Journal of Pharmaceutical Analysis 13 (2023) 127–141

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Review paper

Novel insights into histone lysine methyltransferases in cancer therapy: From epigenetic regulation to selective drugs

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ARTICLE INFO

Article history:
Received 25 July 2022
Received in revised form
24 November 2022
Accepted 27 November 2022
Available online 30 November 2022

Keywords:
Cancer
Epigenetics
Histone lysine methyltransferase
Epigenetic inhibitors
Combined therapeutic strategy
Clinical application

ABSTRACT

The reversible and precise temporal and spatial regulation of histone lysine methyltransferases (KMTs) is essential for epigenome homeostasis. The dysregulation of KMTs is associated with tumor initiation, metastasis, chemoresistance, invasiveness, and the immune microenvironment. Therapeutically, their promising effects are being evaluated in diversified preclinical and clinical trials, demonstrating encouraging outcomes in multiple malignancies. In this review, we have updated recent understandings of KMTs' functions and the development of their targeted inhibitors. First, we provide an updated overview of the regulatory roles of several KMT activities in oncogenesis, tumor suppression, and immune regulation. In addition, we summarize the current targeting strategies in different cancer types and multiple ongoing clinical trials of combination therapies with KMT inhibitors. In summary, we endeavor to depict the regulation of KMT-mediated epigenetic landscape and provide potential epigenetic targets in the treatment of cancers.

1. Introduction

Eukaryotic gene expression is a complex process regulated by an interplay of genetic and epigenetic events at many different levels, including chromatin accessibility, transcription, RNA processing and stability, translation, and protein activity. Epigenetics is a branch of biology, and its concept was proposed by a British biologist named Conrad Waddington: inheritable changes in gene expression without alterations in DNA sequences [1]. With the expansion of the field of epigenetics, epigenetic alterations are divided into four levels of regulation of gene expression: DNA methylation, histone modification, chromatin remodeling, and regulation of noncoding RNAs [2].

Genomic DNA is wrapped around histone octamers (H2A, H2B, H3, and H4) to form nucleosomes, which are further folded and compressed to form chromatin at each level. Histone methyltransferases (HMTs) are epigenetic "writers" that affect gene transcription, DNA replication, and DNA repair, playing an important role in maintaining chromatin stability and regulating gene expression by catalyzing methyl transfer to specific lysine side chains at the ends of histones H3 and H4. HMTs can be classified into two families, namely, histone lysine methyltransferases (KMTs) and protein arginine methyltransferases, depending on their target residue. Most biological methylation reactions are catalyzed by methyltransferases with *S*-5′-adenosyl-L-methionine (SAM) as the methyl donor and there are approximately 100 putative KMT candidates encoded by the human genome [3]. KMTs are a class of enzymes that catalyze the transfer of the methyl group from the cofactor SAM to lysine residues of histone and nonhistone substrates [4,5]. Depending on the number of methyl groups added to the ε-nitrogen of a lysine side chain, mono-, di-, and tri-methylated groups are formed in the lysine residue (Kme1, Kme2, and Kme3), and trimers with trimethylation are generally regarded as the most mechanistically effective markers [6].

The first biochemically characterized lysine methyltransferase was described in 2000 [7]. To date, 49 HMTs have been known in

Peer review under responsibility of Xi'an Jiaotong University.
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https://doi.org/10.1016/j.jpha.2022.11.009

the human genome, and the most extensively studied histone methylation sites include histone H3 at lysine 4 (H3K4), lysine 9 (H3K9), lysine 27 (H3K27), lysine 36 (H3K36), lysine 79 (H3K79), and histone H4 at lysine 20 (H4K20) [8]. Methylation of different amino acid residues on histone 3 is associated with distinct transcriptional effects. H3K4me2/3, H3K36me3, and H3K79me1/2 are generally associated with transcriptional activation, whereas H3K9me2/3, H3K27me2/3, and H4K20me are linked to transcriptional repression [7]. These modifications have important functions in many biological processes, including heterochromatin formation, transcriptional regulation, DNA damage repair, cell growth, metabolism, and signal transduction [9]. In addition to histone methylation, other posttranslational histone modifications (e.g., acetylation and ubiquitination) also play critical roles in transmitting various regulatory signals in biological processes. H3K79 methylation by disruptor of telomeric silencing 1 (DOT1) is a prominent example of *trans*-histone crosstalk, a process in which one histone modification affects another histone modification [10,11]. The study claimed that acetylation of lysine 16 (H4K16ac) and histone H2B ubiquitination (H2BUb) allosterically stimulated H3K79 methylation by DOT1 [11,12].

HMTs have been reported to be closely associated with the development of many cancers and diseases, and their potential for use as therapeutic targets is unlimited [5]. Over the past decade, significant advances have been made in developing drugs to target KMTs involved in histone methylation and epigenetic regulation, but these drugs are at a relatively early stage compared with other therapeutic drugs. In 2020, the United States Food and Drug Administration (US FDA) approved the first KMT inhibitor (tazemetostat for epithelioid sarcoma (ES) and subsequently for follicular lymphoma) [13].

In this review, we provide an overview of the biologically relevant role of KMTs in cancers and the development in the therapeutic use of their inhibitors, highlighting the recent findings and values in combination with other therapeutic approaches (Fig. 1). First, we discuss the classification of KMTs, several of which are most widely described as drug targets in previous studies, such as enhancer of zeste homolog 2 (EZH2), disruptor of telomeric silencing 1-like (DOT1L), and euchromatic histone lysine methyltransferase 2 (also known as G9a). Then, we describe the research progress on inhibitors targeting these targets, focusing on the possible significance of KMT inhibitors in combination with other drugs in cancer treatment. The clinical application of EZH2 is a major advance in KMT inhibitor research, suggesting that EZH2 and other KMT inhibitors have great potential for cancer treatment. They can also be used in combination with conventional therapeutic modalities [14]. In addition to acting synergistically with classical chemotherapeutic agents and small molecule-targeted drugs, EZH2 and G9a can assist in achieving better efficacy with immune checkpoint therapy [15].

2. Biological function of KMTs in cancers

The regulatory effects of KMTs on tumor biological characteristics have been extensively studied by independent groups over the past two decades. Studies of KMTs have revealed an elaborate network connecting epigenetic regulation with tumorigenesis and metastasis. Thus, several KMTs have been pursued as therapeutic targets, including EZH2, DOT1L, and G9a [16,17]. A better understanding of KMTs would allow the development of a potent cancer management strategy.

2.1. EZH2 in cancer

EZH2 is a catalytic component of polycomb repressive complex 2 (PRC2), whose HMT activity catalyzes the trimethylation of lysine at position 27 of histone H3. PRC2 is a polycomb group protein core complex composed of three subunits: the catalytic subunit EZH2 or EZH1, scaffolding subunit suppressor of zeste 12, and embryonic ectoderm development (EED), which mediate chromatin compaction and gene repression [18]. The function of EZH2 as an HMT is performed mainly by its su(var)3-9, enhancer-of-zeste, trithorax (SET) domain. EZH2 can suppress or coactivate transcription in a PRC2-dependent or PRC2-independent manner. In addition to histones, it can also methylate nonhistone proteins [19,20]. For instance, PRC2-independent functions of EZH2 are involved in the interaction of EZH2 with non-PRC2 partners, such as the androgen receptor and nuclear factor-κB, in prostate cancer and breast cancer [21,22].

To date, numerous experimental results have indicated that EZH2 acts as a crucial epigenetic regulator governing diverse gene expressions in many aspects of human cancers. Elevated levels of EZH2 expression have been reported in lymphoma [23], lung cancer [24], prostate cancer [25], breast cancer [26], melanoma [27,28], retinoblastoma [29], and glioblastoma [30]. Notably, EZH2 temporarily silences genes involved in B-cell differentiation and withdraws them from the germinal centers of lymph nodes. When the EZH2 gene is mutated, B cells are unable to exit the germinal centers of the lymph nodes and eventually become tumorigenic, as in the case of follicular lymphoma [31]. Jin et al. [32] revealed that EZH2 is overexpressed in uveal melanoma (UM), where it increases the percentage and self-renewal of cancer stem cells (CSCs) via the microRNA (miR)-29c-disheveled segment polarity protein 2-β-catenin signaling pathway and facilitates migration and invasion via the Rho-GDP-dissociation inhibitor-gamma-Rac1 axis. They claimed that EZH2 can be an attractive and promising therapeutic target in UM [33,34]. Khan et al. [29] found that EZH2 is overexpressed in retinoblastoma (RB) tissues and cell lines by analyzing immunohistochemistry of 43 human RB tumor sections. EZH2 inhibition specifically impairs intracellular ATP production in RB cells in a time- and dose-dependent manner. Smith et al. [26] found a hitherto unknown c-Src/mechanistic target of rapamycin complex 1/PRC2 axis, which is pivotal in human epidermal growth factor receptor 2 (ErbB2)-mediated mammary tumorigenesis. In addition, Yang et al. [30] indicated that E2F transcription factor 7 promotes cell proliferation, cell metastasis, and tumorigenesis by regulating the EZH2-mediated phosphatase and tensin homolog (PTEN)/protein kinase B (AKT)/mechanistic target of rapamycin pathway in glioblastoma. However, prolonged EZH2 inhibition in glioblastoma may promote tumorigenesis, which indicates a dual role for EZH2 in tumorigenesis [35].

Interestingly, EZH2 does not always function as an oncogene, and its tumor-suppressive roles have been reported in many studies. For example, Shimizu et al. [36] found that deletion of EZH2 can synergize with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. Wang et al. [37] revealed that EZH2 loss dramatically promotes Kirsten rat sarcoma viral oncogene homologue (KRAS)-driven lung adenocarcinoma formation in a KRAS-driven antibody-drug conjugate mouse model, which demonstrated that EZH2 might act as a tumor suppressor. Mechanistically, EZH2 loss activates AKT and extracellular signal-regulated kinase by derepressing its target insulin-like growth factor 1. Therefore, pharmacological inhibition of EZH2 should be considered with caution because EZH2 may also have antitumor effects.

Tumors with enhanced EZH2 activity often exhibit an immunosuppressive tumor microenvironment (TME) and immunotherapy resistance. Recent studies have demonstrated that EZH2 might contribute to immune evasion by suppressing intratumoral antigen presentation and immune cell migration and enhancing $CD4^+$ T regulatory cells' suppressive activity [38]. In contrast, inhibition of

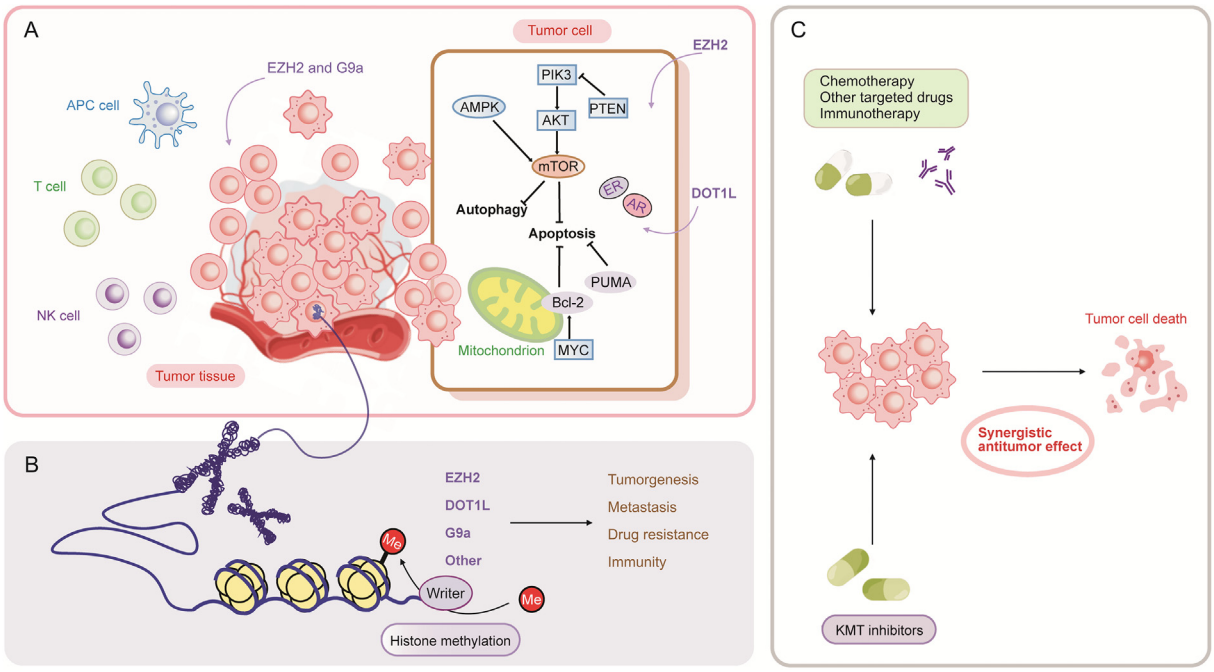

Fig. 1. The potential epigenetic targets and combination strategies in the treatment of cancers. (A) The tumor biological functions of histone lysine methyltransferases (KMTs). (B) The potential epigenetic targets of KMTs. (C) Combination strategies with conventional oncology treatments (chemotherapy, targeted therapy, immunotherapy). APC cell: antigen-presenting cell; NK cell: natural killer cell; EZH2: enhancer of zeste homolog 2; G9a: euchromatic histone lysine methyltransferase 2; PIK3: phosphatidylinositol 3-kinase; AKT: protein kinase B; AMPK: AMP-activated protein kinase; PTEN: phosphatase and tensin homolog; mTOR: mechanistic target of rapamycin; ER: estrogen receptor; AR: androgen receptor; DOT1L: disruptor of telomeric silencing 1-like; PUMA: p53-upregulated modulator of apoptosis; Bcl-2: B-cell lymphoma-2; MYC: myelocytomatosis.

EZH2 may stimulate the presentation of tumor antigens and increase the trafficking and function of immune effector cells in the TME [39]. Notably, many studies have proven that EZH2 can modulate the immune response to tumor cells by reducing their immunogenicity [40,41]. In summary, advancing the development of complementary therapeutic approaches combining immunotherapy with epigenetic reprogramming may be a valuable option.

EZH2 is also associated with targeted drug resistance. EZH2 induces acquired resistance to gefitinib in non-small cell lung cancer (NSCLC) by activating the phosphoinositide 3-kinase (PI3K)/AKT pathway [42]. In addition, Quan et al. [43] revealed that EZH2 was negatively correlated with resistance to epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs). Sun et al. [44] demonstrated that EZH2 regulates cisplatin resistance in ovarian cancer via the avian myelocytomatosis virus oncogene cellular homolog (c-Myc)-miR-137-EZH2 axis.

2.2. DOT1L in cancer

DOT1L is the only known HMT that catalyzes H3K79 methylation in mammals and plays crucial roles in embryogenesis and hematopoiesis. DOT1L is implicated in several processes, including transcription elongation by RNA polymerase II, DNA damage repair, and cell cycle checkpoint activation [45]. High expression of DOT1L was also observed in breast cancer [46], colorectal cancer [47], ovarian cancer [48], retinoblastoma, and mixed lineage leukemia (MLL) [49]. It is undisputed that DOT1L methyltransferase plays an essential role in MLL fusion-mediated leukemogenesis [50,51]. Moreover, the interaction between MLL fusion partners and DOT1L promotes the overexpression of MLL target genes, such as the *HOXA9* cluster and myeloid ecotropic viral integration site 1 (MEIS1), by inducing aberrant recruitment of DOT1L and ectopic H3K79 methylation [52]. The crosstalk between H4Kac/H2Bub/H3K79me may improve the previous understanding of the role that DOT1L plays in developmental processes and disease, including MLL1/KMT2A (*MLL*-rearranged) leukemia [11,53].

In addition to leukemia, DOT1L has been reported as a potential therapeutic target in other solid tumors, including ovarian cancer, breast cancer, and prostate cancer [54,55]. Chava et al. [48] revealed that DOT1L promoted ovarian cancer cell proliferation by regulating apoptotic and metabolic pathways as well as natural killer (NK)-cell-mediated eradication of ovarian cancer. Recently, Kurani et al. [46] found that DOT1L emerged as a key CSC regulator both in triple-negative breast cancers (TNBCs) and colorectal cancer. Nassa et al. [54] revealed that DOT1L blockade reduces the proliferation of hormone-responsive breast cancer cells by including estrogen receptors alpha and forkhead box protein A1 gene silencing. Another study showed that DOT1L selectively regulates the tumorigenicity of androgen receptor (AR)-positive prostate cancer cells, which suggests a more general role for DOT1L in regulating nuclear receptors [55]. Overall, this evidence provides a rationale for the use of DOT1L inhibitors with hormone therapy in cancer. Interestingly, DOT1L plays a protective role in ultraviolet radiation-induced melanoma development, in contrast to its oncogenic role in *MLL*-rearranged leukemia [56].

2.3. G9a in cancer

G9a is an HMT that specifically mediates the monomethylation and dimethylation of H3K9, which plays an essential role in the repression of gene transcription [57]. Multiple studies have reported elevated levels of G9a expression in various cancers, including hepatocellular carcinoma, bladder cancer, melanoma, neuroblastoma, and aggressive ovarian carcinoma [17,58,59]. Overexpression of G9a is linked to malignant behaviors of cancer cells (e.g., aberrant proliferation, metastasis, and drug resistance) by silencing tumor suppressors or activating epithelial-to-

mesenchymal transition programs [60–63]. Liu et al. [64] highlighted the important role of G9a in suppressing endogenous retroviruses in ovarian cancer cells. Ke et al. [65] revealed that G9a controlled glioblastoma cell proliferation and autophagy by transcriptionally regulating oncogene c-Myc expression in glioblastoma. Knockdown of the G9a gene induces chromosomal instability and inhibits cancer cell proliferation and invasion, supporting the idea that G9a is a promising drug target.

2.4. Other potential targets and their roles in cancer

SET domain bifurcated histone lysine methyltransferase 1 (SETDB1) reversibly catalyzes the di- and tri-methylation of H3K9 in euchromatic regions of chromosomes [66]. SETDB1 was identified as the mediator of immune escape after performing in vivo clustered regularly interspaced short palindromic repeats-associated protein 9 screens targeting 936 chromatin regulators in mouse tumor models treated with immune checkpoint blockade (ICB). In addition, amplification of SETDB1 was found to be related to immune exclusion and resistance to ICB in human tumors [67]. As is shown by previous studies, SETDB1 represents a novel candidate target for immunotherapy, and its inhibition may enable more patients to respond to immunotherapy.

SET domain-containing protein 2 (SETD2) is the only known HMT that can trimethylate the K36 residue of histone H3 in mammals. SETD2 is involved in various cellular processes and has been proven to mutate in a range of solid cancers, including renal cancer, prostate cancer, gastrointestinal cancer, and pancreatic cancer. Interestingly, SETD2-catalyzed H3K36me3 modification participates in crosstalk with other chromatin markers, including antagonizing H3K4me3 and H3K27me3 [68,69]. Furthermore, Yuan et al. [70] revealed that the tumor-suppressive function of SETD2 in prostate cancer is largely dependent on EZH2 modification and the AMP-activated protein kinase signaling pathway.

SET domain-containing protein 8 (SETD8) is the only lysine methyltransferase that can specifically monomethylate H4K20, which is involved in transcriptional regulation [71]. Multiple studies have proven the crucial functions of SETD8 associated with tumorigenesis and related signaling pathways and active factors, including p53 [72], Twist [73], hypoxia-inducible factor [74], and the wingless/integrated (Wnt)/β-catenin signaling pathway [75]. Given the remarkable roles of SETD8 in cancer and the potential of small-molecule inhibitors, the development of SETD8-selective inhibitors has resulted in powerful chemical probes as well as highly effective antitumor lead compounds. Unfortunately, no SETD8 inhibitor has advanced into clinical application [71].

3. Drugs targeting KMTs

To date, multiple inhibitors have demonstrated encouraging outcomes in dealing with diversified cancers, especially for the

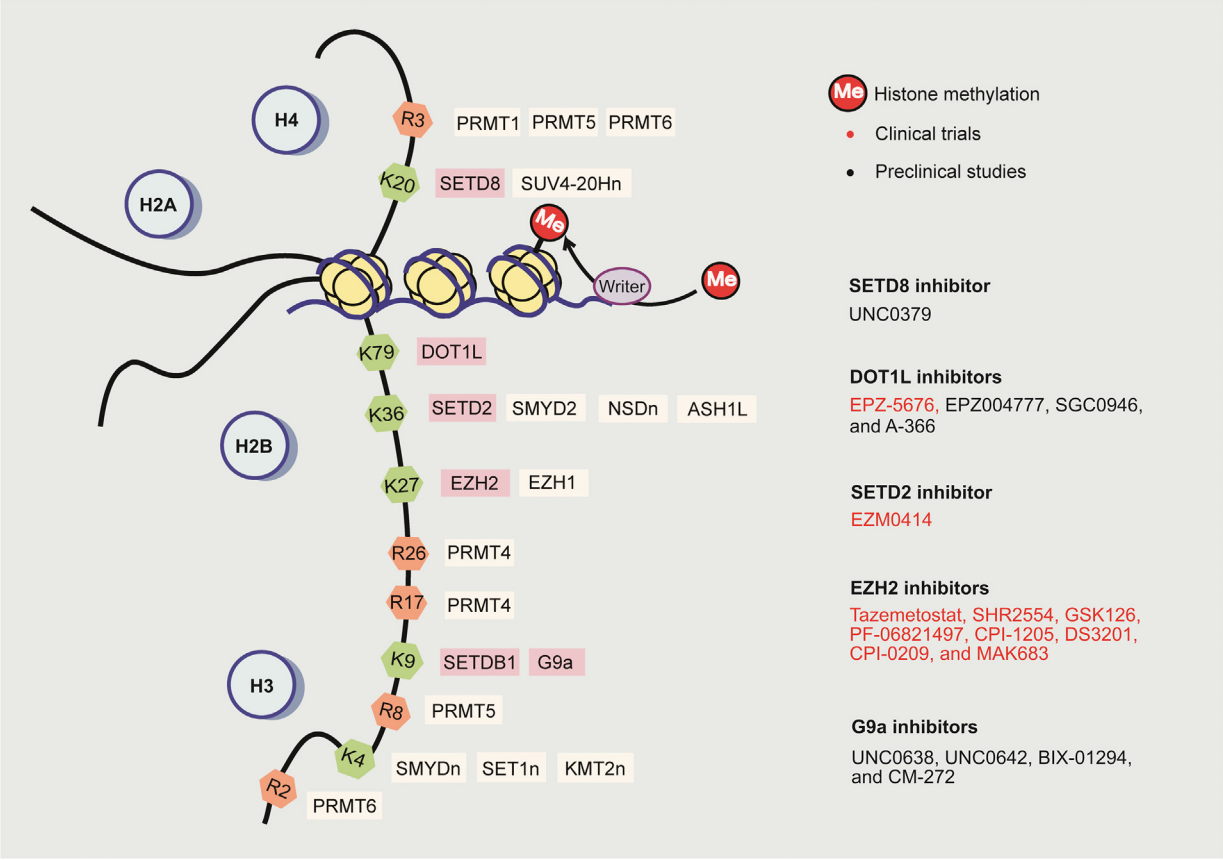

Fig. 2. The current situation of preclinical studies and clinical trials of EZH2, DOT1L, G9a, SETD8, and SETD2 inhibitors. The preclinical studies are marked in black, and the clinical trials are marked in red. PRMT1: protein arginine methyltransferase 1; PRMT5: protein arginine methyltransferase 5; PRMT6: protein arginine methyltransferase 6; SET: su(var)3-9, enhancer-of-zeste, trithorax; SETD8: SET domain-containing protein 8; SUV4-20Hn: structure of the histone H4K20 methyltransferase family; DOT1L: disruptor of telomeric silencing 1-like; SMYDn: SET and MYND domain-containing protein family; NSDn: nuclear receptor-binding SET domain protein family; ASH1L: absent, small, or homeotic-like 1; EZH2: enhancer of zeste homolog 2; EZH1: enhancer of zeste homolog 1; PRMT4: protein arginine methyltransferase 4; SETDB1: SET domain bifurcated histone lysine methyltransferase 1; G9a: euchromatic histone lysine methyltransferase 2; KMT2n: histone-lysine *N*-methyltransferase 2 family.

inhibition of EZH2, DOT1L, G9a, and SETDB1. Herein, we have listed these epi-drugs of anti-KMTs in Fig. 2.

3.1. Targeting EZH2 in cancer

Small molecule inhibitors targeting EZH2 can be broadly classified into three categories according to their modes of action: 1) The largest class covers the small molecules that inhibit the enzymatic activity of EZH2. On January 23, 2020, the EZH2 inhibitor tazemetostat (EPZ-6438), an oral bioavailable SAM-competitive inhibitor of EZH2 enzyme activity, was approved by the US FDA for the treatment of locally advanced or metastatic ES ineligible for complete resection [76,77]. Furthermore, on June 18, 2020, the US FDA granted accelerated approval for the use of tazemetostat in the treatment of follicular lymphoma [78]. Between July 29, 2016, and June 2, 2017, a multicenter, open-label, phase II study (NCT02860286) recruited 74 patients with malignant pleural mesothelioma and the results have been reported [79]. The 12-week disease control rate was 51% (95% confidence interval (CI) 40%–63%) in the overall cohort and 54% (95% CI 42%–67%) in the cohort with *BAP1* inactivation, which far exceeded the 35% threshold set by the protocol. In addition, tazemetostat showed favorable safety and tolerability in patients with relapsed or refractory malignant pleural mesothelioma [79]. Tazemetostat is also being studied in other molecularly driven hematological malignancies, lymphomas, prostate cancer, and other solid tumors (e.g., NCT02601950, NCT04179864, and NCT04204941) [80]. There are many other SAM-sensitive inhibitors with therapeutic potential, including GSK126, CPI-1205 [81], PF-06821497 [82], SHR2554 [83], and valemetostat [84]. A clinical trial of GSK126 was terminated because the maximum dose was achieved and the schedule of GSK126 showed insufficient evidence of clinical activity to justify further clinical investigation (NCT02082977). CPI-0209 is currently undergoing phase I/II clinical trials in patients with advanced solid tumors and lymphomas (NCT04104776). Other EZH2 SAM-competitive inhibitors, including PF-06821497, SHR2554, and CPI-1205, are currently in clinical trials (e.g., NCT03460977, NCT03741712, and NCT02395601). 2) The second category works by disrupting the PRC2 complex and inhibiting its interaction with H3K27. MAK683 is a small-molecule EED inhibitor that blocks the binding of EED to H3K27, consequently preventing EZH2 activation, and is in phase I/II trials for diffuse large B-cell lymphoma (DLBCL) (NCT02900651) [85]. 3) The remaining group involves a promising new avenue for triggering EZH2 degradation [19]. As was mentioned before, the non-PRC2-related activities of EZH2 rely on acting as a coactivator for critical transcription factors, including the AR, instead of methyltransferase catalytic activity [86]. Thus, most current EZH2 inhibitors, which only inhibit the catalytic activity of EZH2, may fail to suppress its noncanonical activities [87]. To develop a strategy that suppresses the noncatalytic functions of EZH2, the discovery of a first-in-class EZH2-selective degrader 56 (MS1943) was reported in 2018 [88]. Furthermore, the EZH2-targeting proteolysis-targeting chimera (PROTAC) degrader MS177 was designed in 2021. The study showed that the cancer-killing effects of MS177 are more potent in leukemia than EZH2 catalytic inhibitors and an EED degrader [89].

Collectively, there are numerous key observations pointing to potential roles of EZH2 inhibition in tumor treatment. However, EZH2 inhibitors may exert either oncogenic or carcinogenic effects through different modes of action in various types of tumors, and further assessment is required for targeted therapeutic applications.

3.2. Targeting DOT1L in cancer

Based on the catalytic mechanism, most DOT1L inhibitors are mimetics of the SAM molecule that inhibit DOT1L activity by displacing or blocking SAM from its binding site, such as EPZ-5676 (also known as pinometostat), EPZ004777, and the brominated analog SGC0946 [90]. Unfortunately, the very low pharmacological properties of EPZ004777 prevented its adoption in therapy [91]. In contrast, EPZ-5676 had superior selectivity and pharmacokinetics and was validated as an 'orphan drug' for *MLL*-rearranged leukemia by the US FDA in 2013 [92]. Furthermore, two completed clinical trials of EPZ-5676 (NCT02141828 and NCT01684150) revealed that the clinical benefit was very modest, with formal clinical responses in only two out of 51 patients, and combining EPZ-5676 with other antileukemia agents is warranted [93]. EPZ-5657 is also undergoing investigation in several preclinical trials in other solid tumors. Kurani et al. [46] revealed that EPZ-5676 significantly reduces tumor growth and metastasis by regulating malignant stem cell maintenance or expansion in TNBC. In addition, Chava et al. [48] in 2021 proved that EPZ-5676 significantly inhibits the growth of subcutaneous ovarian tumors in mouse xenografts. In addition, Wu et al. [94] revealed that protein-protein interactions (PPIs) between DOT1L and fusion partners are a potential anticancer drug target. Recently, Cao et al. [95] revealed roles of DOT1L, independent of H3K79 methylation, in modulating cell fate determination and in transcriptional elongation control. Therefore, it has been proposed that degrading DOT1L instead of solely inhibiting its catalytic activities may be more beneficial for cancer therapeutics. Subsequently, several researchers explored the design of an effective PROTAC or molecular glue to degrade DOT1L, which can be an alternative approach to enzymatic inhibitors in *MLL*-rearranged leukemias [96]. A significant number of researchers are exploring and synthesizing some small-molecule PPI inhibitors, such as MLL1-menin and MLL1-WDR5 interaction inhibitors [97].

3.3. Targeting G9a in cancer

The antitumor effects of G9a inhibitors have been extensively studied by multiple independent groups over the last decade. As a result, several small-molecule inhibitors of G9a HMTase activity have been developed, such as the quinazoline-core-based BIX-01294 [98], UNC0638 [99], and UNC0642 [100], which block the H3 substrate binding site of G9a. Studies have found that G9a inhibitors can inhibit tumor growth by blocking cell cycle progression and triggering apoptosis or inducing autophagic cell death [101]. However, BIX01294 exhibits selectivity for glucagon-like peptide (GLP) over G9a, and UNC0638 is not suitable for animal studies due to its poor pharmacokinetic properties [100]. In contrast, UNC0642 is shown to have excellent selectivity, low cell toxicity, and high in vitro cellular potency, as well as improved in vivo pharmacokinetic properties [102]. The indole core molecule A-366 was also identified as another competitive inhibitor of the H3 peptide of G9a, being more selective for G9a than GLP (i.e., euchromatic histone lysine methyltransferase 1/GLP). Recently, the novel drug CM-272 (a dual G9a/DNA methyltransferase (DNMT) inhibitor) was suggested to induce hematopoietic stem cell differentiation and growth inhibition, underscoring the potential of differentiation therapy in hepatocellular carcinoma [103]. Many preclinical studies have indicated that both G9a and EZH2 are frequently upregulated simultaneously in several human tumors and synergistically induce apoptosis via the interleukin (IL)-24-ER stress axis in cancer cells [104]. Spiliopoulou et al. [105] found in 2022 that dual inhibition of G9a and EZH2 can induce more robust chemokine induction than blocking either one alone. Moreover, histone lysine methyltransferase inhibitor-1-005 was the first pharmacologic inhibitor that targets both enzymes simultaneously, which alters the immune microenvironment and reduces tumor growth in ovarian high-grade serous carcinoma.

3.4. Small-molecule compounds for other targets

Multiple studies have demonstrated that SETDB1 depletion decreases cell cycle progression, cell proliferation, migration in vitro, and reduced tumor growth in vivo. Drugs available today that affect SETDB1 function include SET-domain HMT inhibitors (chaetocin, 3′-deazaneplanocin A, 3-deazaneplanocin A (DZNep), and miR-381-3p) and H3K9 methylation inhibitors (mithramycin A) and miRNAs (miR-621 and miR-29) [106,107]. Unfortunately, the SETDB1 inhibitors that have undergone preclinical testing are mostly nonselective compounds. Notably, in 2021, the US FDA granted Fast Track status to the first oral SETD2 inhibitor, EZM0414, as a treatment option for adult patients with relapsed or refractory DLBCL. The safety of this agent is being evaluated in a recently initiated phase I/Ib study (NCT05121103). Veschi et al. [108] revealed that UNC0379 (SETD8 inhibitor) confers a significant survival advantage in high-risk neuroblastoma models by activating the p53 canonical pathway. However, Herviou et al. [109] found that UNC-0379 can trigger p53-independent replicative stress in p53-deficient myeloma cells. Furthermore, the study found that SETD8 inhibition is strongly synergistic with melphalan, and the combined strategy might be used to reverse resistance to this alkylating agent widely used in multiple myeloma treatment.

4. KMT inhibitor combination strategy

As is mentioned above, EZH2 and DOT1L inhibitors have demonstrated therapeutic efficacy against tumors in clinical trials, and G9a and other KMTs have shown full potential as cancer therapeutic targets in preclinical trials [110]. To date, the main treatment options for cancer include conventional chemotherapy, targeted therapy, and immunotherapy [14]. A number of clinical trials and preclinical studies of combination therapeutic strategies are also progressively underway, demonstrating their unique benefits in reversing drug resistance and potentiating the effectiveness of treatment. Thus, we highlight the cooperation between epigenetic therapy and other therapeutic regimens in cancer treatment.

4.1. Combination of KMT inhibitors with chemotherapeutic agents

As several epigenetic therapies have only limited efficacy as single agents, combinations with conventional chemotherapy have shown promising synergy in a series of clinical studies (Fig. 3, Tables 1 and 2 [111–118]). Chemotherapeutic agents can be commonly divided into several classes: alkylating agents, platinum-based drugs, antimetabolites, antibiotics, etc. [119,120]. Common conventional chemotherapy drugs, including cisplatin, 5-fluorouracil (5-FU), and doxorubicin, are widely used in clinic with remarkable therapeutic efficacy and a clear mechanism of action [111,121,122].

Ramakrishnan et al. [112] found that EPZ011989 alone and in combination with cisplatin can increase NK-cell infiltration and thereby promote interferon (IFN)-γ release in HT1376-derived xenografts. Further study found that the EZH2 inhibition-mediated increase in NK-cell activity increases the response to cisplatin therapy through increased cytotoxicity of pluripotent cells or tumor cell

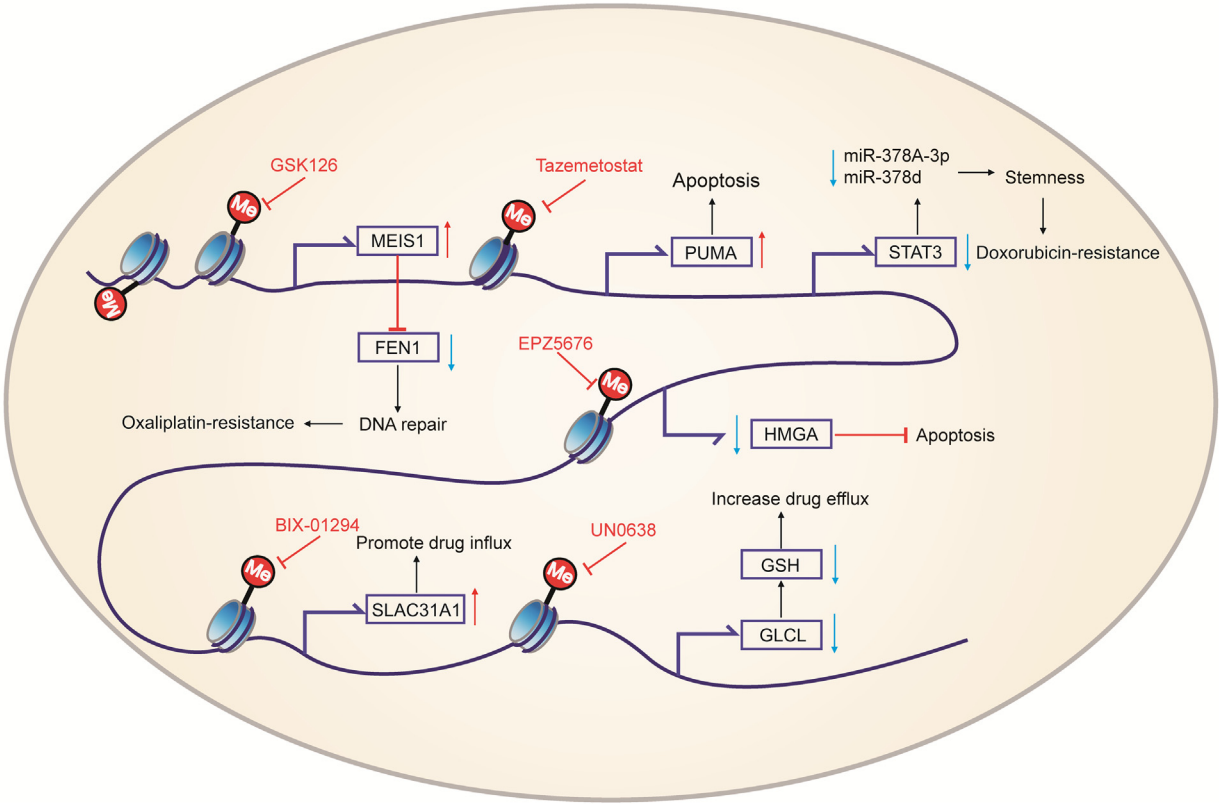

Fig. 3. Molecular regulatory mechanisms of histone lysine methyltransferase (KMT) inhibitors in increasing the sensitivity of chemotherapeutic drugs. MEIS1: myeloid ecotype virus insertion site 1; FEN1: flap structure-specific endonuclease 1; PUMA: p53-upregulated modulator of apoptosis; STAT3: signal transducer and activator of transcription 3; miR: microRNA; HMGA: high-mobility gene group A; SLC31A1: copper transporter 1; GCLC: glutamate-cysteine ligase catalytic subunit; GSH: glutathione.

Table 1
Clinical trials of histone lysine methyltransferases (KMTs) in combination with chemotherapeutic agents in different types of cancer.

Strategy	Trial registration	Phase	Status	Tumor	Drug combination
EZH2i + topoisomerase inhibitor	NCT04204941	Ib/III	Recruiting	Advanced epithelioid sarcoma	Tazemetostat + doxorubicin
EZH2i + chemotherapy	NCT03460977	I	Recruiting	Small cell lung cancer/castration resistant prostate cancer/follicular lymphoma	PF-06821497 + standard of care
EZH2i + topoisomerase inhibitor	NCT03879798	I/II	Recruiting	Small cell lung cancer	DS3201 + irinotecan
EZH2i + R-CHOP	NCT02889523	Ib/II	Recruiting	Lymphoma/diffuse large B-cell lymphomas/Follicular Lymphoma	Tazemetostat + R-CHOP
DOT1Li + demethylating agent	NCT03701295	Ib/II	Completed	Relapsed, refractory, or newly diagnosed acute myeloid leukemia with 11q23 rearrangement	EPZ-5676 + Azacitidine

EZH2i: enhancer of zeste homolog 2 inhibitors; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; DOT1Li: disruptor of telomeric silencing 1-like inhibitors.

differentiation. Tan et al. [111] indicated that tazemetostat combined with 5-FU exhibits synergistic antitumor function both in vitro and in vivo in colorectal cancer cells. The study found that tazemetostat can enhance the therapeutic effect of 5-FU through the upregulation of p53-upregulated modulator of apoptosis. Previous evidence showed that the EZH2/signal transducer and activator of transcription 3 pathway is activated after chemotherapy and induces the secretion of exosomes enriched in miR-378a-3p and miR-378d. Furthermore, exosomes produced by cancer cells after stimulation with chemotherapeutic agents deliver miR-378a-3p and miR-378d to neighboring cells to activate Wnt and Notch signaling pathways and induce drug resistance. Yang et al. [113] demonstrated that the cytotoxic drug doxorubicin combined with the EZH2 inhibitor tazemetostat can effectively reverse chemotherapy-elicited exosome-induced drug resistance in a mouse tumor xenograft model. Li et al. [114] claimed that tumorigenesis and oxaliplatin resistance in colorectal cancer are associated with the downregulation of MEIS1 mediated by the extracellular leucine-rich repeat fibronectin containing 1-antisense RNA 1/EZH2/DNA methyltransferases 3A axis. The study found that GSK126 and oxaliplatin can significantly reverse the resistance of drug-resistant cells to oxaliplatin, impairing cell survival, and inhibiting tumor growth in mice. Lee et al. [123] revealed that SETDB1 depletion in colonic cancer cells combined with cytotoxic drugs, such as 5-FU, irinotecan, and oxaliplatin, promotes the differentiation of cancer stem cells into postmeiotic normal-like cells.

In a multicenter, double-blind, placebo-controlled, randomized phase III study, the efficacy and safety of tazemetostat plus doxorubicin for patients with advanced ES is being evaluated with approximately 150 patients enrolled (NCT04204941) [77]. PF-06821497 in combination with standard of care is under evaluation in an open-label, multicenter phase I study in patients with relapsed/refractory small cell lung cancer (SCLC) and castration-resistant prostate cancer (CRPC) (NCT03460977). The safety of DS3201 in combination with irinotecan is being assessed in a nonrandomized phase I/II trial in patients who have recurrent SCLC (NCT03879798). Cotreatment with tazemetostat and rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisolone (R-CHOP) for patients with newly diagnosed DLBCL and follicular lymphoma is still under evaluation in a phase Ib/II clinical trial (NCT02889523). Phase I is designed to evaluate the safety of the combination and to identify the recommended phase II dose of tazemetostat. Seventeen participants were enrolled in the phase Ib study, and preliminary efficacy data were encouraging, indicating the necessity of further investigations in phase II trials. Furthermore, phase II of the study was designed to determine the safety and efficacy of tazemetostat in patients treated with eight cycles of R-CHOP [124].

The study confirmed the synergistic antiproliferative activity of EPZ-5676 combined with azacitidine or decitabine in *MLL*-rearranged leukemia [115]. A phase Ib/II trial investigating the ability of EPZ-5676 and azacytidine to cotreat patients with relapsed, refractory, or newly diagnosed acute myeloid leukemia with 11q23 rearrangement has been completed (NCT03701295). In addition, Mao et al. [116] provided preclinical evidence demonstrating that cotreatment with EPZ-5676 and etoposide significantly improves the therapeutic efficacy in murine orthotopic xenografts of retinoblastoma. The dual role of DOT1L targeting was validated in RB cells, interfering with the immediate engagement of DOT1L in the early DNA damage response and downregulating the expression of high mobility group AT-hook 2, whose depletion can augment the drug sensitivity of RB cells.

Liu et al. [117] found that G9a elevates glutathione (GSH) by regulating H3K9me1 levels at the glutamate-cysteine ligase catalytic subunit (GCLC) transcriptional start site in head and neck squamous cell carcinoma (HNSCC). GSH has been shown to be a major contributing factor to drug resistance due to its ability to induce drug conjugation and eliminate radical scavenging. The use of the G9a inhibitor UNC0638 significantly reduces GCLC expression in cisplatin resistant HNSCC, thereby reversing drug resistance. Ovarian cancer is one of the diseases known as 'female killers', and platinum drugs are used as first-line treatments. However, most patients eventually generate acquired resistance against platinum drugs, and nearly 80% of them develop recurrent tumors within 2 years [44]. The latest study found that ZNF711 downregulation promotes cisplatin resistance in epithelial ovarian cancer (EOC) patients. Mechanistically, the expression of ZNF711 decreases the level of H3K79me2, which leads to the activation of SLC31A1 transcription, a transmembrane protein that can promote the influx of cisplatin. Thus, cotreatment with BIX-01294 greatly augments cisplatin sensitivity in ZNF711-downregulated EOC cells [118].

4.2. Combination of KMT inhibitors with other targeted drugs

Although mutation or aberrant upregulation of KMTs occurs frequently in multiple cancers, there are still limitations in the therapeutic application of KMT epi-drugs (Tables 3 and 4 [125–137]). For instance, Hirukawa et al. [125] revealed that EZH2 inhibitors can potentiate the IFN-driven immune response to anti-ErbB2 mAb therapy in trastuzumab-resistant breast cancer models of mice. Mechanistically, derepression of retrotransposons by EZH2 inhibition triggers a type-I IFN response and an influx of cytotoxic leukocytes that enhances the efficacy of trastuzumab. In addition, the efficacy of the AR inhibitor SHR3680 combined with the EZH2 inhibitor SHR2554 is under evaluation in a phase Ib/II study in patients with refractory metastatic TNBC (NCT03805399). As is mentioned before, phosphorylated EZH2 directly activates AR gene transcription in prostate cancer, suggesting that EZH2 inhibition and AR-targeted therapies may be effective in CRPC [86].

Table 2
Preclinical studies of histone lysine methyltransferases (KMTs) in combination with chemotherapeutic agents in cancers.

Strategy	Mechanism	Tumor	Drug combination	Year	Refs.
EZH2i + cytotoxic drugs	Inducing PUMA upregulation and mitochondrial apoptosis pathway	Colorectal cancer	Tazemetostat + 5-FU	2020	[111]
EZH2i + cytotoxic drugs	Increasing NK cell infiltration and thereby promoting IFN-γ release	Bladder cancer	EPZ011989 + cisplatin	2019	[112]
EZH2i + cytotoxic drugs	Inhibiting exosome expression of miR-378a-3p and miR-378d via EZH2/STAT3 pathway	Breast cancer	Tazemetostat + doxorubicin	2021	[113]
EZH2i + platinum drugs	Downregulating of MEIS1 mediated by ELFN1-AS1/EZH2/DNMT3a axis	Colorectal cancer	GSK126 + oxaliplatin	2022	[114]
DOT1Li + demethylating drugs	Displaying synergistic antiproliferative activity	*MLL*-rearranged leukemia	EPZ5676 + azacitidine/decitabine	2014	[115]
DOT1Li + topoisomerase inhibitor	Downregulating the expression of HMGA2 and augmenting the drug sensitivity	Retinoblastoma	EPZ-5676 + etoposide	2021	[116]
G9ai + cytotoxic drugs	Reducing GCLC expression to reverse resistance	Head and neck squamous cell carcinoma	UNC0638 + cisplatin	2017	[117]
G9ai + cytotoxic drugs	Upregulating the transcriptional expression of SLC31A1 and promoting the influx of cisplatin	Epithelial ovarian cancer	BIX-01294 + cisplatin	2021	[118]

EZH2: enhancer of zeste homolog 2; EZH2i: EZH2 inhibitors; PUMA: p53-upregulated modulator of apoptosis; 5-FU: 5-fluorouracil; NK: natural killer; IFN: interferon; STAT3: signal transducer and activator of transcription 3; MEIS1: myeloid ecotype virus insertion site 1; ELFN1: extracellular leucine-rich repeat fibronectin containing 1; AS1: antisense RNA 1; DNMT3a: DNA methyltransferases 3A; DOT1L: disruptor of telomeric silencing 1-like; HMGA2: high-mobility gene group A2; GCLC: glutamate-cysteine ligase catalytic subunit; G9ai: euchromatic histone lysine methyltransferase 2 inhibitor; SLC31A1: copper transporter 1.

There are two ongoing phase Ib/II clinical trials to evaluate the effects of EZH2 inhibitors in combination with either enzalutamide (an AR antagonist) or abiraterone/prednisone on metastatic CRPC (mCRPC) (NCT04179864 and NCT03480646). In addition to novel AR-targeted therapies, poly(ADP-ribose) polymerase inhibitors have antitumor activity in mCRPC due to DNA damage response alterations occurring in approximately 23% of mCRPC [138]. The safety, tolerability, and preliminary clinical activity of the combination of talazoparib with tazemetostat are being tested in a phase Ib/II study in mCRPC patients (NCT04846478).

The possible benefits and side effects of tazemetostat in combination with dual BRAF/MEK inhibition are being evaluated in a phase I/II study in patients with BRAF-mutated metastatic melanoma who progressed on prior BRAF/MEK inhibitor therapy (NCT04557956) [139]. In addition, Fu et al. [126] found that using dabrafenib/selumetinib and tazemetostat ultimately enhance the differentiation of papillary thyroid cancer (PTC) cells harboring $BRAF^{V600E}$ and overcome resistance to BRAF inhibitor treatment by synergistically suppressing the trimethylation of H3K27.

The safety and efficacy of tazemetostat in combination with rituximab are under evaluation in patients with relapsed/refractory follicular lymphoma (R/R FL), previously treated with at least two standard prior systemic treatment regimens where at least one anti-CD20-based regimen was used (NCT04762160). A multicenter, double-blind, active-controlled, randomized three-stage study was designed to evaluate the efficacy and safety of tazemetostat in combination with lenalidomide plus rituximab in patients with R/R FL who completed at least one prior course of systemic chemotherapy, immunotherapy, or chemoimmunotherapy (NCT04224493). The study showed that preliminary efficacy with the combination of umbralisib (a PI3K-δ inhibitor) plus ublituximab (an anti-CD20 monoclonal antibody) in lymphoma patients is promising and warrants further investigation [140]. Therefore, the phase I/II trial is designed to estimate the safety and tolerability of a regimen combining tazemetostat, umbralisib, and ublituximab in R/R FL patients (NCT05152459).

Huang et al. [127] revealed that the application of bromodomain-containing protein 4 (BRD4) inhibitors can overcome resistance to EZH2i by interferring H3K27 acetylation recognition. However, as the EZH2-BRD4 inhibitor combination differentially activates mitogen-activated protein kinase (MAPK) in some cancers, a triple combination plus MAPK pathway inhibitors

Table 3
Clinical trials of histone lysine methyltransferases (KMTs) in combination with other targeted drugs in different types of cancer.

Strategy	Trial registration	Phase	Status	Tumor	Drug combination
EZH2i + AR antagonist	NCT03805399	Ib/II	Recruiting	Triple-negative breast cancer	SHR2254 + SHR3680
EZH2i + AR antagonist	NCT04179864	Ib/II	Recruiting	Metastatic castration resistant prostate cancer	Tazemetostat + enzalutamide/ abiraterone/prednisone
EZH2i + AR antagonist	NCT03480646	Ib/II	Active, not recruiting	Metastatic castration resistant prostate cancer	CPI-1205 + enzalutamide/ abiraterone/prednisone
EZH2i + PARPi	NCT04846478	Ib/II	Recruiting	Metastatic castration resistant prostate cancer	Tazemetostat + talazoparib
EZH2i + BRAFi + MEKi	NCT04557956	I/II	Recruiting	Metastatic melanoma	Tazemetostat + dabrafenib + trametinib
EZH2i + monoclonal anti-CD20 antibody	NCT04762160	II	Recruiting	R/R FL	Tazemetostat + rituximab
EZH2i + anti-CD20 monoclonal antibody + immunomodulatory agent	NCT04224493	III	Recruiting	R/R FL	Tazemetostat + lenalidomide + rituximab
EZH2i + PI3K-δ inhibitor + anti-CD20 monoclonal antibody	NCT05152459	I/II	Not yet recruiting	R/R FL	Tazemetostat + umbralisib + ublituximab

EZH2i: enhancer of zeste homolog 2 inhibitor; AR: androgen receptor; PARPi: poly(ADP-ribose) polymerase inhibitor; BRAFi: BRAF inhibitor; MEKi: mitogen-activated protein-extracellular signal-regulated kinase inhibitor; R/R FL: relapsed/refractory follicular lymphoma; PI3K: phosphoinositide 3-kinase.

Table 4
Preclinical studies of histone lysine methyltransferases (KMTs) in combination with other targeted drugs in cancers.

Strategy	Mechanism	Tumor	Drug combination	Year	Refs.
EZH2i + anti-HER2 antibodies	Promoting interferon-driven immune responses	Breast cancer	GSK126/EPZ-6438 + trastuzumab	2019	[125]
EZH2i + MAPKi	Enhancing differentiation of papillary thyroid cancer cells	Papillary thyroid cancer	Tazemetostat + dabrafenib/ selumetinib	2020	[126]
EZH2i + BRD4i + MAPKi	Impairing H3K27ac-medicated transcription and blocking MAPK pathway	Solid tumors	EPZ-6438 + OTX015 + GDC-0994	2018	[127]
EZH2i + RTKi	Modifying sunitinib resistance resulting from adaptive kinase reprogramming	Clear cell renal cell carcinoma	EPZ011989 + sunitinib	2017	[128]
EZH2i + EGFR-TKIs	Downregulating the phosphorylation of EGFR and AKT and inducing cell apoptosis	Non-small cell lung cancer	GSK343/DZNep + gefitinib	2020	[129]
	Snergistically inducing autophagy and inhibiting the activity of mTOR signaling pathway	Colon cancer	UNC1999 + gefitinib	2017 and 2014	[130,131]
	Synergistically inducing autophagy and increasing apoptosis	Gastric cancer	GSK126 + gefitinib	2017 and 2014	[130,131]
G9ai + EGFR-TKIs	Regulating the PTEN/AKT pathway	Non-small cell lung cancer	UNC0638 + erlotinib	2018	[132]
G9a/DNMTi + Bcl-2i	Activating endogenous retroelement recognition and reversing the Warburg effect	Acute myeloid leukemia	CM-272 + venetoclax	2021	[133]
DOT1L + SIRT1	Depletion of Sirt1 selectively inhibiting the accumulation of H3K9me2 at *MLL*-AF9 targets after DOT1L inhibition	*MLL*-rearranged leukemia	EPZ4777 + RT1720	2015	[134]
DOT1L + LSD1i	Synergistically downregulating expression of several leukemia-relevant genes and inducing apoptosis	*MLL*-rearranged leukemia	SYC-522 + cyclopropylamine-based LSD1 inhibitors	2016	[135]
DOT1L + MLL-menin interaction inhibitor	Suppressing the target genes of MLL-fusion proteins and MYC	*MLL*-rearranged leukemia	EPZ004777 + MI-2-2	2017	[136]
DOT1Li + SHP2i	Promoting *PREX1* expression and feedback activation of MAPK signaling	Pancreatic cancer	SGC0946 + SHP099	2021	[137]

EZH2i: enhancer of zeste homolog 2 inhibitor; MAPK: mitogen-activated protein kinase; MAPKi: MAPK inhibitor; BRDi: bromodomain-containing protein inhibitor; RTKi: RTK inhibitor; EGFR-TKIs: epidermal growth factor receptor-tyrosine kinase inhibitors; G9ai: euchromatic histone lysine methyltransferase 2 inhibitor; PTEN/AKT: phosphatase and tensin homolog/protein kinase B; DNMTi: DNA methyltransferase inhibitor; Bcl-2i: B-cell lymphoma-2 inhibitor; DOT1L: disruptor of telomeric silencing 1-like; SIRT1: sirtuin 1; MLL: mixed lineage leukemia; LSD1: lysine-specific demethylase 1; LSD1i: LSD1 inhibitor; MYC: myelocytomatosis; DOT1Li: DOT1L inhibitors; SHP2i: SHP2 inhibitor.

may display robust efficacy with very tolerable toxicity. Adelaiye-Ogala et al. [128] provided a combination strategy with an EZH2 inhibitor and sunitinib, which can overcome/delay sunitinib resistance in clear cell renal cell carcinoma (ccRCC) patients. EZH2 expression correlates with metastatic disease and decreases sunitinib response in ccRCC patients via adaptive kinase reprogramming.

The usage of targeted therapies has greatly increased the survival of NSCLC patients. In particular, EGFR-TKIs, including gefitinib and erlotinib, have been used as first-line treatments for advanced NSCLC patients harboring activating EGFR mutations [141,142]. However, similar to other anticancer drugs, one-third of patients over a period of 9–13 months develop acquired resistance to EGFR-TKIs [143]. Gong et al. [129] demonstrated that the coadministration of EZH2 inhibitors (GSK343 or DZNep) with gefitinib can reverse EGFR-TKI resistance by downregulating the phosphorylation of EGFR and AKT and by inducing cell apoptosis in NSCLC. Several studies also found that the inhibition of EZH2 and EGFR exerts a stronger inhibitory effect on tumor growth and cell migration than single-drug administration in colon cancer and gastric cancer [130,131] (Fig. 4A).

In addition, G9a was also found to be associated with EGFR-TKI resistance in NSCLC. Wang et al. [132] demonstrated that G9a activated the AKT signaling pathway by increasing the level of the PTEN promoter region H3K9me2 and reducing acetylation, leading to drug resistance. The combination of the indicated G9a inhibitor UNC0638 and erlotinib can robustly inhibit cell growth and induce apoptosis in EGFR-TKI-resistant NSCLC cells by regulating the PTEN/AKT pathway. Fresquet et al. [133] revealed that epigenetic therapy in combination with drugs targeting B cell lymphoma 2 (Bcl2) family proteins yielded synergistic responses in cancer treatment. CM-272 plus venetoclax was found to activate endogenous retroelement recognition, increasing ATP hydrolysis, thereby reversing the Warburg effect, inducing tumor necroptosis, and increasing sensitization to Bcl-2 inhibitors (Fig. 4B).

Studies have demonstrated that *MLL*-rearranged acute lymphoblastic leukemia is characterized by strong epigenetic dysregulation and sensitivity to epigenetic perturbators [144]. In 2015, Chen et al. [134] found that the activation of regulator 2 related enzyme 1 (sirtuin 1, SIRT1), an NAD-dependent protein deacetylase, might enhance the efficacy of a DOT1L inhibitor. Mechanistically, inhibition of DOT1L leads to a SIRT1-dependent decrease in H3K9 acetylation, increase in H3K9 methylation, and loss of chromatin accessibility at *MLL* fusion target genes, which highlights the necessity of SIRT1 for acquisition of a repressed chromatin state upon DOT1L inhibition. Furthermore, RT1720, a small-molecule activator of SIRT1, has been demonstrated to sensitize *MLL*-rearranged leukemias and to show enhanced antiproliferative activity in combination with DOT1L inhibitors. In addition, Feng et al. [135] found that combination treatments of LSD1 inhibitors with a DOT1L inhibitor can display strong synergism against the proliferation of *MLL*-rearranged leukemia cells. It is presumed that H3K79 hypermethylation is closely related

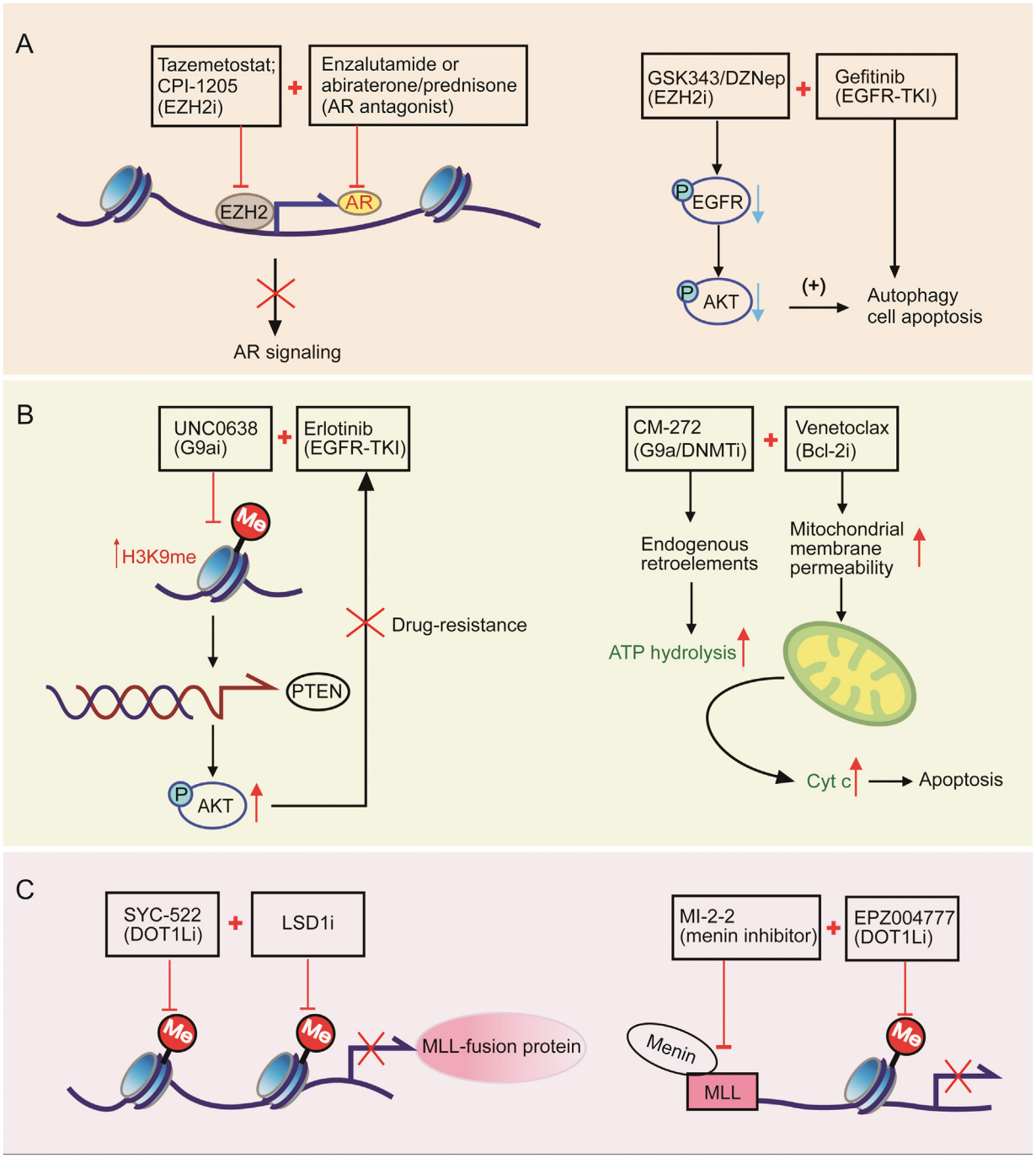

Fig. 4. The mechanisms of histone lysine methyltransferases (KMTs) in combination with other targeted drugs in cancers. (A) Enhancer of zeste homolog 2 inhibitor (EZH2i) synergized with androgen receptor (AR) antagonist and epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) drug for the targeted treatment of tumors. (B) Euchromatic histone lysine methyltransferase 2 inhibitors (G9ai) synergized with EGFR-TKI and B-cell lymphoma-2 inhibitor (Bcl-2i) for the targeted treatment of tumors. (C) Disruptor of telomeric silencing 1-like inhibitors (DOT1Li) synergized with other epigenetic drugs for the targeted treatment of leukemia. AKT: protein kinase B; PTEN: phosphatase and tensin homolog; DNMTi: DNA methyltransferase inhibitor; Cyt c: cytochrome c; LSD1i: lysine-specific demethylase 1 inhibitor; MLL: mixed lineage leukemia.

to imbalanced H3K4 methylation in *MLL*-rearranged leukemia. Recently, the study revealed that combining the DOT1L inhibitor EPZ004777 with the MLL-menin interaction inhibitor (MI-2-2) markedly enhances the induction of differentiation and cell killing in various *MLL* disease models. Gene expression analysis revealed that target genes of MLL-fusion proteins and myelocytomatosis are suppressed more powerfully upon combination treatment [136]. In addition, Liu et al. [137] revealed that the DOT1Li-SHP2i combination exhibits a synergistic effect in inhibiting tumor growth in patient-derived xenograft models. The study found that DOT1Li-induced loss of H3K79 methylation can facilitate H3K79 acetylation on the *PREX1* gene locus, promoting *PREX1* expression and feedback activation of MAPK signaling. Notably, the study found that the DOT1Li-SHP2i combination apparently affects different responsive subsets among KRAS mutant cancers by analyzing 18 KRAS mutant cell lines similarly tested (Fig. 4C).

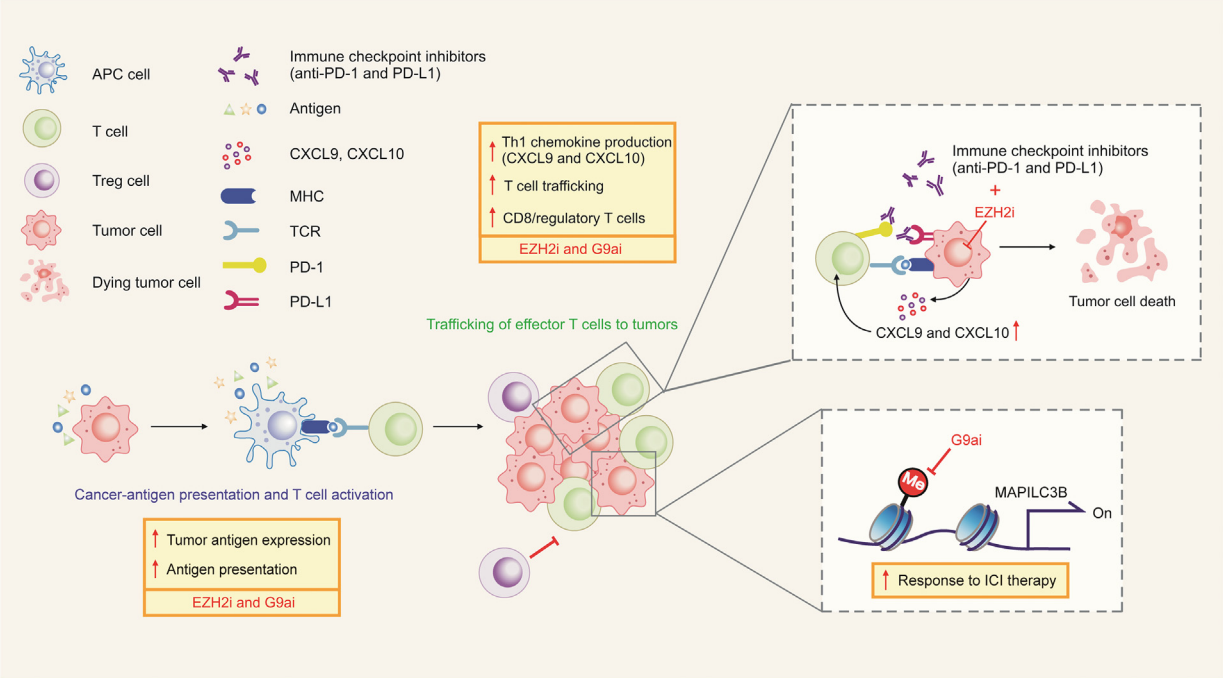

Fig. 5. The mechanisms of enhancer of zeste homolog 2 inhibitor (EZH2i) and euchromatic histone lysine methyltransferase 2 inhibitor (G9ai) that enhance immune checkpoint inhibitor (ICI) responses. APC cell: antigen-presenting cell; Treg cell: regulatory T cell; CXCL: C-X-C motif chemokine ligand; MHC: major histocompatibility complex; TCR: T cell receptor; PD-1/PD-L1: programmed cell death protein 1/programmed cell death ligand 1; EZH2i: EZH2 inhibitors; G9ai: G9a inhibitor; MAPILC3B: microtubule-associated protein 1 light chain 3 beta.

Table 5
Clinical trials of histone lysine methyltransferases (KMTs) in combination with immunotherapy in different types of cancer.

Strategy	Trial registration	Phase	Status	Tumor	Drug combination
EZH2i + anti-PD-1/PD-L1	NCT03854474	I/II	Recruiting	Advanced urothelial carcinoma	Tazemetostat + pembrolizumab
EZH2i + anti-PD-1/PD-L1	NCT04624113	I/II	Recruiting	Head and neck squamous cell carcinoma	Tazemetostat + pembrolizumab
EZH2i + anti-PD-1/PD-L1	NCT03525795	I/II	Completed	Advanced solid tumors	CPI-1205 + ipilimumab
EZH2i + anti-PD-1/PD-L1	NCT04388852	Ib	Recruiting	Metastatic prostate urothelial and renal cell cancers	DS3201 + ipilimumab
EZH2i + anti-PD-1/PD-L1	NCT02220842	Ib	Recruiting	Relapsed/Refractory diffuse large B-cell lymphoma	Tazemetostat + atezolizumab
EZH2i + anti-PD-L1/TGF-βRII	NCT04407741	I/II	Recruiting	Advanced solid tumors/B-cell lymphomas	SHR2554 + SHR1701

EZH2i: enhancer of zeste homolog 2 inhibitor; anti-PD-1/PD-L1: anti-programmed cell death protein 1/programmed cell death ligand 1; TGF-βRII: transforming growth factor type II receptor.

4.3. Combination of KMT inhibitors with immunotherapy

Immunotherapies, such as immune checkpoint inhibitors (ICIs; anti-programmed cell death protein 1 (anti-PD-1) and anti-cytotoxic T lymphocyte associated antigen-4 (anti-CTLA-4) antibodies), have shown clinical benefits in patients with different types of cancers. However, in most cases, the efficacy of ICI monotherapy is not satisfactory. Therefore, finding more effective and less toxic strategies to improve the efficacy of tumor immunotherapies has attracted much attention from researchers (Fig. 5, Tables 5 and 6 [58,145–148]).

In 2015, Peng et al. [41] revealed that EZH2 gene expression is negatively associated with the number of tumor-infiltrating lymphocytes by silencing the Th1-type chemokines CXCL9 and CXCL10.

Table 6
Preclinical studies of histone lysine methyltransferases (KMTs) in combination with immunotherapies in cancers.

Strategy	Mechanism	Tumor	Drug combination	Year	Refs.
G9ai + anti-PD-1/PD-L1	Enhancing antitumor activity of ICB by modulating autophagy and IFN signaling	Melanoma	UNC0642 + anti-PD-1 antibody	2021	[58]
EZH2i + anti-PD-1/PD-L1	Enhancing antigen presentation	Head and neck squamous cell carcinoma	GSK126 + anti-PD-1 antibody	2020	[145]
EZH2i + anti-PD-1/PD-L1	Increasing effector-like T cell responses	Bladder cancer/melanoma	CPI-1205 + ipilimumab	2018	[146]
G9a/DNMTi + anti-PD-1/PD-L1	Increasing the host immune reactivation against tumors and metastasis	Bladder cancer	CM-272 + anti-PD-L1 antibody	2019	[147]
EZH2i + immunomodulatory agent	Inducing B-cell maturation and apoptosis	Diffuse large B-cell lymphomas	GSK126 + pomalidomide	2020	[148]

G9ai: euchromatic histone lysine methyltransferase 2 inhibitor; anti-PD-1/PD-L1: anti-programmed cell death protein 1/programmed cell death ligand 1; ICB: immune checkpoint blockade; IFN: interferon; EZH2i: enhancer of zeste homolog 2 inhibitor; DNMTi: DNA methyltransferase inhibitor.

As epigenetic silencing of the expression of CXCL9 and CXCL10 limits effector T-cell tumor trafficking to the tumor, ICB in combination with EZH2 inhibitors and DNMT inhibitors significantly inhibits cancer progression in ID8 ovarian cancer. In addition, Ennishi et al. [149] in 2019 suggested a significant enrichment of EZH2 mutations in both major histocompatibility complex (MHC)-I- and MHC-II-negative primary lymphomas and a functional link between EZH2 mutations and loss of MHC expression. Of clinical relevance, EZH2 inhibitors significantly restore MHC expression in EZH2-mutated human DLBCL cell lines, a crucial contributor to their synergy with ICIs in patients. In addition, Zingg et al. [40] found that the expression of EZH2 increases in all three melanoma models treated with anti-CTLA-4 or IL-2 immunotherapy, which in turn silences their immunogenicity and antigen presentation. Further research showed that EZH2 inhibition reverses this resistance and synergizes with immunotherapy to suppress melanoma growth [40]. As previous studies provided a strong rationale for novel combination strategies targeting EZH2 with ICIs, many clinical trials have already begun [150].

In a phase I/II clinical trial, the synergistic effect of EZH2 inhibition and anti-PD-1 treatment was investigated in patients with advanced urothelial carcinoma (NCT03854474). Additionally, the phase I/II study of the EZH2 inhibitor SHR2554 in combination with the anti-PD-L1/transforming growth factor-beta antibody SHR1701 in patients with advanced solid tumors and B-cell lymphomas is ongoing (NCT04407741). The phase Ib study of the safety and pharmacology of atezolizumab administered with tazemetostat in patients with relapsed/refractory diffuse large B-cell lymphoma has ended, but thus far, the results are not posted (NCT02220842). In addition to the clinical trials mentioned above, many researchers are still exploring novel combination strategies. In 2020, Zhou et al. [145] found that combining GSK126 with anti-PD-1 therapy may improve the low response rate and increase therapeutic susceptibility in anti-PD-1-resistant HNSCC. Mechanistic studies revealed that EZH2 inhibition upregulates target tumor cell antigen presentation by reducing histone H3K27me3 modification on the β-2-microglobulin promoter. At the same time, the efficacy of patients with pembrolizumab- or nivolumab-resistant, PD-L1 positive, recurrent or metastatic HNSCC treated with tazemetostat and pembrolizumab is under evaluation in a phase I/II clinical trial (NCT04624113).

Goswami et al. [146] confirmed that pharmacologic inhibition of EZH2 by CPI-1205 increases effector-like T-cell responses in vitro and enhances the effectiveness of anti-CTLA-4 therapy in murine bladder cancer and melanoma, which provided a powerful rationale for a combination trial of CPI-1205 plus ipilimumab. However, the phase I/II multicenter, open-label study of CPI-1205 and ipilimumab in patients with advanced solid tumors was stopped prior to proceeding to phase II, and there were no patients enrolled in phase II (NCT03525795). In addition, DS3201 plus ipilimumab is under evaluation in a phase Ib clinical trial in patients with metastatic prostate, urothelial, or renal cell cancer, which is recruiting participants (NCT04388852).

Multiple studies have proven that G9a inhibition can also enhance the antitumor activity of checkpoint inhibitor blockade by re-expressing endogenous retroviruses and thereby inducing viral mimicry and IFN signaling to increase tumor immunogenicity and recognition in preclinical cancer models [10,17]. The study found robust tumor growth reduction and tumor regression in an anti-PD-1-resistant B16F10 mouse melanoma model treated with both a G9a inhibitor and an anti-PD-1 antibody. Mechanistically, G9a inhibitors induce microtubule-associated protein 1 light chain 3 beta expression and thereby modulate autophagic processes. Therefore, Kelly et al. [58] proposed that G9a inhibition either improves the efficacy or extends the proportion of patients with melanoma who respond to checkpoint inhibitor treatment. Patients with advanced bladder cancer that progresses after platinum-based chemotherapy have a poor prognosis and limited treatment options. Anti-PD-L1 immune checkpoint inhibitor treatment as a second-line therapy for cisplatin-ineligible patients has been shown to increase survival, with responses in 20%–30% of patients [151,152]. Paramio and co-workers [147] suggested novel and promising opportunities for the treatment of bladder cancer using a combination of epigenetic inhibitors and ICB. They found that CM-272 can augment an endogenous antitumor immune response and induce immunogenic cell death, along with an increased proportion of CD8/regulatory T cells within the tissues. These effects suggested the conversion of a cold immune tumor into a hot tumor, which can be more susceptible to respond to ICIs.

Approximately 22% of patients with the germinal center B-cell-like subtype of DLBCL carry EZH2 gain-of-function mutations. Park et al. [148] demonstrated that the combination of GSK126 and pomalidomide synergistically inhibits tumor growth by promoting B-cell differentiation and apoptosis in DLBCL with EZH2 gain-of-function mutations.

5. Conclusions and future perspectives

This review summarizes the most recent and promising advances in KMT inhibitor therapy with an emphasis on the likely implications of the application of KMT inhibitors combined with other therapies for treating tumors. As a novel potential target for cancer therapy, KMTs have become a topic of intense research interest. An increasing number of functions and roles of KMTs in different types of cancers have been revealed. Multiple new drugs targeting KMTs are under development and evaluation in clinical trials. Although most of them are still on the stage of preclinical study or phase I/II clinical trials, the two US FDA approvals in less than six months for tazemetostat are a milestone event for epigenetic drugs and bodes well for the use of EZH2 inhibitors and even other KMT inhibitors in oncology treatment.

In addition, the studies explored in this review show synergies between KMT inhibitors and chemotherapy, targeted drugs and immune-based therapies that could mitigate the resistance development and toxic effects associated with individual treatment regimens. Notably, histone methylation has shown the most potential to overcome ICB resistance mechanisms by counteracting immune escape mechanisms of the tumor and the TME. As with current immunotherapies, the effect of KMT inhibitors on the TME may vary not only among different cancers but also among individuals within the same cancer type, and this must be taken into account when designing combination trials.

We expect efforts to identify new targets and explore other potential therapeutic applications of the drugs against KMTs to rapidly increase over the next decade. In addition, precision therapy in oncology patients may be improved if the crosstalk between different posttranslational histone modifications and even other epigenetic modifications has been further explored. These breakthroughs may ultimately benefit not only oncology patients but also patients with diverse diseases. However, there is still much uncertainty about KMT inhibitors and other epigenetic drugs, and more clinical trials are required to validate their efficacy and safety. Furthermore, the specific sites and mechanisms of action of EZH2 in several cancers are still not fully elucidated and remain to be explored. Many studies have demonstrated the regulatory function of G9a in tumor immunity, and combining G9a inhibitors with ICIs may be a potential strategy, which deserves further clinical trials. Combination strategies involving epigenetic drugs may be a further stepping stone to cancer treatment.

CRediT author statement

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Declaration of competing interest

The authors declare that there are no conflicts of interests.

Acknowledgments

This work was supported by the Science and Technology Commission of Shanghai, China (Grant Nos.: 20DZ2270800 and 19JC1410200), Innovative Research Team of High-Level Local Universities in Shanghai, China (Grant No.: SHSMU-ZDCX20210900), and the National Natural Science Foundation of China (Grant No.: 82073889).

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