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DRUGGING HISTONE METHYLTRANSFERASES IN CANCER

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HIGHLIGHTS

- Chromatin-related genes are often de-regulated in cancers, targeting them constitutes a promising therapeutic strategy.
- More than 50 HMTs exist in mammalian cells; however, inhibitors are only available for a small subset.
- The EZH2 axis is de-regulated in multiple cancer types, where it can play tumor suppressor or pro-oncogenic roles.
- Small-molecule inhibition of EZH2 has shown promising results in the treatment of some blood and solid tumors of specific mutational background.
- Identifying novel synthetic interactions, synergistic drug combinations, and mechanisms of resistance can help expanding the therapeutic niche of EZH2 inhibitors.

ABSTRACT

Targeting chromatin modifying enzymes is a promising strategy for cancer treatment. The anti-tumor effectivity of compounds inhibiting histone methyltransferases (HMTs) -mainly EZH2- is currently being tested in Phase I/Phase II clinical trials; some of them showing positive results in haematological malignancies and solid tumors of specific mutational background. In this review we aim at highlighting the recent advances in the field of HMT inhibitors and describe the challenges that need to be addressed for their successful implementation in the clinics.

INTRODUCTION

Cell identity is controlled by a network of chromatin regulators including histone modifiers, DNA methyltransferases/demethylases and chromatin remodelling complexes. Disruption of one node of this carefully orchestrated network can have profound effects on the epigenetic landscape and thus alter gene expression, genomic stability and/or replication.

Systematic characterization of cancer genomes revealed a high prevalence of mutations in chromatin-related genes, some of which constitute drivers of oncogenic transformation [1,2]. Aberrant proliferation, metabolism reprogramming and mutations in metabolic enzymes also contribute to the disrupted epigenome observed in cancer cells [3]. This has prompted the rational design of compounds that modulate the epigenetic landscape: The so-called *epidrugs* have shown promising anti-cancer properties in pre-clinical models, their application in the clinical setting being currently investigated. Due to the limited success and toxicity of the first generation of *epidrugs* (inhibitors of histone deacetylases and DNA demethylating agents), a second generation of more selective and mechanism-based compounds is under development.

The diversity and high specificity of histone methyltransferases (HMTs) makes them very attractive targets for therapeutic intervention. The number of HMT inhibitors is still limited, but some have reached clinical testing (**Figure 1**). Given the attention they have sparked, we will dedicate the current review to inhibitors of HMTs, focusing largely on those targeting the Polycomb complex PRC2.

PRC2

Polycomb-repressive complex 2 (PRC2) is a multisubunit enzymatic complex that catalyses methylation of H3K27 (H3K27me1/me2/me3) to maintain transcriptional silencing. Within PRC2, EZH1/2 contains a conserved SET domain with methyltransferase activity that adopts an inactive conformation unless it associates with EED and SUZ12 [4]. RBBP4/7 are also core subunits of PRC2 and are believed to facilitate nucleosome binding. In addition, a variety of cofactors bind to PRC2 and modulate its recruitment and activity [5[•],6].

The PRC2 axis in cancer

Early associations between PRC2 and oncogenesis were based on the apparent overexpression of *EZH2* in a wide range of cancer types [7-10[•]]. However, high levels of EZH2 do not necessarily translate into increased dependency on the complex, and loss of EZH2 does not prevent tumor development in mouse models of carcinogenesis [11,12[•]-14]. Hence, EZH2 overexpression in solid tumors is unlikely to play a causative role and probably stems from a need to prevent H3K27me dilution during cell division in highly proliferative tumors [15].

More recently, high-throughput sequencing efforts have identified mutations in PRC2 that enhance or decrease the activity of the complex. These mutations often translate into changes in both the levels and the distribution of H3K27me2/3 and have been proven to predispose to or even be sufficient for tumor development. The underlying mechanisms are often cell type-specific, from aberrant transcriptional activation of developmental regulators, to increased vulnerability to cancer signalling pathways [16[•],17[•], 13]. The context specificity of PRC2 alterations may reflect the *cell-of-origin*, the mutational landscape, and the history of the tumor among other factors [18].

1. EZH1/2 *Gain-of-function* (GOF):

EZH1 and EZH2 are present in similar PRC2 complexes with overlapping targets, but mutational events are far more prevalent in EZH2. Divergent expression patterns (EZH2 is linked to proliferative tissues), functional roles, and enzymatic activity [19] could account for the differences in mutation frequency.

Heterozygous missense mutations of Y641 in the catalytic SET domain of EZH2 have been identified in follicular lymphoma, germinal centre (GC) diffuse large B cell lymphoma (DLBCL), and melanoma [20[•],21]. The mutant PRC2 shows altered substrate specificity, with a preference to methylate H3K27me2 over H3K27me0/1, and cooperates with wild-type PRC2 to yield a global increase in H3K27me3 [22[•],23]. While the oncogenic potential of this mutation is well established -it drives lymphoma and melanoma formation when expressed in mouse B cells and melanocytes, respectively [24,25[•]]- the underlying mechanisms are less well understood. An excess of H3K27me3 on tumor suppressors and bivalent genes normally activated during B cell differentiation could account for the differentiation blockade [26,23-25[•],27]. However, caution should be exercised when ascribing the effect of genome-wide changes to a handful of genes. Whether no other mechanisms than aberrant activity of PRC2 are in place to promote H3K27me3 deposition on tumor suppressor genes remains to be demonstrated.

More recently, EZH1 GOF mutations (Q571R) have been detected in autonomous thyroid adenomas and are expected to translate into a more active PRC2 complex [28], but whether similar oncogenic mechanisms to EZH2 GOF are in place is not known.

2. PRC2 *Loss-of-function* (LOF):

LOF mutations on PRC2 genes have been reported in myeloid malignancies, malignant peripheral nerve sheath tumors (MPNSTs), and T cell acute lymphoblastic leukemia (T-ALL) [29-31]. EZH2 is the major hit in myeloid cancers; SUZ12 and EED are exclusively targeted in MPNSTs; and LOF mutations in EZH2, SUZ12, and EED occur in equal proportions in T-ALL.

Disruption of the PRC2 axis can also result from alterations on its substrate or co-factors. Mutation of K27 in the gene encoding histone H3.3 (H3K27M) has been reported in ~80% of paediatric diffuse intrinsic pontine gliomas (DIPGs) [32,33] and in 6% of secondary acute myeloid leukaemia (AML) [34]. This mutation behaves as a dominant negative inhibitor of EZH2 and results in genome-wide loss of H3K27me3 [35[•]]. The tumor suppressor role of PRC2 in the central nervous system is further supported by the observation that EZHIP, a co-factor that limits PRC2 activity through a mechanism similar to that of H3K27M, is abnormally expressed in a subtype of ependymomas [36-38].

Little is known about the mechanisms whereby loss of function of PRC2 promotes tumorigenesis. Inactivation of PRC2 could decrease the threshold for transcriptional activation of oncogenes and their targets and thus amplify oncogenic signalling (e.g. NOTCH in T-ALL; RAS in MPNSTs) [39,17[•]]. Alternatively, it might increase transcriptional noise and prime for stochastic processes that drive tumor growth and resistance to therapy. The tumor specificity of PRC2-targeting mutations suggests different functional consequences. A simple model is that each of these mutations alters PRC2 activity to a variable extent, hence resulting in de-regulation of a different set of genes.

3. Mutations affecting the essentiality of PRC2:

Another set of therapeutically relevant mutations are those targeting chromatin modifiers that promote transcriptional activation and therefore antagonize PRC2. Disrupting the activity of these enzymes leads to aberrant gene expression, drives tumorigenesis, and potentially renders cells susceptible to PRC2 inhibition. Whether the increased sensitivity to PRC2 inactivation results from loss of epigenetic antagonism or synthetic lethality is still a matter of debate. This is best exemplified by rhabdoid tumors (RT). RTs are deadly paediatric cancers with virtually no genomic instability and a very low mutational burden; the sole recurring event being inactivation of SMARCB1 [40]. Loss of SMARCB1 compromises recruitment of the SWI/SNF chromatin remodelling complexes BAF/PBAF to key developmental genes, which are subsequently targeted and repressed by PRC2 [41]. The essentiality of PRC2 in a SMARCB1-null background has been shown *in vitro*, *in vivo* (in both mice and xenograft models of RT), and was recently confirmed in a clinical trial [42[•],43-45[•]]. Mutations in SWI/SNF subunits other than SMARCB1 have also been associated with higher PRC2 addiction: inhibition of EZH2 was reported to be synthetic lethal in

ARID1A mutant ovarian cancer cell lines [46,47]. However, this notion was recently contested in a panel of ovarian cancer cell lines [48]; the discrepancies between studies potentially arising from differences in the employed small-molecules (GSK126 vs. tazemetostat), genetic strategies (shRNA-mediated silencing of ARID1A vs. ARID1A-mutant cancer cell lines), or cell growth protocols (3D cultures and xenograft assays vs. 2D cultures).

Tumors harbouring mutations in other PRC2-antagonizing enzymes such as the H3K27 demethylases UTX/JMJD3, the H2AK119 deubiquitinase BAP1, and the H3K4 mono-methyltransferase MLL3, were also proposed to be dependent on PRC2 activity [49-52]. However, this awaits clinical validation since, at least in the case of BAP1, synthetic lethality appears to be cell type-dependent [53].

Targeting PRC2

Pharmaceutical intervention of PRC2 addiction is an area that sparks a great deal of interest. High-throughput screens have yielded a collection of pyridone and indole-based small molecule inhibitors that compete with the SAM cofactor for selective binding to the SET domain of EZH1/2 (**Table 1**). A few residues within the SET and SRM domains differ between EZH1 and EZH2 that determine the preferential responsiveness of PRC2-EZH2 to these compounds [54]. EZH1 is more abundant in non-proliferating tissues and has weaker HMT activity when compared to EZH2 [19]; however, it can contribute to the maintenance of H3K27me3 levels in some tissues [55]. These data suggest that simultaneous targeting of EZH1 and EZH2 may be advantageous over EZH2-selective strategies, as proven by the dual inhibitor DS-3201 [56,57]. More recently, molecules that target the H3K27me3-binding pocket of EED, and thus block its ability to activate PRC2 (allosteric antagonists), have shown promising results on DLBCL cell lines, even in those made resistant to SAM-competitive inhibitors [58-60]. Similarly to SAM-competitive inhibitors, this group of compounds displays more robust inhibition of PRC2-EZH2 [54]. A third PRC2 inhibition strategy consists on targeting specific interfaces within the PRC2 complex with synthetic peptides, including that of EZH2-EED [61]. Overall, PRC2 inhibitors selectively and dose-dependently reduce the levels of higher order H3K27 methylation (me2/3), but do not interfere with H3K27me1 [62].

Six PRC2 inhibitors have so far reached clinical trials (**Table 1**). Tazemetostat and CPI-1205 are well tolerated and demonstrated preliminary benefits in B cell lymphomas [45[•],63]. Only a subset of patients showed an objective response (Tazemetostat: 38%); nonetheless, most of them were in very advanced stages of their disease, having either relapsed or become refractory to conventional chemotherapy [45[•]]. Results are less encouraging with GSK126 given its toxicity and limited efficiency at maximal tolerated dose [64]. As monotherapy, EZH2 inhibitors have shown virtually no positive effects in solid tumors with the exception of cases with mutations in components of SWI/SNF [45[•]] and potentially malignant mesotheliomas [65].

Combining EZH2 inhibitors with other therapies can potentially boost their efficacy by promoting synergistic re-activation of tumor suppressors and/or limiting the emergence of resistance and side effects. Several efforts have been done in this line with promising results in preclinical models (**Table 2**). Targeting EZH2 is proposed to sensitize cancer cells to chemotherapy and to boost their immunogenicity, thus rendering immunotherapy strategies more effective [66-69].

PRC2 LOF mutations are more difficult to target pharmacologically since they often destabilize PRC2. In those cases where the mutant protein is synthesized and stable, development of molecules aimed at correcting the impact of the mutation may still be feasible. As proof-of-concept, Suh and colleagues (2019) [70] reported the design of agonists that selectively stimulate the activity of a PRC2 complex containing EED-I363M, a LOF mutation present in myeloid disorders that impairs EED binding to H3K27me3. Alternatively, the epigenetic imbalance triggered by loss of PRC2 could be partially reverted by targeting chromatin modifiers involved in promoting transcription. In this line, bromodomain inhibitors show anti-tumoral activity against DIPGs expressing H3K27M [71] and NF1, SUZ12 double

mutant cancer cell lines [17[•]]. Recently-developed small molecule inhibitors of SWI/SNF subunits [72,73] could also be potentially used to treat PRC2 LOF mutations.

Finally, it was reported that inhibiting the residual PRC2 activity in DIPG could be therapeutically relevant [74], yet further investigations are required to confirm this strategy.

DISCUSSION

HMT inhibitors have shown promising preliminary results in the treatment of human malignancies with otherwise dismal therapeutic opportunities. Yet their application remains limited, several research axes could be developed to favour their implementation in the clinic. Among others:

1. *Expanding the collection of epidrugs:*

Whereas more than 50 HMTs have been identified in mammals, chemical inhibitors are available for a small subset (**Figure 1**), with one target -EZH2- retaining most of the attention. A more systematic characterization of their substrates and function, together with the identification of novel synthetic lethal interactions could stimulate the development of new HMT inhibitors. Data mining from DepMap, a collaborative project that intersects data from genome-wide LOF screens, cellular models and drug screens [75,76], could aid the discovery of novel therapeutically relevant genetic interactions. As proof-of-concept, exploration of DepMap data suggests that PRDM1 is highly expressed and behaves as an essential gene in multiple myeloma in an exclusive manner (**Figure 2**). To date, PRDM1 has not been assessed as a druggable target in this cancer type, but was recently identified as a prognostic marker [77]. Tissue-specific information on gene essentiality is particularly relevant when balancing potency versus undesired toxicity. Of note, a big effort has been dedicated at developing inhibitors against PRMT1 and PRMT5 [78-90]. Both genes are however essential in all cell types assessed by DepMap, suggesting that if the available inhibitors were to be applied in a clinical setting, they would most likely elicit undesired side-effects (**Figure 2**).

Traditional drug design has focused on targeting enzymatic activity. The more recent development of small molecules that exploit the cell's destruction machinery to selectively degrade oncogenic drivers (PROTACs: PROteolysis-TArgeting Chimeras) could also expand the number of druggable targets [91].

2. *Prediction of responders:*

In line with the cell-specific gene targeting of chromatin modifiers, response to *epidrugs* is highly context-dependent. This highlights the need for precise patient stratification with rationale-based *biomarkers* or (epi)genetic determinants that are predictive of drug response. Retrospective epi-transcriptomic profiling of cancer samples could facilitate this process. In this regard, characterization of the activation status of the K-Ras pathway could help predict the response to PRC2 inhibitors, since hyperactivity of this pathway has been associated with resistance to EZH2i [17[•],92,93].

3. *Battling resistance:*

The efficacy of treatments with *epidrugs* is limited by both primary and acquired resistance.

Primary resistance is often detected in solid tumors, which are mostly resilient to *epidrugs* mono-therapy [45[•]]. Their unresponsiveness likely stems from a combination of factors, including (epi)genomic features: mutations in chromatin enzymes can drive tumor formation in the haematopoietic compartment, but they often are permissive or secondary events in solid tumors. Differences in the microenvironment of blood and solid tumors could also account for the differential effectiveness of *epidrugs*. Aberrant proliferation coupled with inefficient vascularization often creates a hypoxic environment in the centre of solid tumors that can lead to acidosis, metabolic reprogramming, and drug resistance [94]. Furthermore, the tissue structure of solid tumors can affect the pharmacokinetics and successful delivery of *epidrugs*. Resistance can also result from the aberrant activation of pro-survival

pathways (e.g. IGF-1R, PI3K, or RAS/MEK/ERK) as reported in DLBCL and SWI/SNF mutant cancer cells [47,92]. Alternatively, it can arise from the inherent interplay between chromatin modifiers. Accordingly, systematic analysis of ~100 cell lines revealed that resistance to EZH2 inhibition was associated with up-regulation of H3K27Ac and transcriptional activation of oncogenic pathways. Resistance was reversed by treatment with BRD4/p300 inhibitors [93]. In ARID1A mutant cancer cells, a switch in the composition of SWI/SNF remodelling complexes (SMARCA4 to SMARCA2) accounts for the resistance to EZH2 inhibitors [94]. As for acquired resistance, secondary mutations on EZH2 have been reported in an *in vitro* model [92], but it is not yet clear whether this is a major phenomenon in the clinics.

Deciphering the underlying molecular events will help broaden *epidrugs* effectiveness and therapeutic niche.

HMT inhibitors have shown promising anti-tumoral activity in clinical trials as single or multi-drug therapies in specific tumor types and mutational backgrounds, increasing in some cases life expectancy with fewer side effects than conventional chemotherapy. Nonetheless, they will need to overcome (some of) the aforementioned limitations for their successful implementation. Furthermore, thorough, extended surveillance of patients exposed to such drugs should be in place since their long-term effects remain unknown.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest.

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FIGURE AND TABLE CAPTIONS

Figure 1. Available small-molecule inhibitors against human HMTs. Inhibitors in current clinical trials are highlighted with a star.

Figure 2. Essentiality of HMTs over a panel of cell lines. Bubble chart plotting the average dependency scores and mRNA levels for known human HMTs (values retrieved from the DepMap portal; DepMap 19Q3 Public) [75,76]. A dependency score of -1 is indicative of essentiality. The number of cell lines per tumor type is indicated between parenthesis.

Table 1. EZH2 inhibitors.

Table 2. EZH2 inhibitors in combination therapies.

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Figure 1.

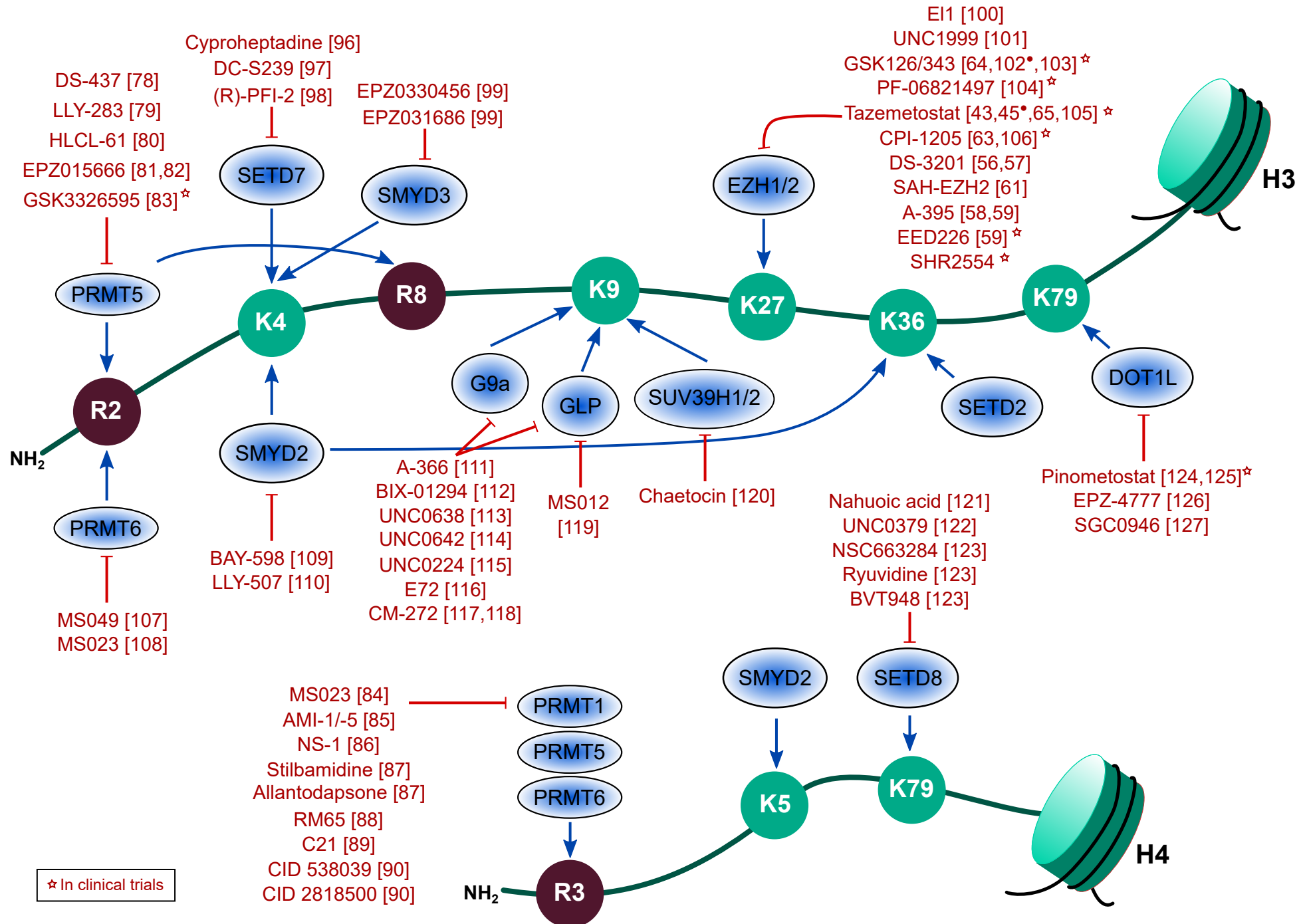


Figure 2.

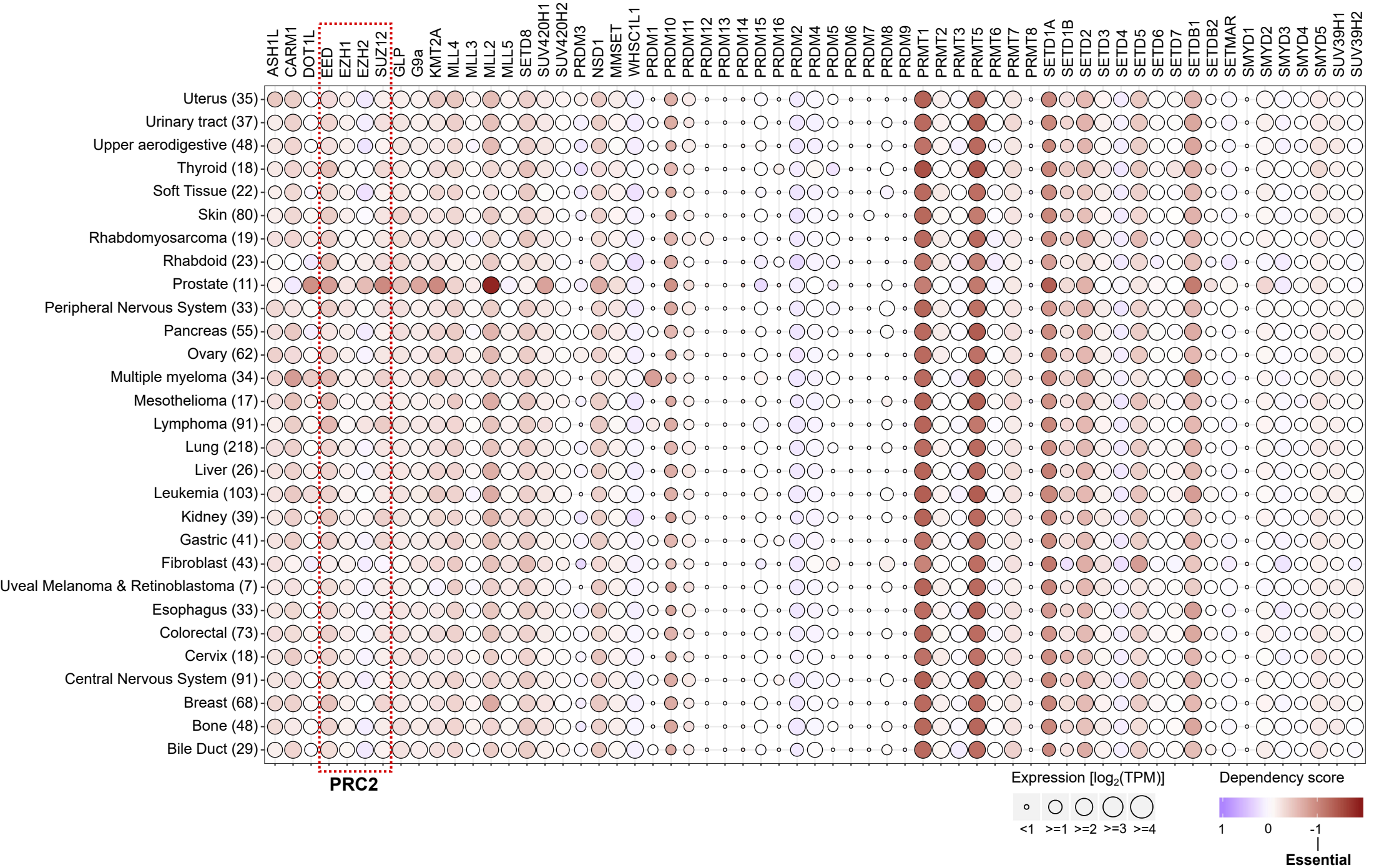


Table 1. EZH2 inhibitors.

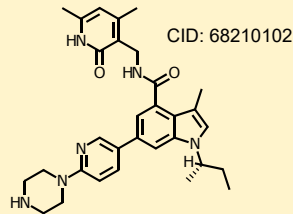
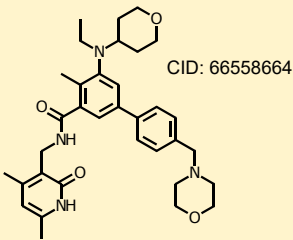
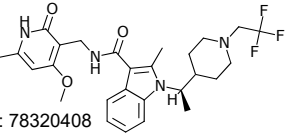
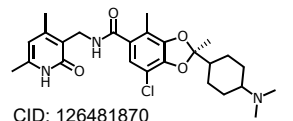
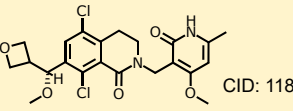
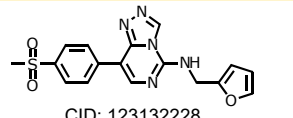
Compound	Mechanism	Selectivity EZH1/EZH2	Structure	Pre-clinical	Clinical	References
EI1	SAM competitive inhibitor	EZH2 > EZH1 (90X)		DLBCL		[100]
UNC1999	SAM competitive inhibitor	EZH2 > EZH1 (10X)		DLBCL		[101]
GSK126	SAM competitive inhibitor	EZH2 > EZH1 (150X)		SCLC	B cell lymphomas Advanced solid tumors Multiple myeloma	[64,102*]
GSK343	SAM competitive inhibitor	EZH2 > EZH1 (60X)		Breast cancer Prostate cancer		[103]
Tazemetostat	SAM competitive inhibitor	EZH2 > EZH1 (35X)		SWI/SNF* rhabdoid tumors EZH2* GOF lymphomas	B cell lymphomas DLBCL, FL SWI/SNF* tumors BAP1* mesothelioma EZH2* GOF tumors Advanced solid tumors Histiocytic disorders	[43,45*, 65,105]
CPI-1205	SAM competitive inhibitor	EZH2 > EZH1		B cell lymphomas	B cell lymphomas Advanced solid tumors	[63,106]
CPI-0209	SAM competitive inhibitor	EZH2 > EZH1		Lymphoma Bladder cancer		[128]
DS-3201		EZH1/2 dual inhibitor		DLBCL, AML Multiple myeloma Solid tumors		[56,57]
PF-06821497		EZH2 > EZH1			SCLC DLBCL, FL Prostate cancer	[104]
SAH-EZH2	Disruptor of EZH2-EED	EZH2 > EZH1		Leukemia		[61]
A-395	PRC2 allosteric antagonist	EZH2 > EZH1		DLBCL		[58,59]
EED226 (MAK683)	PRC2 allosteric antagonist	EZH2 > EZH1		DLBCL	DLBCL	[59]

Table 2. EZH2 inhibitors in combination therapies.

<i>Epidrug</i>	<i>Co-treatment</i>		<i>Evaluated tumor types</i>		<i>References</i>
	<i>Mechanism</i>	<i>Drug</i>	<i>Pre-clinical</i>	<i>Clinical</i>	
Tazemetostat	Standard chemotherapy	Prednisolone Dexamethasone	Non-Hodgkin Lymphoma		[129]
DZNep GSK126	Standard chemotherapy	Etoposide	NSCLC ⁽¹⁾		[130]
GSK126	Proteasome inhibitor	Bortezomib Nimesulide	NSCLC		[131]
Tazemetostat	Standard chemotherapy	R-CHOP 21: Rituximab Cyclophosphamide Vincristine Doxorubicin Prednisolone		DLBCL	NCT02889523
CPI-1205	Anti-androgen therapy	Enzalutamide Abiraterone/Prednisone		Prostate Cancer	NCT03480646
SHR2554	Anti-androgen therapy	SHR3680		Prostate Cancer	NCT03741712
DZNep Tazemetostat	DNMT ⁽²⁾ inhibitor Immunotherapy	5-AZA dC B7-H1 (αPD-L1 mAb)	Ovarian Cancer Hepatocellular Carcinoma		[66,69]
GSK503 CPI-1205	Immunotherapy	IL-2cx Ipilimumab (αCTL-4 mAb)	Melanoma Bladder Cancer		[67,68]
CPI-1205	Immunotherapy	Ipilimumab		Advanced solid tumors	NCT03525795
Tazemetostat	Immunotherapy	Atezolizumab (αPD-L1 mAb) Obinutuzumab (αCD20 mAb)		FL and DLBCL	NCT02220842
Tazemetostat	Immunotherapy	Pembrolizumab (αPD-1 mAb)		Urothelial Carcinoma	NCT03854474
CPI-0209	Standard chemotherapy	Irinotecan		Advanced solid tumors	NCT04104776

(1) NSCLC: Non-Small Cell Lung Cancer

(2) DNMT: DNA Methyltransferase