anti-tumor activity of EBI-2511 at the same dose is better than Tazemetostat, which provides a new compound skeleton to EZH2 inhibitors [83]. UNC1999 is a selective inhibitor of EZH2 and EZH1 with high oral bioavailability. In the cell-free test, the  $IC_{50}$  for EZH2 and EZH1 is 2 nM and 45 nM. UNC1999 is an effective autophagy inducer. It specifically inhibits H3K27me3/2 and selectively kills the diffuse large B-cell lymphoma cell line while carrying the EZH2 (Y641N) mutant [84]. Valemetostat (DS-3201, DS-3201b) is a selective EZH1/2 dual inhibitor ( $IC_{50} \leq 10$  nM), which could significantly inhibit the H3K27Me3 in adult T-cell leukemia lymphoma cells (ATL cells). At the same time, SLA and PAG1 genes are reactivated and normal immune function is restored by Valemetostat [85]. (R)-OR-S1 is a SAM-competitive, highly selective, oral EZH1/2 inhibitor. It has a good inhibitory effect on EZH2 (Y641F). The  $IC_{50}$  of EZH1 and EZH2 is 7.4 nM and 10 nM and the  $IC_{50}$  of H3K27Me3 is 0.47 nM. (R)-OR-S1 could significantly inhibit the growth of KARPAS-422 cells [86]. PF-06821497 is a lactam EZH2 inhibitor designed based on ligands and physicochemical properties. It has a good inhibitory effect on EZH2 (Y641N), and the  $K_i$  is 1.15 nM. It is suitable for Karpas-422 xenotransplantation. PF-06821497 also has a good inhibitory effect on tumor growth in mice [87]. DZNeP is an adenosine analogue and competitive S-adenosylhomocysteine hydrolase (SAH) hydrolase inhibitor. It inhibits the activity of EZH2 by increasing the content of SAH. DZNeP globally inhibits histone methylation.

Strictly speaking, DZNeP is not a selective for the methylation process regulated by EZH2 [88]. Oxetinib (AZD9291) is a highly effective selective EGFR mutant inhibitor for the treatment of advanced non-small cell lung cancer. Interestingly, Oxetinib exhibits an affinity for EZH2 and can disrupt the interaction between EZH2 and EED, ultimately leading to impaired PRC2 function and reduced EZH2 expression [89] (Table 1).

### PRC2 inhibitors

As the catalytic subunit of PRC2, EZH2 regulated epigenetic. It must be combined with EED and SUZ12. Therefore, destroying the structure of PRC2 or inhibiting the function of EED or SUZ12 can also indirectly inhibit the function of EZH2. However, if EZH2 does not play a regulatory role through PRC2, the effectiveness of inhibitors needs further research. Proteolysis targeting chimeras (PROTACs) have become a new technology in drug development. Researchers have synthesized two active compounds with the function of inhibiting PRC2 through this technology, PROTAC EED Degradator-1 and PROTAC EED Degradator- 2. The  $IC_{50}$  is 8.17 nM and 8.11 nM, both of them could inhibit the function of PRC2 by binding to EED (pKD1 is  $9.02 \pm 0.09$  nM, pKD2 is  $9.27 \pm 0.05$  nM) and could inhibit the proliferation of DLBCL cell lines with EZH2 mutations in vitro [90]. UNC6852 is a selective degradation agent of PRC2. The  $IC_{50}$  of this compound to EED is 247 nM. UNC6852 could block the histone methyl transfer of EZH2 and reduce the level of H3K27me3 in HeLa cells and diffuse large B-cell lymphoma (DLBCL) cells which contain mutations in EZH2 [91]. A-395 could interact with the H3K27me3 binding pocket in EED to prevent the allosteric activation of PRC2 catalytic activity. It can effectively inhibit the activity of PRC2 in vitro, and the  $IC_{50}$  is  $18 \pm 2$  nM. A-395's ability of regulation of H3K27Me3 is equivalent to GSK126 in Pfeiffer and Karpas-422 DLBCL cell lines [92]. MS1943 is an oral EZH2 selective degradation agent, which can effectively reduce EZH2 levels in cells. Its  $IC_{50}$  value of inhibiting EZH2 methyltransferase activity is 120 nM, which can effectively reduce the level of EZH2 in BT549, HCC70, MDA-MB-231, TNBC cells, KARPAS-422, SUDHL8 lymphoma cells, and PNT2 non-cancerous prostate cells [93]. Chemical resistance in prostate cancer can be overcome by inhibitors designed with EED as a target. LG1980 has been discovered to bind to EED, inhibiting the activity of EZH2 without affecting its catalytic function. LG1980 and EED have a  $K_d$  value of 2.71  $\mu$ M, and LG1980 significantly inhibited the in vitro viability of ARCaPE-shEPLIN cells ( $IC_{50} = 0.26$   $\mu$ M) and C4-B-TaxR cells ( $IC_{50} = 6.87$   $\mu$ M) [94]. Astemizole, an FDA-approved drug, is a small-molecule inhibitor of the PRC2 EZH2-EED interaction. As

tinidazole inhibits the activity of the EZH2-EED interaction, the stability of the PRC2 complex in cancer cells, and the activity of its methyltransferase. It can significantly inhibit the proliferation of DLBCLs and works well in combination with EPZ005687 [95]. The PRC2 cofactor Jarid2 can bind to EED with a  $K_d$  value of 3.0 mM following trimethylation of the lysine residue at position 116. This can result in allosteric stimulation of the PRC2 enzyme activity. Subsequent investigation revealed that Jarid2's amino acid residues 114–118 were crucial for the protein's ability to bind EED. Researchers created Jarid 2<sub>114–118</sub>-K116me3, which has a  $K_d$  value of 8.82  $\mu$ M, as a result of these discoveries. Two more potent peptide mimicking ligands, UNC5114 and UNC5115, were eventually found through more thorough SAR investigation. The  $K_d$  values of UNC5114 and UNC5115 for EED in the ITC experiment were 0.68  $\mu$ M and 1.14  $\mu$ M, respectively. Unlike Jarid 2-K116me3, UNC5114 and UNC5115 prevent the binding of H3K27me3 to EED, which is how PRC2 is inhibited [96].

EED226 could change the conformation of PRC2 by binding to the H3K27Me3 pocket, therefore EED226 could inhibit the methyltransferase activity of PRC2 and reduce the level of H3K27Me3 in the cell. EED226 also exhibits inhibiting effects in Y641N EZH2 mutant cells. In the 14-day anti-proliferative activity experiment of KARPAS-422, the  $IC_{50}$  value of EED226 was 80 nM. By constructing the Karpas-422 cell xenograft model, EED226 has a good pharmacokinetic parameter [97]. Despite the fact that EED226 has demonstrated good tumor treatment, EZH2 selectivity, and bioavailability, there are still issues, such as modeling solubility and durability, as well as the presence of the mono-substituted electron-rich furan ring. Researchers optimized the structure of EED226 and obtained MAK683. Researchers evaluated the performance of candidate compounds by assessing PRC2 activity, H3K27 methylation status, and Pfeiffer cell proliferation. The values of MAK683 are  $9 \pm 4$  nM,  $3 \pm 2$  nM, and 4 nM ( $GI_{50}$ ). MAK683 also showed good therapeutic effects on Karpas-422-derived xenograft mouse model [98]. Based on the structure–activity relationship of EED 226, researchers discovered a new EED inhibitor ZJH-16 by changing the substituent type. ZJH-16 directly binds to the H3K27me3 binding pocket of EED with high affinity (HTRF  $IC_{50} = 2.72$  nM, BLI  $K_d = 4.4$  nM), and ZJH-16 demonstrates volatile pharmaceutical (PK) properties and robust antagonist efficiency in a KARPAS-422 xenograft model [99]. The EED inhibitor BR001, which demonstrated significant EED targeting activity in competitive binding tests and had an  $IC_{50}$  value of 4.5 nM, was found based on the structure of EED226 and utilizing the scaffold picking approach. Inhibiting cell growth and dramatically lowering the expression level of H3K27me3



**Table 1** Development status of EZH2/PRC2 inhibitors

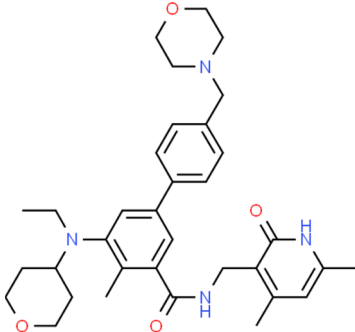
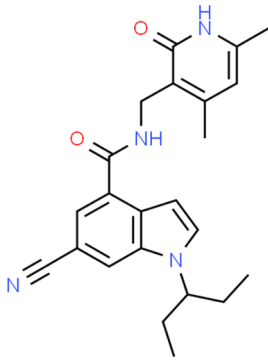
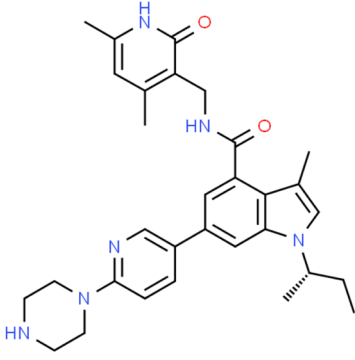
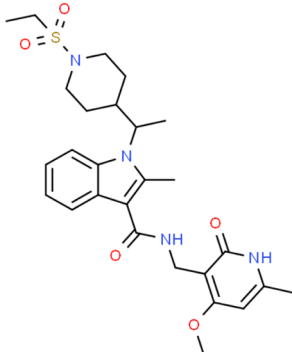
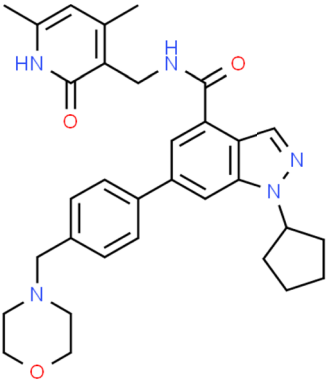
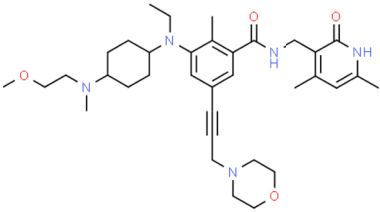
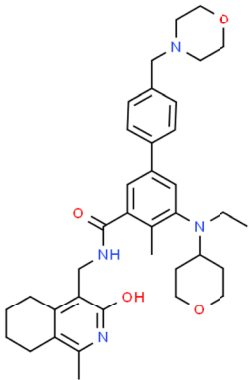
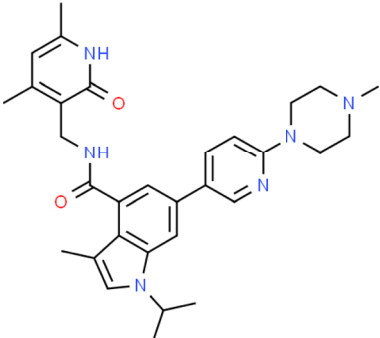
Inhibitor name	Compound structure	Targets of Action & Diseases	Inhibitory activity
Tazemetostat		EZH2 Epithelioid Sarcoma	Ki =2.5 nM IC <sub>50</sub> =11 nM
EL1		EZH2 EZH2 (Y641) Diffuse large B cell lymphoma	IC <sub>50</sub> =15 nM IC <sub>50</sub> =13 nM
GSK126		EZH2 EZH2 (mutation)	EZH2 IC <sub>50</sub> =9.9 nM
CPI-169		EZH2 EZH2 Y641N EZH1 NHL	IC <sub>50</sub> =0.24 nM IC <sub>50</sub> =0.51 nM IC <sub>50</sub> =6.1 nM

Table 1 (continued)

EPZ005687	 <p>The structure of EPZ005687 features a central benzimidazole ring system. It is substituted with a cyclopentyl group at the 2-position, a 4-(benzyl(morpholin-2-yl)amino)phenyl group at the 5-position, and a 2-(2,4-dimethyl-5-oxo-1H-imidazol-1-yl)ethyl group at the 6-position.</p>	EZH2 EZH2 (Tyr641) EZH2 (Ala677) Lymphoma	EZH2, $K_i=24$ nM
EPZ011989	 <p>The structure of EPZ011989 consists of a central benzimidazole core. It is substituted with a 2-(2-(2-methoxyethyl)ethylamino)ethyl group at the 2-position, a 4-(2-(2-methoxyethyl)ethylamino)phenyl group at the 5-position, and a 2-(2,4-dimethyl-5-oxo-1H-imidazol-1-yl)ethyl group at the 6-position.</p>	EZH2 EZH2 (Y641F) B cell lymphoma	EZH2, $K_i<3$ nM
ZLD10A	 <p>The structure of ZLD10A features a central benzimidazole ring system. It is substituted with a 2-(2-(2-methoxyethyl)ethylamino)ethyl group at the 2-position, a 4-(2-(2-methoxyethyl)ethylamino)phenyl group at the 5-position, and a 2-(2,4-dimethyl-5-oxo-1H-imidazol-1-yl)ethyl group at the 6-position.</p>	EZH2 EZH2 (Y641F) EZH2 (A677G) NHL	$IC_{50}=18.6$ nM $IC_{50}=27.1$ nM $IC_{50}=0.9$ nM
GSK503	 <p>The structure of GSK503 features a central benzimidazole ring system. It is substituted with a 2-(2-(2-methoxyethyl)ethylamino)ethyl group at the 2-position, a 4-(2-(2-methoxyethyl)ethylamino)phenyl group at the 5-position, and a 2-(2,4-dimethyl-5-oxo-1H-imidazol-1-yl)ethyl group at the 6-position.</p>	EZH2 Diffuse large B cell lymphoma	$K_i\approx 3\sim 27$ nM

**Table 1** (continued)

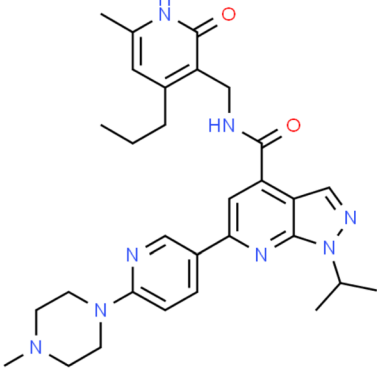
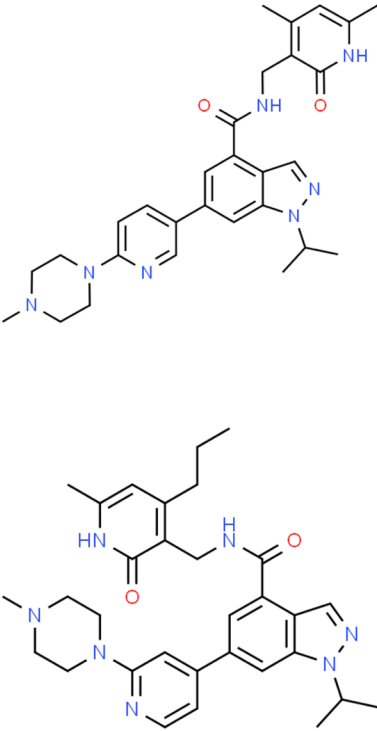
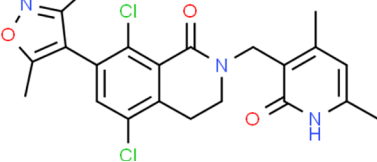
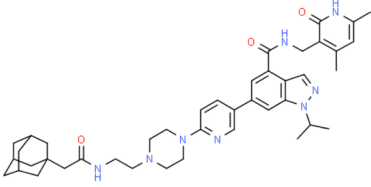
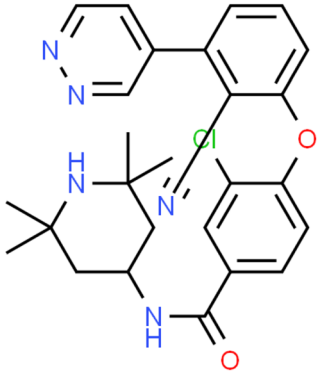
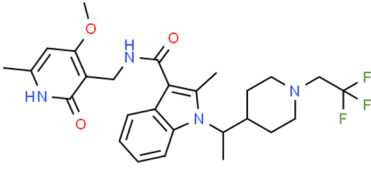
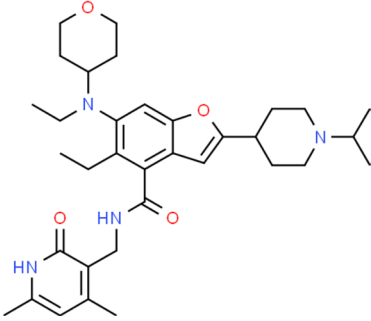
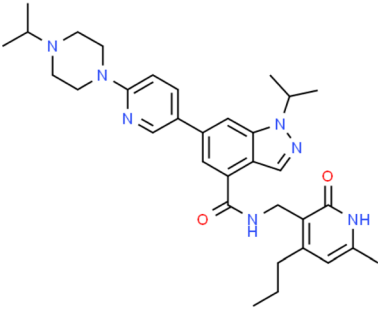
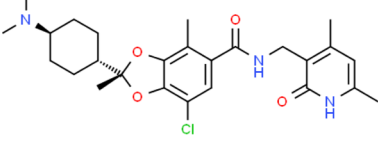
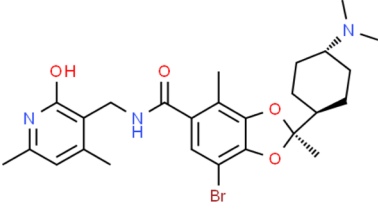
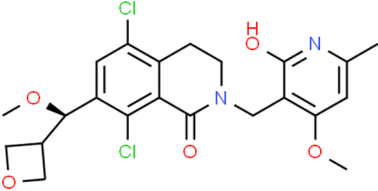
<p>JQEZ5</p>		<p>EZH2 CML</p>	<p>IC<sub>50</sub>=11.1 nM</p>
<p>GSK926 GSK343</p>		<p>EZH2 Breast cancer Prostate cancer</p>	<p>IC<sub>50</sub>=324±174 nM IC<sub>50</sub>=174±84 nM</p>
<p>PF-06726304</p>		<p>EZH2 EZH2(Y641N) B cell lymphoma</p>	<p>Ki=0.7 nM Ki=3 nM</p>

Table 1 (continued)

MS1943		EZH2 Prostate cancer Breast cancer Lymphoma	IC <sub>50</sub> =120 nM
EZH2-IN-3		EZH2 EZH1 Diffuse large B cell lymphoma	IC <sub>50</sub> =0.032±0.019 nM
CPI-1205		EZH2 EZH1 Multiple myeloma Plasma cell tumor	IC <sub>50</sub> =2 nM IC <sub>50</sub> =52 nM
EBI-2511		EZH2(A667G) NHL	IC <sub>50</sub> =4 nM

**Table 1** (continued)

<p>UNC1999</p>	 <p>The structure of UNC1999 features a central benzimidazole ring system. It is substituted with a piperazine ring at the 2-position, a pyridine ring at the 4-position, and a 2-ethyl-5-methyl-1H-imidazole-4-carbonyl group at the 5-position.</p>	<p>EZH2 EZH1 Diffuse large B cell lymphoma</p>	<p>IC<sub>50</sub>=2 nM IC<sub>50</sub>=45 nM</p>
<p>Valemetostat</p>	 <p>Valemetostat consists of a central benzimidazole core. It is substituted with a 4-(dimethylamino)cyclohexane ring at the 2-position, a 4-chlorophenyl ring at the 4-position, and a 2-(2,4-dimethyl-1H-imidazol-5-yl)ethyl group at the 5-position.</p>	<p>EZH1/2 T cell leukemia lymphoma</p>	<p>IC<sub>50</sub> ≤ 10 nM</p>
<p>(R)-OR-S1</p>	 <p>(R)-OR-S1 features a central benzimidazole ring. It is substituted with a 2-hydroxy-5-methylpyridin-3-ylmethyl group at the 2-position, a 4-bromophenyl ring at the 4-position, and a 1-(2,2-dimethylcyclohexyl)ethyl group at the 5-position.</p>	<p>EZH1 EZH2 Diffuse large B cell lymphoma</p>	<p>IC<sub>50</sub>=7.4 nM IC<sub>50</sub>=10 nM</p>
<p>PF-06821497</p>	 <p>PF-06821497 has a central benzimidazole core. It is substituted with a 2,4-dichlorophenyl ring at the 2-position, a 4-(2-methoxyphenyl)ethyl group at the 4-position, and a 2-(2-methoxyphenyl)ethyl group at the 5-position.</p>	<p>EZH2 (Y641N) Diffuse large B cell lymphoma</p>	<p>Ki=1.15nM</p>





**Table 2** Clinical trials of EZH2/PRC2 inhibitors, the data comes from ClinicalTrials.gov

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT05018975	Tazemetostat for the Treatment of Moderate to Severe COVID-19 Infection	Not yet recruiting	<ul style="list-style-type: none"> <li>• COVID-19 Acute Respiratory Distress Syndrome</li> <li>• Cytokine Release Syndrome</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 2
NCT02875548	Tazemetostat Rollover Study (TRuST): An Open-Label, Rollover Study	Active, not recruiting	<ul style="list-style-type: none"> <li>• Diffuse Large B cell Lymphoma (DLBCL)</li> <li>• Follicular Lymphoma (FL)</li> <li>• Synovial Sarcoma</li> <li>• Epithelioid Sarcoma (ES)</li> <li>• Mesothelioma</li> <li>• Advanced Solid Tumors</li> <li>• Renal Medullary Carcinoma</li> <li>• Non-Hodgkin Lymphoma (NHL)</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 2
NCT03009344	A Phase I Study of Tazemetostat in Patients With Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma	Completed	<ul style="list-style-type: none"> <li>• Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 1
NCT05023655	Phase II Study of Tazemetostat in Solid Tumors Harboring an ARID1A Mutation	Not yet recruiting	<ul style="list-style-type: none"> <li>• Solid Tumor</li> <li>• ARID1A Gene Mutation</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 2
NCT04590820	Oral Tazemetostat in Combination With Rituximab in R/R FL	Terminated	<ul style="list-style-type: none"> <li>• Follicular Lymphoma</li> <li>• Non-Hodgkin Lymphoma</li> <li>• Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> <li>• Drug: Rituximab</li> </ul>	Phase 2
NCT02860286	Study of the EZH2 Inhibitor Tazemetostat in Malignant Mesothelioma	Completed	<ul style="list-style-type: none"> <li>• Mesothelioma</li> <li>• BAP1 Loss of Function</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 2

Table 2 (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT03010982	Open-Label, Multi-Center, Two-Part, Phase I Study to Characterize the PKs of an Intravenous Micro-Dose of [ <sup>14</sup> C]-Tazemetostat (EPZ 6438) and the ADME of an Oral [ <sup>14</sup> C]-Labeled Dose of Tazemetostat in Subjects With B-Cell Lymphomas or Adv Solid Tumors	Completed	<ul style="list-style-type: none"> <li>• Diffuse Large B Cell Lymphoma</li> <li>• Primary Mediastinal Lymphoma</li> <li>• Mantle Cell Lymphoma</li> <li>• Follicular Lymphoma</li> <li>• Marginal Zone Lymphoma</li> <li>• Advanced Solid Tumors</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat and [<sup>14</sup>C] Tazemetostat</li> </ul>	Phase 1
NCT04917042	Tazemetostat in Malignant Peripheral Nerve Sheath Tumors	Recruiting	<ul style="list-style-type: none"> <li>• Peripheral Nerve Sheath Tumor</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 2

activity in Pfeiffer and KARPAS-422 cell lines which carrying EZH2 mutations, the IC<sub>50</sub> is 20 pM and 0.5 nM. EEDi5285 is also possess an excellent pharmacokinetic data and achieve complete tumor treatment effects on the KARPAS-422 xenograft model in mice [101]. Furthermore, EEDi-5273, a class of highly effective EED inhibitors with oral bioavailability greater than EEDi5285, was discovered through conformal restriction and system structure–activity relationship studies. With an IC<sub>50</sub> value of 0.2 nM, EEDi-5273 has a high affinity for EED. With an IC<sub>50</sub> value of 1.2 nM, EEDi-5233 effectively inhibits the growth of KARPAS-422 cells and achieves good tumor treatment effects in xenotransplantation animal models without side effects [102] (Table 1).

### The impact of natural products on EZH2 and PRC2

Natural products derived from plants have been proven that has good anti-cancer activity, such as paclitaxel and vincristine. There are also a large number of natural products that can target EZH2. Gambogic acid (GNA) is derived from *Garcinia hanburyi* (*Garcinia hanburyi Hook.f*). It could bind to Cys668 in the EZH2 SET domain specifically and trigger ubiquitination of EZH2. According to the structure of GNA, the researchers have designed GNA002, which has much better activity. Through a xenograft tumor model confirm that when the anti-tumor activity is close, GNA002 has better safety than Cisplatin [103]. 16-Hydroxycyclohexa-3,13-dien-15,16-olide (PL3) is a diterpene compound isolated from *Polyalthia longifolia*, which could significantly inhibit EZH2 and SUZ12 in the PRC2 complex in dose-dependent manner, reduce the inhibition of Msk1, Set7, and Src1 genes by PRC2, and finally induce apoptosis of K562 cells [104]. Tanshinone I, a diterpene compound derived from *Salvia miltiorrhiza*. Tanshinone I could directly bind to EZH2 (Kd = 94.475 μM), thereby inhibiting the activity of PRC2 and the level of H3K27Me3. Tanshinone I could also increase the expression of MMP9 and ABCG2 and limit the growth of leukemia cells and function of malignant hematopoietic [105]. Curcumin is a natural polyphenol component, mainly derived from the rhizomes of turmeric (*Curcuma longa Linn*). Curcumin could inhibit the expression of EZH2, ASXL1, H3K27me3, and HOXA9 significantly. Curcumin could inhibit the proliferation and induce apoptosis in MDS cell lines from human and show anti-tumor activity in xenograft model mice [106]. Others like Triptolide, Ursolic acid, and Resveratrol are shown inhibitory to EZH2 [107].

Table 1 (continued)

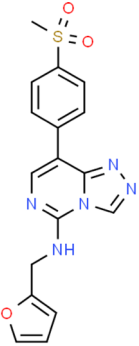
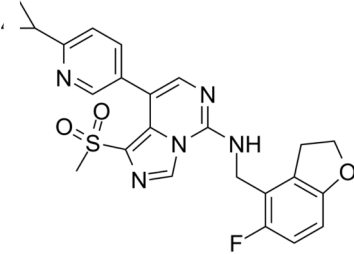
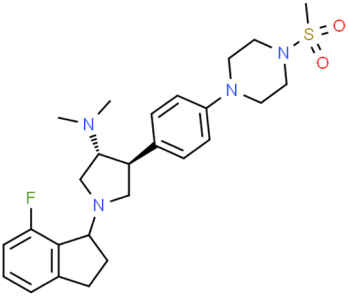
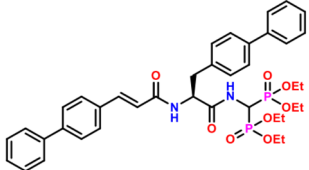
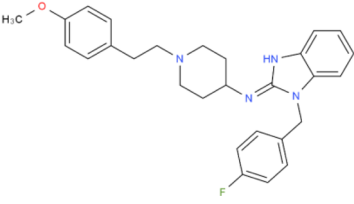
<p>EED226</p>		<p>EED EZH2 (Y641N ) Diffuse large B cell lymphoma</p>	<p>Anti-KARPAS-422 proliferation: IC<sub>50</sub>=80 nM</p>
<p>EEDi5285</p>		<p>EED Diffuse large B cell lymphoma</p>	<p>IC<sub>50</sub>=0.2 nM</p>
<p>A-395</p>		<p>EED Diffuse large B cell lymphoma</p>	<p>IC<sub>50</sub>=18±2 nM</p>
<p>LG-1980</p>		<p>EED Chemoresistant prostate cancers</p>	<p>ARCaP<sub>E</sub>-shCtrl (IC<sub>50</sub>=0.26 μM) C4-B-TaxR (IC<sub>50</sub>=6.87 μM).</p>
<p>Astemizole</p>		<p>Inhibited the EZH2 binding to EED  GCB-DLBCL cell lines</p>	<p>Ki=23.01 μM  IC<sub>50</sub>=3.0 μM</p>

Table 1 (continued)

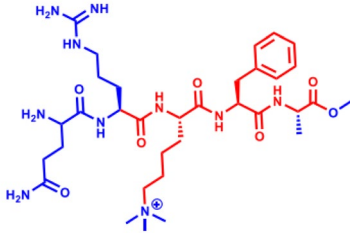
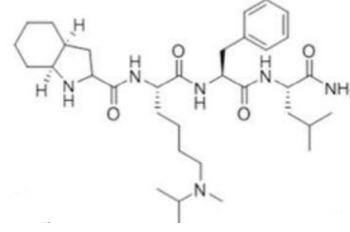
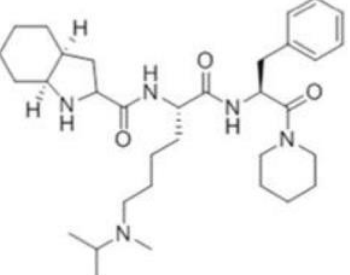
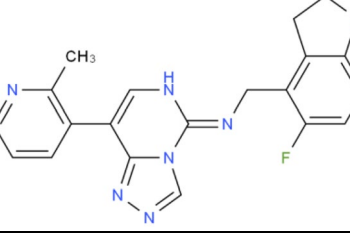
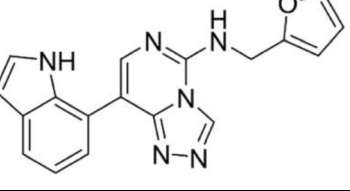
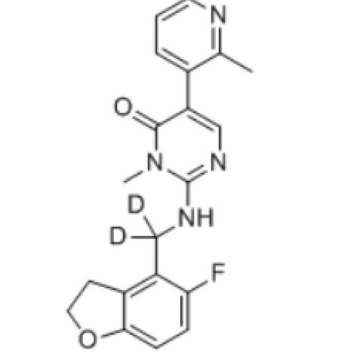
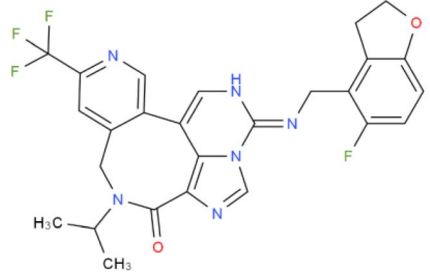
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UNC5114		EED	Kd=0.68 $\mu$ M
UNC5115		EED	Kd=1.14 $\mu$ M
MAK683		EED Pfeiffer cells	IC <sub>50</sub> =60nM GI <sub>50</sub> =4 nM GI <sub>90</sub> =8 nM
ZJH16		EED KARPAS422 Pfeiffer cells	IC <sub>50</sub> =0.22 $\mu$ M IC <sub>50</sub> =0.13 $\mu$ M
BR-001		EED KARPAS422 cells	IC <sub>50</sub> =4.5 nM in competition binding assay



Table 1 (continued)

EEDi-5273		EED  KARPAS422 cells	EEDi-5273 binds to EED with an $IC_{50}=0.2$ nM  $IC_{50}=1.2$ nM
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### Analysis of the current situation and prospects for EZH2 inhibitors

EZH2 encourages carcinogenesis and malignant transformation and is highly expressed in a variety of human cancers. Therefore, EZH2-targeted inhibition is a perfect drug design target. According to the aforementioned research, there is still a ton of flexibility in the structure and choice of targets for EZH2 inhibitors. By examining the structures of the “Deep Pocket,” “Aromatic Cage,” and “Tail,” researchers have continuously improved the main skeleton of EZH2 and EED inhibitors. They have also adopted strategies like changing substituents to improve the physicochemical characteristics and affinity of EZH2 inhibitors.

At this point, the main emphasis is on the EZH2 SET domain and disabling the interaction between EZH2-EED, which will allow for the correction of epigenetic flaws and the elimination of cancer. This approach has performed well in the treatment of some hematological tumors, but the effectiveness of EZH2-related inhibitors in treating some solid tumors has not been satisfactory [108]. It is well known that EZH2 can be used as a single active molecule to extensively regulate physiological activities and that its role does not only depend on the activity of the PRC2-dependent methyltransferase. However, the non-classical function of EZH2 is largely ignored, and the design of existing inhibitors is only restricted to the pertinent domains of the methyltransferase function. Only by defining the non-classical function of EZH2's mode of action will it be possible to develop EZH2 inhibitors with more thorough functionality. For instance, scientists have shown that EZH2's N-terminal has a transcriptional activation region that mediates the binding of EZH2 to

c-Myc, p300, and SMARCA4, thereby accelerating the growth of tumors. Based on this, scientists have developed a new class of EZH2 small-molecule degrading agent called MS177 that can significantly inhibit c-Myc-mediated gene upregulation in addition to targeting the catalytic and non-catalytic functions of EZH2. This agent can also degrade the components of the PRC2 complex. This scenario indicates that it is impossible to overlook EZH2's non-classical catalytic function [109]. Existing inhibitors have functional flaws as a result of the diversity of EZH2 activities. At the same time, it is important to keep in mind that using EZH2 inhibitors might actually increase cell proliferation and improve DNA damage repair capabilities, which promotes the growth of tumors [110]. According to research, EZH2 inhibitors can activate H3K27ac, which makes cells more resistant to drugs. The therapeutic efficacy of tumors can be improved by giving BRD4 inhibitors to cells that have lost their sensitivity to EZH2 inhibitors [111]. In addition, CDK1, HSP90, or proteasome inhibitors prevent EZH2 from being degraded in AML cells, restoring the cells' sensitivity to medicines [112].

In summary, EZH2 plays a role in metabolic pathways, inflammation, and tumor growth [113, 114]. In addition, Tazemetostat or similar substance has brought EZH2 to the public notice as a crucial regulatory molecule of epigenetics, which gives us significant confidence that blocking EZH2 will eradicate the tumor. However, we should also be aware of the EZH2 inhibitors' current drawbacks, such as their single target function and the risk of drug resistance and illness aggravation associated with overusing them. This necessitates a thorough assessment of EZH2's structure and function, as well as the utilization of numerous pharmacological combination therapies to control the dangers associated with EZH2 inhibitors.

Table 2 (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT02601950	A Phase II, Multicenter Study of the EZH2 Inhibitor Tazemetostat in Adult Subjects With INI1-Negative Tumors or Relapsed/Refractory Synovial Sarcoma	Recruiting	<ul style="list-style-type: none"> <li>• Malignant Rhabdoid Tumors (MRT)</li> <li>• Rhabdoid Tumors of the Kidney (RTK)</li> <li>• Atypical Teratoid Rhabdoid Tumors (ATRT)</li> <li>• Selected Tumors With Rhabdoid Features</li> <li>• Synovial Sarcoma</li> <li>• INI1-negative Tumors</li> <li>• Malignant Rhabdoid Tumor of Ovary</li> <li>• Renal Medullary Carcinoma</li> <li>• Epithelioid Sarcoma</li> <li>• Poorly Differentiated Chordoma (or Other Chordoma With Sponsor Approval)</li> <li>• Any Solid Tumor With an EZH2 GOF Mutation</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 2
NCT04624113	Tazemetostat and Pembrolizumab in Patients With Pembrolizumab- or Nivolumab-Resistant, Recurrent, or Metastatic Head and Neck Squamous Cell Carcinoma	Recruiting	<ul style="list-style-type: none"> <li>• Head and Neck Squamous Cell Carcinoma</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> <li>• Drug: Pembrolizumab</li> </ul>	<ul style="list-style-type: none"> <li>• Phase 1</li> <li>• Phase 2</li> </ul>
NCT04241835	A Study of Oral Tazemetostat in Subjects With Moderate and Severe Hepatic Impairment With Advanced Malignancies	Recruiting	<ul style="list-style-type: none"> <li>• Hepatic Impairment</li> <li>• Advanced Malignant Solid Tumor</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tazemetostat</li> </ul>	Phase 1

**Table 2** (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT01897571	Open-Label, Multicenter, Phase 1/2 Study of Tazemetostat (EZH2 Histone Methyltransferase [HMT] Inhibitor) as a Single Agent in Subjects With Adv. Solid Tumors or With B-cell Lymphomas and Tazemetostat in Combination With Prednisolone in Subjects With DLBCL	Active, not recruiting	<ul style="list-style-type: none"> <li>B-cell Lymphomas (Phase 1)</li> <li>Advanced Solid Tumors (Phase 1)</li> <li>Diffuse Large B-cell Lymphoma (Phase 2)</li> <li>Follicular Lymphoma (Phase 2)</li> <li>Transformed Follicular Lymphoma</li> <li>Primary Mediastinal Large B-Cell Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	<ul style="list-style-type: none"> <li>Phase 1</li> <li>Phase 2</li> </ul>
NCT02601937	A Phase 1 Study of the EZH2 Inhibitor Tazemetostat in Pediatric Subjects With Relapsed or Refractory IN11-Negative Tumors or Synovial Sarcoma	Active, not recruiting	<ul style="list-style-type: none"> <li>Rhabdoid Tumors</li> <li>INI1-negative Tumors</li> <li>Synovial Sarcoma</li> <li>Malignant Rhabdoid Tumor of Ovary</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	Phase 1
NCT04762160	SYMPHONY-2, A Trial to Examine Combination of Tazemetostat and Rituximab for Patients With Relapsed/Refractory Follicular Lymphoma	Recruiting	<ul style="list-style-type: none"> <li>Follicular Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	Phase 2
NCT03456726	Study of Tazemetostat in Participants With Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma With EZH2 Gene Mutation	Active, not recruiting	<ul style="list-style-type: none"> <li>Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	Phase 2
NCT03217253	Tazemetostat in Treating Patients With Metastatic or Unresectable Solid Tumors or B-Cell Lymphomas With Liver Dysfunction	Withdrawn	<ul style="list-style-type: none"> <li>Ann Arbor Stage III B-Cell Non-Hodgkin Lymphoma</li> <li>Ann Arbor Stage IV B-Cell Non-Hodgkin Lymphoma</li> <li>Metastatic Malignant Solid Neoplasm</li> <li>Stage III Hepatocellular Carcinoma AJCC v7</li> <li>Stage IIIA Hepatocellular Carcinoma AJCC v7</li> <li>Stage IIIB Hepatocellular Carcinoma AJCC v7</li> <li>Stage IIIC Hepatocellular Carcinoma AJCC v7</li> <li>Stage IV Hepatocellular Carcinoma AJCC v7</li> <li>Stage IVA Hepatocellular Carcinoma AJCC v7</li> <li>Stage IVB Hepatocellular Carcinoma AJCC v7</li> <li>Unresectable Solid Neoplasm</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	Phase 1

Table 2 (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT03213665	Tazemetostat in Treating Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With EZH2, SMARCB1, or SMARCA4 Gene Mutations (A Pediatric MATCH Treatment Trial)	Active, not recruiting	<ul style="list-style-type: none"> <li>Advanced Malignant Solid Neoplasm</li> <li>Ann Arbor Stage III Hodgkin Lymphoma</li> <li>Ann Arbor Stage III Non-Hodgkin Lymphoma</li> <li>Ann Arbor Stage IV Hodgkin Lymphoma</li> <li>Ann Arbor Stage IV Non-Hodgkin Lymphoma</li> <li>Ewing Sarcoma/Peripheral Primitive Neuroectodermal Tumor</li> <li>Low-Grade Glioma</li> <li>Recurrent Ependymoma</li> <li>Recurrent Ewing Sarcoma</li> <li>Recurrent Glioma and 31 more</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	Phase 2
NCT04537715	A Two-part Study to Characterize Drug-Drug Interaction Effects on Steady-State Pharmacokinetics of Oral Tazemetostat	Recruiting	<ul style="list-style-type: none"> <li>All Malignancies</li> <li>Advanced Malignancies</li> <li>Hematologic Malignancy</li> <li>Solid Tumor</li> <li>Follicular Lymphoma (FL)</li> <li>Non-Hodgkin Lymphoma (NHL)</li> <li>Diffuse Large B-Cell Lymphoma (DLBCL)</li> <li>Epithelioid Sarcoma (ES)</li> <li>Synovial Sarcoma</li> <li>Renal Medullary Carcinoma</li> <li>Mesothelioma</li> <li>Rhabdoid Tumor</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> <li>Drug: Itraconazole</li> <li>Drug: Rifampin</li> </ul>	Phase 1
NCT04204941	Tazemetostat in Combination With Doxorubicin as Frontline Therapy for Advanced Epithelioid Sarcoma	Recruiting	<ul style="list-style-type: none"> <li>Advanced Soft Tissue Sarcoma</li> <li>Advanced Epithelioid Sarcoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> <li>Drug: Placebo</li> <li>Drug: Doxorubicin HCl</li> </ul>	Phase 3
NCT04846478	Phase Ia/Ib Talazoparib + Tazemetostat for mCRPC	Recruiting	<ul style="list-style-type: none"> <li>Metastatic Prostate Cancer</li> <li>Metastatic Castration-resistant Prostate Cancer</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Talazoparib</li> <li>Drug: Tazemetostat</li> </ul>	Phase 1
NCT03854474	Tazemetostat and Pembrolizumab in Treating Patients With Locally Advanced or Metastatic Urothelial Carcinoma	Recruiting	<ul style="list-style-type: none"> <li>Locally Advanced Urothelial Carcinoma</li> <li>Metastatic Urothelial Carcinoma</li> <li>Stage III Bladder Cancer AJCC v8</li> <li>Stage IIIA Bladder Cancer AJCC v8</li> <li>Stage IIIB Bladder Cancer AJCC v8</li> <li>Stage IV Bladder Cancer AJCC v8</li> <li>Stage IVA Bladder Cancer AJCC v8</li> <li>Stage IVB Bladder Cancer AJCC v8</li> </ul>	<ul style="list-style-type: none"> <li>Biological: Pembrolizumab</li> <li>Drug: Tazemetostat</li> </ul>	Phase 1 Phase 2

**Table 2** (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT03348631	Tazemetostat in Treating Patients With Recurrent Ovarian or Endometrial Cancer	Suspended	<ul style="list-style-type: none"> <li>FIGO Grade 1 Endometrial Endometrioid Adenocarcinoma</li> <li>FIGO Grade 2 Endometrial Endometrioid Adenocarcinoma</li> <li>Recurrent Endometrial Endometrioid Adenocarcinoma</li> <li>Recurrent Ovarian Carcinoma</li> <li>Recurrent Ovarian Clear Cell Adenocarcinoma</li> <li>Recurrent Ovarian Endometrioid Adenocarcinoma</li> <li>Recurrent Uterine Corpus Cancer</li> <li>Metastatic Prostate Cancer</li> </ul>	<ul style="list-style-type: none"> <li>Other: Laboratory Biomarker Analysis</li> <li>Drug: Tazemetostat</li> </ul>	Phase 2
NCT04179864	Study of Tazemetostat With Enzalutamide or Abiraterone/Prednisone in Subjects With Castration-Resistant Prostate Cancer Who Have Not Received Chemotherapy	Recruiting	<ul style="list-style-type: none"> <li>Metastatic Prostate Cancer</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> <li>Drug: Abiraterone/prednisone</li> <li>Drug: Enzalutamide</li> </ul>	Phase 1 Phase 2
NCT04557956	Testing the Addition of the Anti-cancer Drug, Tazemetostat, to the Usual Treatment (Dabrafenib and Trametinib) for Metastatic Melanoma That Has Progressed on the Usual Treatment	Recruiting	<ul style="list-style-type: none"> <li>Clinical Stage IV Cutaneous Melanoma AJCC v8</li> <li>Metastatic Malignant Neoplasm in the Central Nervous System</li> <li>Metastatic Melanoma</li> <li>Pathologic Stage IV Cutaneous Melanoma AJCC v8</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Dabrafenib mesylate</li> <li>Drug: Tazemetostat</li> <li>Hydrobromide</li> <li>Drug: Trametinib Dimethyl Sulfoxide</li> </ul>	Phase 1 Phase 2
NCT03028103	Open-Label, Multicenter, Two-Part, Phase 1 Study to Characterize Effects of a Moderate CYP3A Inhibitor on PK of Tazemetostat, Effects of Tazemetostat on PK of CYP2C8 and CYP2C19 Substrates, and Effect of Increased Gastric pH on PK of Tazemetostat in B-cell Lymphoma or Advanced Solid Tumor Patients	Completed	<ul style="list-style-type: none"> <li>Diffuse Large B-Cell Lymphoma</li> <li>Primary Mediastinal Lymphoma</li> <li>Mantle Cell Lymphoma</li> <li>Advanced Solid Tumor</li> <li>Marginal Zone Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> <li>Drug: Fluconazole</li> <li>Drug: Omeprazole</li> <li>Drug: Repaglinide</li> </ul>	Phase 1
NCT03874455	Tazemetostat Expanded Access Program for Adults With Solid Tumors	Available	<ul style="list-style-type: none"> <li>Epithelioid Sarcoma (Ex-US Only)</li> <li>Spindle Cell Sarcoma</li> <li>Sinonasal Carcinoma</li> <li>Small Cell Carcinoma of the Ovary Hypercalcemic Type</li> <li>Thoracic Sarcoma</li> <li>Poorly Differentiated Chordoma</li> <li>Epithelioid Sarcoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	
NCT04225429	Tazemetostat Expanded Access Program for Adults With Epithelioid Sarcoma	No longer available	<ul style="list-style-type: none"> <li>Epithelioid Sarcoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> </ul>	



Table 2 (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT04224493	Study in Subjects With Relapsed/Refractory Follicular Lymphoma	Recruiting	<ul style="list-style-type: none"> <li>Relapsed/Refractory Follicular Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> <li>Drug: Placebo oral tablet</li> <li>Combination Product: Lenalidomide</li> <li>Combination Product: Rituximab</li> </ul>	Phase 3
NCT02220842	A Safety and Pharmacology Study of Atezolizumab (MPDL3280A) Administered With Obinutuzumab or Tazemetostat in Participants With Relapsed/Refractory Follicular Lymphoma and Diffuse Large B-cell Lymphoma	Completed	<ul style="list-style-type: none"> <li>Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Atezolizumab</li> <li>Drug: Obinutuzumab</li> <li>Drug: Tazemetostat</li> </ul>	Phase 1
NCT02889523	Study of Tazemetostat in Newly Diagnosed Diffuse Large B-Cell and Follicular Lymphoma Patients Treated by Chemotherapy	Recruiting	<ul style="list-style-type: none"> <li>Lymphoma</li> <li>DLBCL</li> <li>Follicular Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Tazemetostat</li> <li>Drug: Rituximab</li> <li>Drug: Cyclophosphamide</li> <li>Drug: Vincristine</li> <li>Drug: Doxorubicin</li> <li>Drug: Prednisolone</li> </ul>	<ul style="list-style-type: none"> <li>Phase 1</li> <li>Phase 2</li> </ul>
NCT04705818	Combining Epigenetic And Immune Therapy to Beat Cancer	Recruiting	<ul style="list-style-type: none"> <li>Advanced Solid Tumor</li> <li>Advanced Colorectal Carcinoma</li> <li>Advanced Soft tissue Sarcoma</li> <li>Advanced Pancreatic Adenocarcinoma</li> <li>Adult Solid Tumor</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Durvalumab</li> <li>Drug: Tazemetostat</li> </ul>	Phase 2
NCT02082977	A Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics and Clinical Activity of GSK2816126 in Subjects With Relapsed/Refractory Diffuse Large B-Cell Lymphoma, Transformed Follicular Lymphoma, Other Non-Hodgkin's Lymphomas, Solid Tumors and Multiple Myeloma	Terminated	<ul style="list-style-type: none"> <li>Cancer</li> <li>Neoplasms</li> </ul>	<ul style="list-style-type: none"> <li>Drug: GSK2816126</li> </ul>	Terminated
NCT02395601	A Study Evaluating CPI-1205 in Patients With B-Cell Lymphomas	Completed	B-Cell Lymphoma	Drug: CPI-1205	Phase 1
NCT03525795	ORION-E: A Study Evaluating CPI-1205 in Patients With Advanced Solid Tumors	Completed	Advanced Solid Tumors	Drug: CPI-1205 Drug: ipilimumab	Phase 1
NCT03480646	ProSTAR: A Study Evaluating CPI-1205 in Patients With Metastatic Castration-Resistant Prostate Cancer	Active, not recruiting	Metastatic Castration -Resistant Prostate Cancer (mCRPC)	Drug: CPI-1205 Drug: Enzalutamide Drug: Abiraterone/Prednisone	Phase 1 Phase 2
NCT04703192	Valemetostat Tosylate (DS-3201b), an Enhancer of Zeste Homolog (EZH) 1/2 Dual Inhibitor, for Relapsed/Refractory Peripheral T-Cell Lymphoma (VALENTINE PTCL01)	Recruiting	<ul style="list-style-type: none"> <li>Relapsed/Refractory Peripheral T-Cell Lymphoma</li> <li>Adult T-Cell Leukemia/Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>Drug: Valemetostat tosylate</li> </ul>	Phase 2
NCT04842877	Study of Valemetostat Tosylate as a Single Agent in Patients With Relapse/Refractory B-cell Lymphoma	Recruiting	Lymphoma, B-Cell	Drug: Valemetostat tosylate	Phase 2

**Table 2** (continued)

NCT Number	Title	Status	Conditions or disease	Interventions	Phase
NCT04102150	Valemetostat Tosylate (DS-3201b) Phase 2 Study in Relapsed or Refractory Adult T-cell Leukemia/Lymphoma	Active, not recruiting	Adult T-cell Leukemia/Lymphoma	Drug: Valemetostat tosylate	Phase 2
NCT04388852	DS-3201 and Ipilimumab for the Treatment of Metastatic Prostate, Urothelial and Renal Cell Cancers	Recruiting	<ul style="list-style-type: none"> <li>Aggressive Variant Prostate Carcinoma</li> <li>Castration-Resistant Prostate Carcinoma</li> <li>Metastatic Clear Cell Renal Cell Carcinoma</li> <li>Metastatic Malignant Solid Neoplasm</li> <li>Metastatic Prostate Carcinoma</li> <li>Metastatic Urothelial Carcinoma</li> <li>Stage IV Prostate Cancer AJCC v8</li> <li>Stage IV Renal Cell Cancer AJCC v8</li> <li>Stage IVA Prostate Cancer AJCC v8</li> <li>Stage IVB Prostate Cancer AJCC v8</li> </ul>	Drug: Valemetostat	Phase 1
NCT03460977	PF-06821497 Treatment Of Relapsed/Refractory SCLC, Castration-Resistant Prostate Cancer, and Follicular Lymphoma	Recruiting	<ul style="list-style-type: none"> <li>Small Cell Lung Cancer (SCLC)</li> <li>Follicular Lymphoma (FL)</li> <li>Castration-Resistant Prostate Cancer (CRPC)</li> </ul>	Drug: PF-06821497	Phase 1
NCT05773586	A Study to Investigate the Safety, Pharmacokinetics and Efficacy of APG-5918 in Healthy Subjects or Anemic Patients	Not yet recruiting	Anemia	Drug: APG-5918 (EEDj-5273) Drug: Placebo	Phase 1
NCT05415098	Study of Safety, Pharmacokinetic and Efficacy of APG-5918 in Advanced Solid Tumors or Lymphomas	Recruiting	Nasopharyngeal carcinoma castrate-resistant prostate cancer gastric cancer ovarian clear cell carcinoma mesothelioma sarcoma non-hodgkin lymphoma B-cell lymphoma epithelioid sarcoma	Drug: APG-5918	Phase 1
NCT02900651	Safety and Efficacy of MAK683 in Adult Patients With Advanced Malignancies	Active, not recruiting	Diffuse Large B-cell Lymphoma	Drug: MAK683	Phase 1
NCT04413201	AFAMOSI: Efficacy and Safety of Afatinib Folfowed by Osimertinib Compared to Osimertinib in Patients With EGFR-mutated/T790M Mutation-Negative Non-squamous NSCLC	Recruiting	Non-squamous NSCLC	Drug: Afatinib Drug: Osimertinib (AZD9291)	Phase 4
NCT03603951	A Phase 1 Study of SHR2554 in Subjects With Relapsed or Refractory Mature Lymphoid Neoplasms	Recruiting	Relapsed or Refractory Mature Lymphoid Neoplasms	Drug: SHR2554	Phase 1

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## Declarations

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