



The Promise for Histone Methyltransferase Inhibitors for Epigenetic Therapy in Clinical Oncology: A Narrative Review

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ABSTRACT

Epigenetic processes are essential for normal development and the maintenance of tissue-specific gene expression in mammals. Changes in gene expression and malignant cellular transformation can result from disruption of epigenetic mechanisms, and global disruption in the epigenetic landscape is a key feature of cancer. The study of epigenetics in cancer has revealed that human cancer cells harbor both genetic alterations and epigenetic abnormalities that interplay at all stages of cancer development. Unlike genetic mutations, epigenetic aberrations are potentially reversible through epigenetic therapy, providing a therapeutically

relevant treatment option. Histone methyltransferase inhibitors are emerging as an epigenetic therapy approach with great promise in the field of clinical oncology. The recent accelerated approval of the enhancer of zeste homolog 2 (EZH2; also known as histone-lysine *N*-methyltransferase EZH2) inhibitor tazemetostat for metastatic or locally advanced epithelioid sarcoma marks the first approval of such a compound for the treatment of cancer. Many other histone methyltransferase inhibitors are currently in development, some of which are being tested in clinical studies. This review focuses on histone methyltransferase inhibitors, highlighting their potential in the treatment of cancer. We also discuss the role for such epigenetic drugs in overcoming epigenetically driven drug resistance mechanisms, and their value in combination with other therapeutic approaches such as immunotherapy.

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Key Summary Points

Global disruptions in the epigenetic landscape are a key feature of cancer.

Unlike genetic mutations, epigenetic aberrations are potentially reversible through therapy with epigenetic modifiers.

In a rapidly evolving field of research, histone methyltransferases have been targeted with a range of small-molecule inhibitors.

Histone methyltransferase inhibitors are in development for the treatment of both solid and hematologic malignancies.

The success in this field is highlighted by the clinical development of molecules and the recent approval of the methyltransferase inhibitor tazemetostat (EZH2 inhibitor).

INTRODUCTION

In eukaryotic cell nuclei, DNA is packaged and organized in a DNA–protein complex called chromatin. The basic component of chromatin is the nucleosome, consisting of approximately 147 bp of DNA and core histones (H2A, H2B, H3, and H4) [1–4]. Nucleosomes associated with active genes are more accessible than those with inactive genes, suggesting that the degree of chromatin packaging correlates with gene activity [5].

In contrast to inherited genetic variation that remains constant over the lifetime of an individual, epigenetics differ as disease changes over time. This provides the opportunity to employ epigenetic changes as biomarkers for disease and targets for disease modification in many fields of healthcare [6]. Unlike genetic mutations, epigenetic aberrations are potentially reversible. Thus, targeting relevant epigenetic factors with small molecules might provide an efficient approach to “fix”

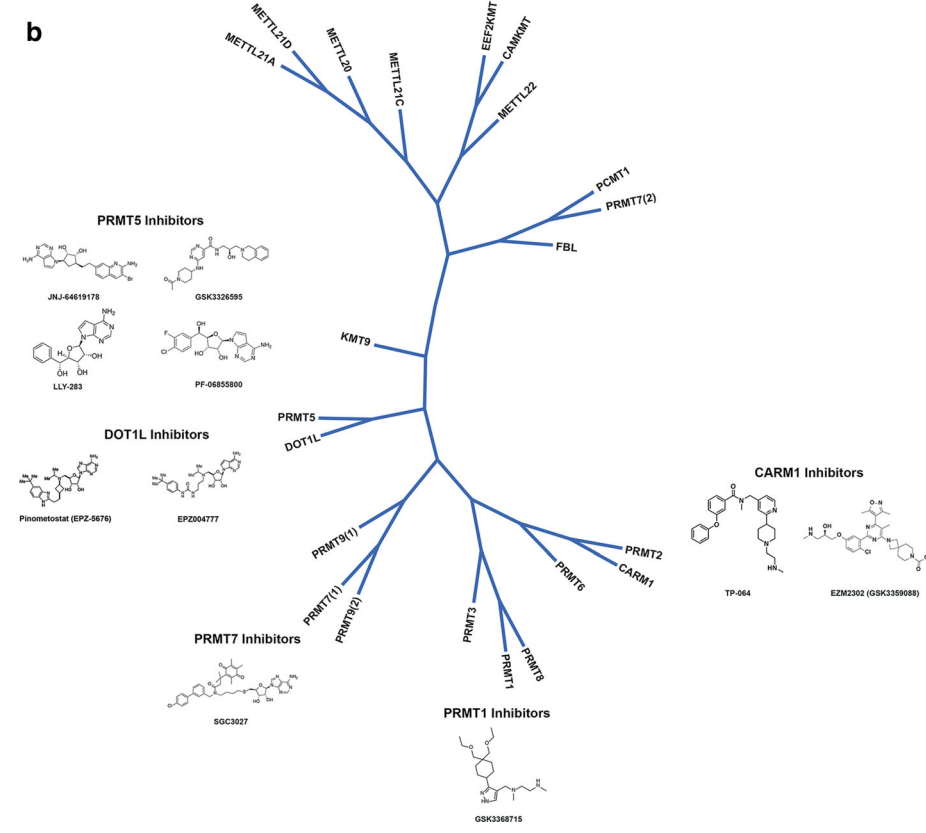
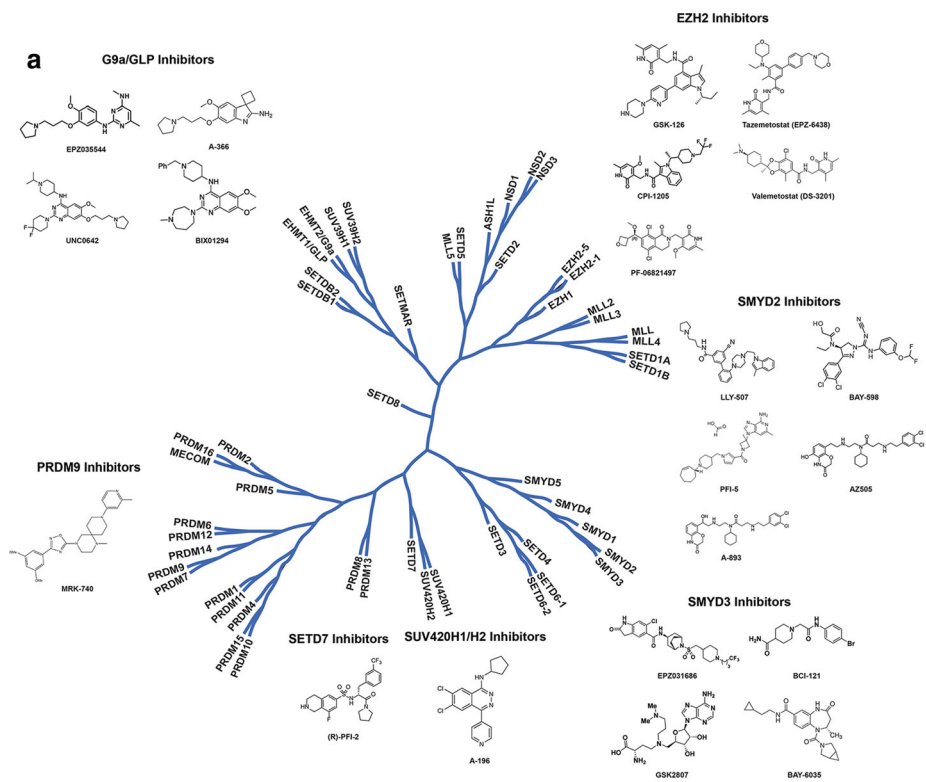
Fig. 1 Phylogenetic tree of protein methyltransferases and molecules in development ▶

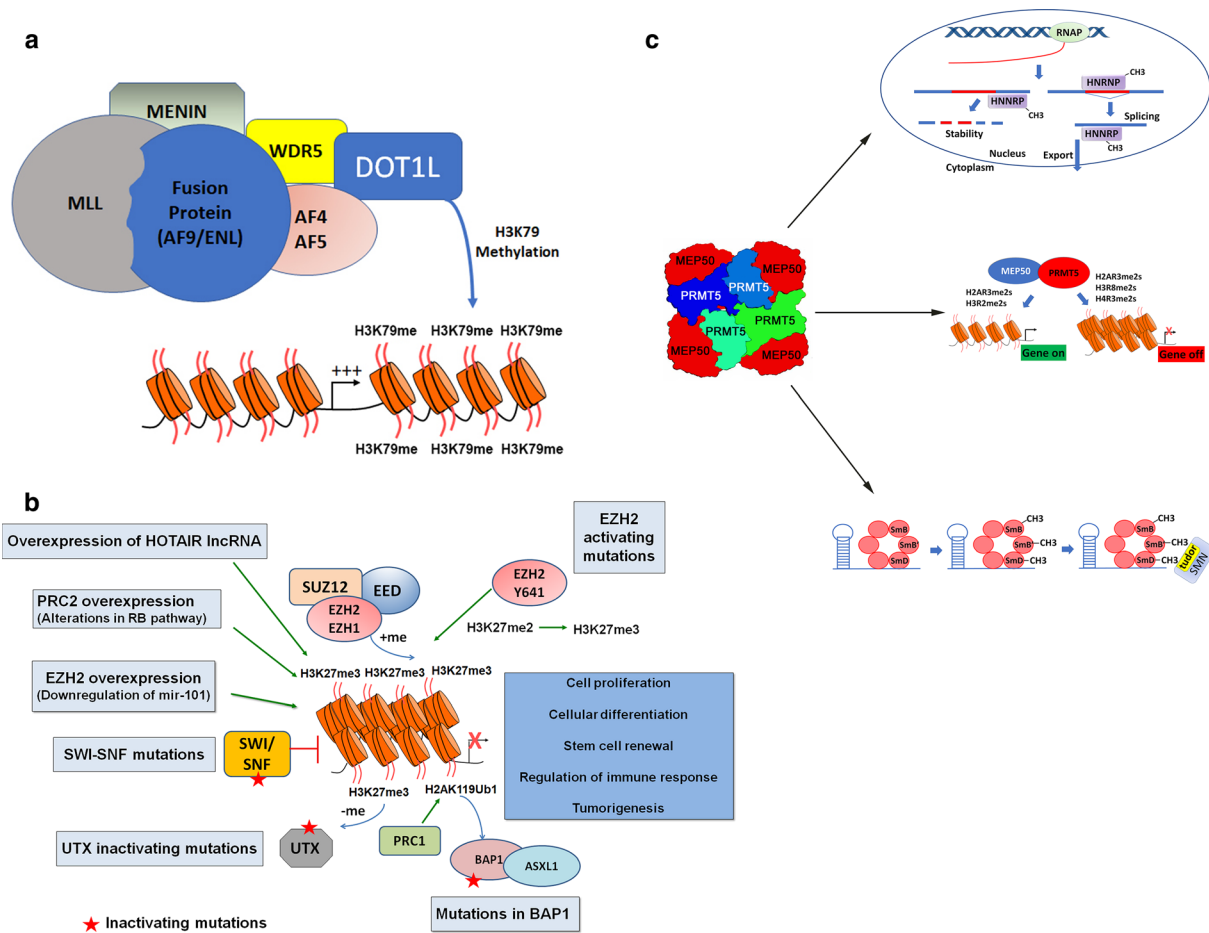
dysregulated gene/chromosome-regulatory systems resulting from aberrant epigenetic profiles. Epigenetic mechanisms are important for the regulation of cell proliferation, differentiation, survival, and other cellular processes [7]. Epigenetic dysregulation results in changes to the transcriptional landscape, affecting multiple pathways which might contribute to tumor pathogenesis [8]. Here we provide an overview of epigenetic modification with relevance to cancer treatment, focusing on histone methyltransferases. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

HISTONE METHYLTRANSFERASE INHIBITORS

According to the histone code hypothesis [9], transcription is in part regulated by methylation of histone proteins resulting in recruitment of other proteins through the specific recognition of modified histone proteins [9]. Lysine as well as arginine residues of the N-terminal tail can be methylated [9]. Lysine methylation has three methyl additions (mono-, di-, and trimethylation). Arginine methylation has two methyl additions (mono- and di-), with dimethylation occurring on either single (asymmetric) or two (symmetric) nitrogen atoms [10]. Key lysine and arginine methyltransferases under investigation for cancer treatment include enhancer of zeste homolog 2 (EZH2), G9a, disruptor of telomeric silencing 1-like protein (DOT1L), and protein arginine methyltransferases (PRMT) 1 and 5. Figure 1 illustrates a phylogenetic tree of protein methyltransferases and the publicly available chemical structures for some of the associated inhibitors.

Genome-wide studies have revealed that different levels of histone methylation of H3K4, which can be mediated by distinct histone methyltransferases [11, 12], have been linked to





specific gene activities: H3K4 monomethylation, together with H3K27 acetylation, marks enhancer activity, whereas H3K4 trimethylation and dimethylation are associated with the transcription start site of active genes [11, 13, 14]. It is also known that specialized proteins such as Jumonji domain-containing 2A (JMJD2A), p53-binding protein 1 (53BP1), SAGA-associated factor 29 (SGF29), and others interact with a broad spectrum of histone methylation marks to facilitate DNA damage repair or regulate transcription [15].

Histone methylation was initially considered relatively stable compared with histone acetylation, but it can also be removed by histone demethylases, e.g., lysine-specific demethylase 1A (LSD1 or KDM1A), the lysine demethylase for H3K4 and H3K9 [16, 17]. The Jumonji C [Jm]C domain-containing protein UTX [ubiquitously transcribed tetratricopeptide repeat, X

chromosome], as well as the related JMJD3 [Jumonji domain containing 3], specifically remove methyl marks on H3K27 [18]. Interplay of these epigenetic mechanisms regulates tissue-specific gene expression patterns and preserves cellular identity. For example, histone methyltransferases (G9a, Suv39h1, and PRMT5) recruit DNA methyltransferases (DNMTs) to specific genomic loci to promote DNA methylation [19–21]. On the other hand, DNMTs can recruit histone deacetylases (HDACs) and other effector methyl-binding proteins to promote gene silencing, highlighting the linkage between DNA methylation and histone modification machinery critical for maintaining the cellular epigenetic landscape.

In an area that has evolved at a rapid pace in recent years, histone methyltransferases have been targeted with a range of small-molecule inhibitors. The success in this field is

◀**Fig. 2** Graphical representation of the mechanisms of selected methyltransferases targeted by protein methyltransferase inhibitors. **a** In acute myeloid leukemia, chromosomal translocations result in formation of oncogenic fusion proteins that combine the chromatin targeting domains of MLL to the protein recruitment domains of fusion partners including AF9 and ENL. These fusion partners initiate the recruitment of DOT1L H3K79 histone methyltransferase function to genes that govern cell proliferation and cell differentiation. At these genes, DOT1L enzyme function increases transcriptional output of these genes, resulting in oncogenic programs. **b** EZH2 or the related protein EZH1 comprise the polycomb repressive complex 2 with partner proteins EED and SUZ12. In normal development and tissue regulation, PRC2 regulates gene expression programs governing cell proliferation, cellular differentiation, stem cell renewal, and immune regulation. In cancer, activation of PRC2 through gain-of-function mutations in EZH2 or overexpression of EZH2 alter the normal regulation of PRC2 function leading to uncontrolled cell growth, survival, and immune evasion. Cancers harboring mutations in epigenetic regulators including SWI/SNF, EED, and BAP1 that antagonize EZH2 and PRC2 function also create opportunities for targeting EZH2 as a synthetic lethal vulnerability in cancer. **c** PRMT5 and MEP50 form a heterooctamer complex catalyzing the symmetric dimethylation of proteins involved in RNA transcription, processing, metabolism, and splicing. PRMT5 works both in the regulation of gene expression through histone methylation as well as alternative splicing of pre-mRNA through methylation of splicing proteins. Both types of regulation impact gene expression and pre-mRNA processing and thus can be considered ‘epigenetic’. The first part of **c** demonstrates the role of PRMT5 in the methylation of HNRNP proteins. These proteins are involved in the regulation of mRNA stability, splicing, and nuclear export. PRMT5 adds symmetric dimethylation to arginine residues on hnRNP proteins, hnRNPA1 and hnRNAH1. The middle of **c** shows PRMT5 methylation of histone proteins. This type of methylation can activate or repress gene expression depending on the arginine residue that is modified. Symmetric dimethylation of histone arginine residues by PRMT5 on H2AR3, H3R2, H3R8, and H4R3 has been demonstrated to impact both transcriptional activation and repression in a context-specific manner. The third part of **c** involves the methylation of Sm proteins by PRMT5. PRMT5 directly methylates Sm proteins including SmB, SmB', and SmD. This methylation is critical for forming key components of the spliceosome through the recruitment of SMN proteins and RNAs for assembly and biogenesis of snRNP core particles required for pre-mRNA splicing that form snRNP complexes

highlighted by the clinical and translational development of molecules discussed in the following paragraphs. Figure 2 is a graphical representation of the major complexes covered in this review (polycomb repressive complex 2 [PRC2], EZH2, DOT1L, and PRMT5).

EZH2

EZH2 is a histone lysine methyltransferase that forms the catalytic subunit of the PRC2 complex, also including EED, SUZ12, and RbAp46. It primarily catalyzes H3K27 methylation which results in gene silencing [22]. It is important in the control of genes involved in the regulation of cell fate decisions, such as self-renewal and differentiation. EZH2 has also been associated with repression of tumor suppressor and epithelial-mesenchymal transition (EMT)-related genes such as p16^{INK4a} and E-cadherin [23]. Apart from its transcriptional repressor activity on chromatin, EZH2 has also been reported to catalyze certain non-histone substrates, including signal transducer and activation of transcription 3 (STAT3), GATA4, androgen receptor (AR), and retinoic acid-related orphan nuclear receptor α [24–27].

Alterations in EZH2 are associated with cancer development and progression. Gain-of-function point mutations at Y641 and A677 in EZH2, which lead to activation of methyltransferase activity and increased H3K27 trimethylation, have been observed in follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL), and associated with disease progression [28–30]. Increased EZH2 activity in such tumors has been associated with suppression of differentiation. EZH2 is overexpressed in solid tumors, including breast, bladder, endometrial, liver, ovarian, prostate, small cell lung cancer (SCLC), melanoma, glioblastoma, and pediatric glioma, as well as lymphomas, correlating with disease progression and poorer prognosis [31–37].

Upregulated EZH2 activity in SCLC, associated with frequent inactivation of the retinoblastoma (RB1) tumor suppression gene, promotes tumor growth and resistance to standard-of-care chemotherapy, resulting in poor

overall survival [38]. EZH2 activity has been associated with tumor progression in prostate cancer, and EZH2 inhibition resulted in antitumor effects both in in vitro and in in vivo prostate cancer preclinical models. Moreover, EZH2 inhibitors synergize with the androgen receptor antagonist enzalutamide in inhibiting growth of adenocarcinoma castration-resistant prostate cancer (CRPC) cells [39–41]. EZH2 activity has also been associated with induction of cellular reprogramming and trans-differentiation of prostate tumors into enzalutamide-resistant neuroendocrine prostate cancer and EZH2 inhibition re-sensitized such NEPC tumors to enzalutamide [42, 43].

In certain cancers, epigenetic factors that interact with EZH2 to regulate cellular transcriptional programs have been shown to harbor mutations. Mutation or loss of INI1 (SMARCB1) and SMARCA4 subunits of the SWI/SNF complex are associated with malignant rhabdoid tumor and ovarian small cell carcinoma of the hypercalcemic type [44–47]. Mutations of *BRCA*-associated protein 1 (BAP1), a deubiquitinating enzyme and a subunit of the polycomb repressive complex containing H2A deubiquitinase activity (PR-DUB) [48], is associated with mesothelioma, uveal melanoma, cutaneous melanoma, renal cell carcinoma, and other cancers [48]. Loss of function mutations in UTX, an H3K27 histone demethylase that antagonizes EZH2 activity, has been reported in multiple myeloma, bladder, medulloblastoma, esophageal, and pancreatic cancers [49, 50]. Preclinical studies imply that such cancer cells are driven by EZH2 activity, with EZH2 inhibition resulting in reduced tumor growth [51–54], prompting their study in the clinical setting.

A number of selective SAM-competitive EZH2 inhibitors have been developed that inhibit both the mutant form of EZH2 as well as the wild-type form [55]. These small molecules mainly inhibit H3K27 di- and trimethylation, leading to reactivation of EZH2 target genes. Six SAM-competitive molecules, CPI-0209, CPI-1205, tazemetostat (EPZ-6438), GSK2816126, PF-06821497, and DS-3201, a dual inhibitor of EZH1 and EZH2, have entered into clinical trials. The clinical trial of GSK2816126 has since been terminated because of modest anticancer

activity at tolerable doses and dosing limitations of the drug [56]. Selective SAM-competitive EZH2 inhibitors presently in clinical trials are shown in Table 1.

Tazemetostat, an orally bioavailable potent and selective SAM-competitive EZH2 inhibitor, was recently approved by the US Food and Drug Administration for epithelioid sarcoma [57], and is currently the most investigated agent in the clinical setting, with antitumor properties demonstrated in preclinical models [58, 59]. A phase 2 study of tazemetostat in patients with relapsed/refractory (r/r) malignant mesothelioma with *BAP1* inactivation (NCT02860286) has also reported favorable safety and tolerability, and promising antitumor activity [60]. Tazemetostat is being tested in an ongoing phase 1/2 clinical trial in patients with r/r B cell non-Hodgkin's lymphoma (NHL) or advanced solid tumors (NCT01897571). The study reported objective responses of 38% ($n = 8/21$) in patients with B cell NHL and 5% ($n = 2/43$) in patients with solid tumors, with favorable safety profiles [61]. Notably, durable objective response or prolonged stable disease was observed in 38% ($n = 5/13$) of patients with INI-negative or SMARCA4-negative solid tumors, whereas responses were not observed in patients with wild-type INI1 or wild-type SMARCA4 proteins [61].

A phase 2 trial of tazemetostat in patients with r/r FL or r/r DLBCL reported clinical activity and durable response with favorable safety profiles in interim analyses. Efficacy for tazemetostat was observed in lymphoma with EZH2 mutations as well as wild-type EZH2. In 39 evaluable patients with r/r FL with an activating EZH2 mutation, the objective response rate (ORR) was 74% with a complete response (CR) rate of 10%. In 53 evaluable EZH2 wild-type patients, the ORR was 34%, with a CR rate of 6% [62]. In patients with r/r DLBCL, the ORR was 17% in both mutant and wild-type subgroups, and a substantially longer duration of response was seen in the mutant arm [63]. A recent update reported an ORR of 77% for 43 patients with EZH2 mutations [64]. The accelerated approval of tazemetostat in epithelioid sarcoma was based on efficacy data from a single-arm cohort (Cohort 5) of a multicenter trial

Table 1 SAM-competitive EZH2 inhibitors in clinical trials

Molecule	Cancer indications in clinical studies	Clinical phase	ClinicalTrials.gov identifier
CPI-1205	B cell lymphoma	1	NCT02395601
	Solid tumor	1/2	NCT03525795 ^a
	mCRPC	1/2	NCT03480646
CPI-0209	Solid tumor	1/2	NCT04104776
DS-3201	AML or ALL	1	NCT03110354
	SCLC	1/2	NCT03879798
	r/r adult T cell lymphoma	2	NCT04102150
Tazemetostat	r/r B cell NHL	2	NCT03456726
	Malignant mesothelioma	2	NCT02860286
	r/r <i>INI1</i> -negative tumors or synovial sarcoma	1	NCT02601937
	r/r FL and DLBCL	1	NCT02220842 ^b
	r/r B cell NHL	1	NCT03009344
	B cell NHL, FL, DLBCL, and solid tumor	1/2	NCT01897571
PF-06821497	SCLC, r/r CRPC, and r/r FL	1	NCT03460977

ALL acute lymphoblastic leukemia, *AML* acute myeloid lymphoma, *CRPC* castration-resistant prostate cancer, *DLBCL* diffuse large B cell lymphoma, *FL* follicular lymphoma, *mCRPC* metastatic castration-resistant prostate cancer, *NHL* non-Hodgkin's lymphoma, *NPC* nasopharyngeal carcinoma, *PD-1* programmed cell death-1, *r/r* relapsed/refractory, *SAM* S-adenosyl-methionine, *SCLC* small cell lung cancer

^a In combination with anti-CTLA-4

^b In combination with anti-PD1 and anti-CD20

(NCT02601950). In this phase 2 trial, tazemetostat was tested across various cohorts of patients with *INI1* deficiency with variable responses. Not all patients with tumors with *INI1* loss responded to therapy, but the epithelioid sarcoma cohort provided evidence for the efficacy of the drug in this setting [65]. Although tazemetostat was tested in other tumors, so far no predictors of response have been identified, even in tumors with *INI1* loss. Although a predictive biomarker for response to EZH2 inhibition is lacking, some data support lower SMARCA2 expression as a biomarker in SWI/SNF mutant cancers [66].

PF-06821497, a potent and selective EZH2 inhibitor with a K_i of less than 0.1 nM against both wild-type and mutant Y641N-EZH2, induced robust tumor growth inhibition in a

dose-dependent manner in DLBCL xenograft models in vivo [67]. This molecule is currently under evaluation in a multicenter, phase 1 study (NCT03460977) in patients with CRPC (in combination with enzalutamide), SCLC (in combination with cisplatin or carboplatin plus etoposide), and FL.

GSK2816126 is a highly selective, SAM-competitive inhibitor of EZH2 methyltransferase activity [68], and was the first commercial EZH2 inhibitor, opening the door for other compounds developed in this space. GSK2816126 efficiently inhibits the growth of EZH2-activating mutant DLBCL cells both in vitro and in vivo [68]; however, a phase 1 study of patients with hematologic malignancies (DLBCL, FL, NKL, and malignant mesothelioma) and solid tumors [69] was terminated

because of the lack of clinical activity, potentially due to poor pharmacokinetic properties.

An ongoing phase 1 study of CPI-1205, a potent, selective SAM-competitive inhibitor [70], in patients with B cell lymphoma reported favorable toxicity, with evidence of antitumor activity and target engagement [71]. In patients with metastatic CRPC, an ongoing randomized phase 1b/2 study (ProSTAR) of CPI-1205 in combination with either abiraterone or enzalutamide reported that CPI-1205 was well tolerated, with a promising pharmacokinetic/pharmacodynamic profile and clinical activity in both arms in second-line treatment [72, 73]. CPI-0209 is a follow-up compound to CPI-1205 with better potency and pharmacokinetics.

Although targeting EZH2 appears to be a promising strategy to treat many types of cancers, when KARPAS422 lymphoma cells were treated with the EZH2 inhibitor EI1 for a prolonged duration, resistant cells carrying the secondary EZH2 mutations Y111L and Y661D emerged [74]. Those mutant cells were not only resistant to EI1 but also to EPZ-6438, which shares the pyridine scaffold with EI1 [74]. Although emergence of such resistant mutations has not yet been observed in the clinic, considering that most potent EZH2 inhibitors contain a common 2-pyridine ring [19, 55], it would be desirable to inhibit EZH2 activity through mechanisms other than a SAM-competitive inhibition. In this context, growing interest in targeting the embryonic ectoderm development (EED) component of the PRC2 complex has evolved as an alternative strategy to inhibit EZH2 [75–79]. For example, Novartis has reported a number of EED binders in development that inhibit basal PRC2 activity, including MAK683, which is an allosteric inhibitor that binds EED and currently in a clinical trial for DLBCL and advanced solid tumors [77].

Some cancers depend on both EZH1 and EZH2 for progression; for example, preclinical studies have shown that the sole inhibition of EZH2 did not suppress MLL-AF9-mediated acute myeloid leukemia (AML), but simultaneous inhibition of EZH2 and EZH1 produced complete suppression [80, 81], and other preclinical studies have shown greater antitumor efficacy for EZH1/2 dual inhibitors compared with EZH2

selective inhibition [82]. The superior antitumor activity of dual inhibition was observed in vitro and in vivo against DLBCL cells harboring gain-of-function mutations in EZH2 [82]. One of these previously reported EZH1/2 dual inhibitors, DS-3201, has since shown clinical activity in patients with NHL, although further clinical data are required to assess if dual inhibition provides a better therapeutic index [83]. Overall, EZH2 inhibitors are emerging as a novel therapeutic option based on their promising clinical data in patients with NHL, especially FL, while their clinical activity in solid tumors is still under investigation. Since oncogenic activity of EZH2 is context-dependent, identification of biomarkers for response along with rational combination approaches might be critical for their successful application in solid tumors. Inactivating EZH2 mutations are also observed in certain tumors, e.g., myelodysplastic syndrome, myelofibrosis, myeloproliferative neoplasm, chronic myelomonocytic leukemia, and T cell acute lymphoblastic leukemia (ALL), so it might play a tumor suppressor role in certain contexts [84–86]. Thus it is important to understand the diverse role of EZH2 in cancer to determine the optimal use of EZH2 inhibitors in the clinic.

DOT1L

The H3K79 methyltransferase DOT1L is involved in gene activation [87, 88]. DOT1L interaction with fusion partners of mixed lineage leukemia (MLL) results in inappropriate DOT1L recruitment and aberrant expression of target genes, leading to MLL-rearranged leukemogenesis [89, 90]. MLL fusion partners AF9, ENL, and AF4 account for over two-thirds of MLL rearrangements [91]. Three compounds (EPZ004777, EPZ-5676 [pinometostat], and SGC0496) have been examined as specific DOT1L inhibitors [92]. Although EPZ004777 demonstrated robust activity in a mouse model of MLL as well as in cultured cells, its pharmacokinetic properties were unsuited to clinical application [92]. EPZ-5676, a DOT1L inhibitor related to EPZ004777, demonstrated better potency and selectivity compared with

EPZ00477 in a rat xenograft model of MLL-rearranged leukemia, with durable complete tumor regression [93].

A phase 1 study of EPZ-5676 (pinometostat) in patients with r/r leukemia involving MLL translocation (NCT01684150) showed that EPZ-5676 was well tolerated with 2 of 51 patients experiencing complete remission [94]. A phase 1 study in children with r/r MLL-rearranged acute leukemia (NCT02141828) demonstrated an acceptable safety profile, though no objective response occurred [95].

The modest clinical response to DOT1L inhibition might be explained by the possibility that residual DOT1L methyltransferase activity is sufficient for cell viability. Further clinical studies, including the ongoing phase 1/2 trial examining EPZ-5676 in combination with chemotherapy agents (NCT03724084) or EPZ-5676 in combination with the DNMT inhibitor azacitidine (NCT03701295), will help evaluate the applicability of DOT1L inhibitors for MLL-rearranged leukemia.

PROTEIN ARGININE METHYLTRANSFERASES

Arginine methylation, mediated by the nine members of the PRMT family, is an important epigenetic mechanism for regulating transcription. The PRMTs are classified as type I, type II, or type III on the basis of the type of methylation made to the target substrates. All three types of PRMT enzymes are capable of catalyzing arginine monomethylation, whereas type I PRMTs catalyze asymmetric dimethylation (me2a) and type II enzymes catalyze symmetric dimethylation (me2s) [96, 97]. PRMTs methylate a variety of substrates, including histones and many non-histone proteins important in cancer signaling pathways.

Coactivator-associated arginine methyltransferase 1 (CARM1 [PRMT4]) is overexpressed in AML and breast, prostate, and liver cancers and is an important regulator of cell differentiation [98–101]. CARM1 asymmetrically dimethylates H3 residues R17 and R26, and also modifies many non-histone proteins. These include members of MLL, SWI/SNF, and

mediator complexes [102], RNA-binding proteins, and some splicing factors [103]. CARM1 has been implicated in both pluripotency [104, 105] and cellular differentiation in multiple lineages [106–108]. It blocks myeloid differentiation of human hematopoietic stem/progenitor cells and is downregulated during myeloid differentiation. CARM1 inhibitors have been reported in preclinical studies from Epi-zyme and Takeda, but these have yet to enter the clinic [109, 110]. For example, depletion of CARM1 reduced the leukemic cell burden in an AML mouse model [98] and pharmacologic inhibition with the CARM1 inhibitor EZM2302 increased survival of AML-transplanted mice [111]. EZM2302 also demonstrated antiproliferative activity of lymphoma cells in vitro and inhibited growth of multiple myeloma tumors in a mouse xenograft model [109].

PRMT5 is overexpressed or upregulated in leukemia, lymphoma, and solid tumors, including glioblastoma and lung cancers [96, 112]. It is the major type II enzyme catalyzing symmetric arginine dimethylation of proteins involved in pathways known to be dysregulated in cancer, including transcription, cell signaling, mRNA translation, DNA damage, receptor trafficking, protein stability, and pre-mRNA splicing [113]. In a colony-forming assay, PRMT5 inactivation inhibited the abilities of cyclin D1, c-MYC, NOTCH1, and MLL-AF9 to induce neoplastic growth [114]. Loss of PRMT5 in primary glioblastoma cells and cell lines resulted in cell-cycle arrest, p53-independent apoptosis, and reduced migration capacity [115]. Genetic knockdown in non-small cell lung carcinoma (NSCLC) cells demonstrated antiproliferative activity that was not rescued by catalytically inactive protein, and it reduced tumor growth in an orthotopic lung cancer mouse model [116]. Taken together, these results suggest that PRMT5 is a potential therapeutic target for many types of cancers.

Preclinical studies have shown the potential for pharmacologic inhibition of PRMT5 in the treatment of cancer. EPZ015666 (GSK3235025), a potent orally bioavailable PRMT5 inhibitor, demonstrated antiproliferation and antitumor activity in vitro and in vivo in mantle cell lymphoma [117] and glioblastoma [118]. The

orally bioavailable PRMT5 inhibitor JNJ-64619178 demonstrated tumor regression in a SCLC xenograft model [119] and has since entered clinical development (NCT03573310). PF-06939999 is a potent and selective orally bioavailable PRMT5 inhibitor with antitumor activity in leukemia and pancreatic cancer xenografts [120]. LLY-283 is a potent and selective PRMT5 inhibitor with demonstrated antitumor effects in a mouse AML xenograft tumor model [121]. CTx-034, developed by a partnership between Merck and Cancer Research Technology (CRT), is an orally bioavailable and potent PRMT5 inhibitor. In mice, administration of CTx-034 was associated with a dose-dependent reduction of the H4R3me2s mark, without a significant reduction in body weight or hematologic changes [122]. Thus far, three PRMT5 inhibitors, GSK3326595 (EPZ015938), JNJ-6461978, and PF-06939999, have entered into clinical studies in patients with hematologic malignancies and solid tumors. A presentation at the European Society for Medical Oncology congress reported data from the METEOR-1 study (NCT02783300) for GSK3326595 in advanced solid tumors [123]. Patients ($n = 54$) received doses of 12.5 mg to 600 mg once daily, and from 50 mg to 200 mg twice daily, with a median time on treatment of 1.8 months (range 1 day to 18.7 months). Grade 3 and 4 adverse events were reported for 35% of patients, and stable disease was reported for 19 patients. Partial responses were observed in patients with human papillomavirus-positive cervical cancer (1 response/1 subject) and adenoid cystic carcinoma (ACC) (3 responses/14 subjects); the responses in ACC were observed at the 200-mg dose. A summary of PRMT inhibitors currently in clinical trials is shown in Table 2.

Research in this field has uncovered potential genetic biomarkers of PRMT5 response. Homozygous deletion of the methylthioadenosine phosphorylase (*MTAP*) gene occurs in many cancer cells because of its location in proximity to *CDKN2A*, a tumor-suppressor gene often deleted in cancers. Viability of *MTAP*-null lines was hampered by shRNA-based inhibition of PRMT5 and its cofactor WDR77 compared with *MTAP*-positive cells [124], though

treatment with the inhibitor EPZ015666 did not demonstrate a significant difference in sensitivity between *MTAP*-null or wild-type cell lines [125, 126]. Similarly, the *TMPRSS2:ERG* fusion gene is commonly found in AR-positive prostate cancer, and PRMT5 shRNA robustly inhibited the growth of AR-positive prostate cancer cells carrying the *ERG* fusion gene, whereas no or only minor effect of growth inhibition was observed in *ERG* fusion-negative cells [127]. GSK3235025-sensitive glioblastoma cell lines demonstrated an altered expression ratio of two PRMT5 cofactors, *CLNS1A* and *RIOK1* [118, 128]. Additionally, AML cell lines with mutations in splicing factors (*SF3B1*, *SRSF2*, and *U2AF1*) demonstrated enhanced sensitivity to GSK591, the first chemical probe of PRMT5 co-developed by Epizyme and GlaxoSmithKline [117, 129]. These results suggest potential biomarkers for preselecting patients who may respond to a PRMT5 inhibitor in clinical studies, though more research is warranted to further refine the responder hypothesis.

PRMT1 is responsible for up to 85% of protein arginine methylation reactions [130] and modifies H4R3, which recruits coactivator p300/CBP-associated factor complex, leading to additional histone acetylation [97, 130]. PRMT1 also methylates many non-histone proteins, including *BRCA1*, *EGFR* [112], and *Ash2L*, a component of H3K4 methyltransferase complexes [131]. Overexpression or upregulation of PRMT1 and its isoforms has been shown in breast cancer, ALL, NSCLC, lung cancer, and glioma [112]. PRMT1 plays an important role in hematologic malignancies [103] and solid tumors [132, 133], although just one PRMT1 inhibitor (GSK3368715) is currently in development. A recent publication reported strong antiproliferative activity for this molecule across a range of solid tumor and hematological models, and synergy with PRMT5 inhibition [134]. Deletion of the *MTAP* gene, which results in accumulation of the metabolite 2-methylthioadenosine, increased sensitivity to GSK3368715 in cell lines [134]. This finding suggests that *MTAP* status may be a useful biomarker for selecting patients that may respond best to GSK3368715. Recently, GSK3368715 entered phase 1 clinical trials for hematologic

Table 2 PRMT inhibitors in clinical trials

Molecules	Cancer indications in clinical studies	Clinical phase	Identifier
PRMT1 inhibitor			
GSK3368715	r/r DLBCL, r/r solid tumors	1	NCT03666988
PRMT5 inhibitor			
GSK3326595	MDS, AML	1/2	NCT03614728 ^a
	r/r solid tumors, NHL	1	NCT02783300
JNJ-64619178	r/r B cell NHL, advanced solid tumors	1	NCT03573310
PF-06939999	Advanced or metastatic solid tumors	1	NCT03854227

AML acute myeloid lymphoma, *DLBCL* diffuse large B cell lymphoma, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin's lymphoma, *PRMT* protein arginine methyltransferases, *r/r* relapsed/refractory

^a In combination with azacitidine and best available care

malignancies or solid tumors with increased incidence of *MTAP* deletion (NCT03666988). Table 3 summarizes various methyltransferase inhibitors under clinical investigation.

EMERGING TARGETS

G9a

G9a (EHMT2) belongs to the Suv39h family, and was initially identified as a histone

methyltransferase that mediates H3K9 and H3K27 methylation in vitro [135]. Subsequent in vivo studies suggest that G9a is a major enzyme for mono- and dimethylation of H3 in euchromatic regions [136–138] and involved in trimethylation of H3K9 [139]. A structurally related methyltransferase, G9a-like protein (GLP/EHMT1), shares the same histone substrate specificities [140–142]. Like many other methyltransferases, G9a (EHMT2) modifies a number of non-histones, including p53 [143]. G9a and GLP (EHMT1) are involved in MYC-

Table 3 Overview of methyltransferase inhibitors under clinical investigation

Target class	Clinical compounds	Manufacturers
Protein lysine methyltransferase inhibitors		
DOT1L inhibitor	Pinometostat (EPZ-5676)	Epizyme
EZH2 inhibitors	Tazemetostat, CPI-0209, CPI-1205, PF-06821497, DS-3201, MAK683	Epizyme, Constellation Pharma, Pfizer, Daiichi Sankyo, Novartis
G9a inhibitor	EZM8266	Epizyme
Protein arginine methyltransferase inhibitors		
PRMT5 inhibitors	GSK3326595, JNJ-64619178, PF-06939999	GlaxoSmithKline, Johnson & Johnson, Pfizer
PRMT1 inhibitor	GSK3368715	GlaxoSmithKline

dependent repression, and G9a inhibition suppresses MYC-dependent tumor growth [144].

G9a is dysregulated in a variety of cancers, including leukemia and ovarian, lung, breast, and other cancers [145]. Pharmacologic inhibition of G9a with BIX-01294, the first-identified G9a inhibitor [146], repressed tumor growth in mouse xenograft models of hepatocellular carcinoma, oral squamous cell carcinoma, and breast cancer [147–149], although cellular toxicity [146] prevented clinical application. Subsequently, several derivatives of BIX-01294 were developed. UNC0638, a potent, selective inhibitor of G9a and glucagon-like peptide-1 (GLP-1), demonstrated a lower toxicity compared with BIX-01294 [150], with an antitumor effect in mouse xenograft models of pancreatic cancer and NSCLC [151, 152]. A-366, a G9a inhibitor structurally unrelated to BIX-01294, demonstrated a 10-fold higher potency for G9a compared with GLP-1 [153], and a lower cytotoxicity compared with other G9a inhibitors, with equivalent cellular activity on H3K9 dimethylation. A recently published report identified G9a as a possible new target for the treatment of bladder cancer [154]. Sustained responses in a mouse model of metastatic bladder cancer were observed with G9a/DNMT inhibition in combination with programmed death ligand 1 (PD-L1) blockade. The study also reported increased G9a levels in bladder cancer patients resistant to PD-L1 inhibition, suggesting potential clinical relevance of this combination approach [154].

Mixed Lineage Leukemia Protein Family

MLL1 is commonly associated with chromosomal translocations in childhood leukemias [155, 156]. In recent years, tumor exome sequencing studies have shown that MLL3 and MLL2 are mutated in a significant proportion of malignancies, particularly solid tumors. The MLL family (MLL1-4) encodes histone methyltransferases for H3K4 that play crucial roles in the regulation of development-related genes, including *Hox* loci. One of the functions of the MLL family of proteins is as a catalytic subunit of the MLL/COMPASS complex, responsible for

mono-, di-, and trimethylation of H3K4 [157]. Although small-molecule inhibitors of the MLL complex catalytic function have not been developed, small-molecule protein–protein interaction inhibitors have been identified for proteins that comprise the MLL/COMPASS complex. Among COMPASS components, menin and WDR5 are of particular interest. Menin is a tumor-suppressor protein that is part of the MLL1/COMPASS or MLL2/COMPASS complex [157] that plays a critical role in H3K4 methylation of *Hox* loci [158]. WDR5 is a common component among all of MLL/COMPASS complexes, and interacts with a number of transcription factors, cofactors, and non-coding RNAs [159]. Like menin, WDR5 is essential for MLL's methyltransferase activities.

The MLL family and their interaction partners are frequently dysregulated in numerous types of cancers. Menin plays important roles in MLL fusion-dependent proliferation, differentiation block, and leukemic transformation [160–162]. WDR5 is overexpressed in solid tumors and leukemia [163–165] and, in patients with leukemia, high WDR5 expression levels are associated with those with high-risk disease [166]. Both menin and WDR5 function as recruiters of MLL fusions to chromatin, and play central roles in leukemic transformation [167, 168], and are thus potential therapeutic targets, with preclinical research underway mostly in MLL-rearranged leukemia.

SMYD2 and SMYD3

A recent report found abundant expression of SMYD2 to be an independent biomarker for poor prognosis of patients with hepatocellular carcinoma, which might imply that SMYD2 overexpression is associated with a more aggressive phenotype [169]. Potent and selective SMYD2 inhibitors have been developed to interrogate this potentially important anti-cancer approach [170–172].

Expressed at high levels in a number of cancers, SMYD3 is a lysine methyltransferase associated with a poor prognosis [173–181]. A causative association between cancer development and SMYD3 function has been shown in

Ras-driven lung and pancreatic adenocarcinomas [182]. Although SMYD3 may be involved in heart development [183] and early development [184], whole-body or organ-specific SMYD3 deletion in mice did not result in aberrant pathology [185]. On the basis of these observations, if developed for the treatment of cancer, one can speculate that SMYD3 inhibition may have few side effects [186–188].

Although there has been extensive preclinical development of small-molecule SMYD3 inhibitors, no clinical trials with these agents are in progress at the time of this writing.

SETD7

Increasing evidence suggests that SET domain-containing (lysine methyltransferase) 7 (SETD7) plays a critical role in a number of physiological and pathological processes, such as metabolism, immunity, and cancer [189]. Histone and non-histone substrates for SETD7 are involved in various distinct cellular operations, including regulation of the cell cycle, response to DNA damage, RNA polymerase II-dependent gene transcription, cell differentiation, and chromatin modulation [12, 190, 191]. Like most lysine methyltransferases, SETD7 contains a SET domain that is responsible for the transfer of a methyl group to a lysine residue [190, 192, 193]. Recently, SETD7 was identified as a potential therapeutic target to inhibit intestinal tumorigenesis, as it is required for Wnt-driven intestinal tumorigenesis and regeneration [194].

SETD8

Current evidence suggests that SETD8 is the only methyltransferase known to catalyze the monomethylation of histone H4 at lysine 20 [195]. Overexpression of SETD8 is observed in some cancers, including pancreatic and bladder cancers, NSCLC, SCLC, chronic myelogenous leukemia, and hepatocellular carcinoma [196]. Various inhibitors of SETD8 have been developed but none have entered into clinical trials at the time of this writing [197, 198].

DRUG RESISTANCE, EPIGENETIC PLASTICITY, AND ANTICANCER IMMUNITY

In 1976, Nowell proposed a well-known tumor evolution model, where the acquired genetic instability combined with clonal selection of tumor cells results in resistance to therapeutic intervention [199]. Inherent variability of cancer cells provides them with the plasticity to survive, even when under the treatment of efficient drugs [200]. Considering a much higher rate of epigenetic “errors” compared with genetic mutations, epigenetic regulators may play an important role in generating new cancer stem/progenitor-like cells as well as in developing cancer heterogeneity, both of which are fundamental mechanisms of developing drug resistance [8, 201].

A certain type of drug resistance caused by epigenetic dysregulation can be reversed by low doses of epigenetic modifiers [201]. Silencing through hypermethylation of the *Schlafen 11* (*SLFN11*) gene is linked to resistance to platinum drugs [38, 202] and treatment of class I (romidepsin, entinostat), but not class II (ricoinostat) HDAC inhibitors increased *SLFN11* expression [203]. A recent study found that combining an EZH2 inhibitor with standard cytotoxic therapies prevented emergence of acquired resistance and improved chemotherapeutic efficacy in chemoresistant and chemosensitive models of SCLC via upregulation of *SLFN11* [38]. These findings suggest the possibility of applying epigenetic modifiers to evade drug resistance, and the study of various drug combinations is warranted in this context.

In addition to tumor intrinsic resistance mechanisms, epigenetic changes in cancer cells have also been associated with a variety of mechanisms mediating escape from immune surveillance [204–208]. These mechanisms include downregulation of cellular antigen presentation machinery, reduced expression of tumor-associated antigens, inhibition of proinflammatory cytokines, and altered expression of checkpoint proteins [209–213]. Epigenetic mechanisms have also been associated with

altered function of immune cells and hence reduced antitumor immunity [205, 206].

Epigenetic reprogramming conferred by EZH2 dysregulation has been identified as a major mechanism of transcriptional repression of genes that confer immunogenicity to tumor cells. This is best exemplified by the role of EZH2 in transcriptional repression of genes involved in antigen-presenting pathways in cancer, and a pivotal role for EZH2-mediated H3K27me3 repressive marks in the maintenance of MHC-I silencing in MHC-I-deficient cancers was recently reported [209]. Interestingly, in neuroendocrine tumors including SCLC and neuroblastoma, EZH2 function appears to hijack a conserved developmental program in neural progenitor cells to potentially confer immune privilege via MHC-I downregulation. Genetic inhibition of EZH2 in a genetically engineered mouse model of SCLC led to MHC-I upregulation and immune-mediated tumor regression [209].

Wang et al. highlighted the critical role of EZH2 activity in mediating T_{reg} cell function in anticancer immunity [214]. Selective upregulation of EZH2 was observed in tumor-infiltrating T_{reg} cells but not in effector T cells and T_{reg} cells in peripheral blood. Genetic deletion or pharmacological inhibition of EZH2 in vitro or in vivo destabilized FOXP3 expression in T_{reg} cells thus promoting immune-mediated rejection of tumors in mouse syngeneic models [214]. These results were further confirmed in a study demonstrating that genetic depletion of EZH2 in T_{reg} cells or EZH2 catalytic inhibition using CPI-1205 elicited phenotypic and functional alterations to T_{reg} cells, along with enhanced activation of effector T cells leading to robust antitumor immunity [215]. However, EZH2 inhibition has also been associated with reduced long-term survival of effector T cells [216, 217], along with induction of immunosuppressive myeloid-derived suppressor cells [218], highlighting the need for further evaluation of its diverse role in mediating immune cell function. Collectively, EZH2-mediated epigenetic programs are essential for the lineage commitment as well as the functional immune suppressive activity of T_{reg} cells, along with tumor escape from immune surveillance via

downregulation of the antigen presentation pathway, providing a rationale for exploring combination therapies of EZH2 inhibitors with immune checkpoint inhibitors.

FUTURE PERSPECTIVE AND CHALLENGES

Although a number of epigenetic modifiers have demonstrated promising results in pre-clinical studies, progressing them for solid tumors has been challenging, and careful consideration of drug combinations may affect their future clinical utility in this respect. A lack of predictive biomarkers has also made progress difficult. However, some traction is being made. For example, recent data suggest that SMARCA2 expression could be predictive for EZH2-targeted approaches in the context of SWI/SNF mutant cancers [66]. It was shown that EZH2 inhibition is effective in SMARCA4 mutant cancers that concurrently transcriptionally silence the paralog helicase SMARCA2. Cellular sensitivity to EZH2 inhibition in SMARCA4 mutant cancer models was associated with lower expression of SMARCA2, independent of tissue derivation.

The combination of epigenetic modifiers with immunotherapy is evolving as another area of great interest, with numerous clinical trials underway in both solid and liquid tumors across a range of epigenetic targets. Other approaches under clinical investigation include combination with treatments such as standard chemotherapy, radiotherapy, angiogenesis inhibition, epidermal growth factor receptor inhibition, and hormone therapy. Methyltransferase inhibitors in combination with checkpoint blockade can play an important role in inhibiting the suppressive function of T_{reg} cells [215], for example, and counter resistance mechanisms that limit the effectiveness of checkpoint inhibitors. As mentioned above, a number of EZH2 inhibitors have now entered into clinical trials, including both CPI-205 and tazemetostat in combination with checkpoint blockade.

CONCLUSIONS

The modulation of epigenetic targets in the treatment of cancer is proving to be a rich area for research, with a plethora of novel epigenetic modifiers in development, some of which are showing early signs of activity in clinical trials. Histone methyltransferase inhibitors are a promising new approach for targeting epigenetic dysregulation in cancer and the recent approval of tazemetostat leads the way for future advances in this field. However, the most optimal approach to using such drugs, including patient selection, the identification of biomarkers, as well as the correct drug combinations, is an area of active research with still many unanswered questions. The outcomes of various ongoing clinical trials will no doubt provide the answers to some of these questions and pave the way to providing novel treatments for patients with an unmet need.

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