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REVIEW

Recent advances in targeting protein arginine methyltransferase enzymes in cancer therapy

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ABSTRACT

Introduction: Exploration in the field of epigenetics has revealed the diverse roles of the protein arginine methyltransferase (PRMT) family of proteins in multiple disease states. These findings have led to the development of specific inhibitors and discovery of several new classes of drugs with potential to treat both benign and malignant conditions.

Areas covered: We provide an overview on the role of PRMT enzymes in healthy and malignant cells, highlighting the role of arginine methylation in specific pathways relevant to cancer pathogenesis. Additionally, we describe structure and catalytic activity of PRMT and discuss the mechanisms of action of novel small molecule inhibitors of specific members of the arginine methyltransferase family.

Expert opinion: As the field of PRMT biology advances, it's becoming clear that this class of enzymes is highly relevant to maintaining normal physiologic processes as well and disease pathogenesis. We discuss the potential impact of PRMT inhibitors as a broad class of drugs, including the pleiotropic effects, off target effects the need for more detailed PRMT-centric interactomes, and finally, the potential for targeting this class of enzymes in clinical development of experimental therapeutics for cancer.

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1. Introduction

Virtually every nucleated cell of the human body contains an identical copy of the human genome. While each cell is intrinsically similar, the ability of a cell to differentiate and specialize is essential to normal embryonic development and survival of the organism. Our genome encodes the full repertoire of genes shared by all humans, but it is the coordinated expression of specified sets of genes that determines the differentiation and commitment of a cell to a distinct lineage. This controlled expression is regulated not by direct changes to the DNA sequence, but by a complex assortment of post-translational modifications that alter nucleoprotein–DNA interactions and ultimately drive changes in gene expression. The field of epigenetics studies chemical modifications of DNA and chromatin that set a dynamic stage to affect cellular phenotype, without changing genotype [1].

The structural unit of the chromosome provides a platform for packaging approximately 1.8 m of DNA into the nucleus no more than 10 µm in diameter. DNA wound about the histone structural proteins form the nucleoprotein supra-structure known as chromatin. Four histone proteins (H2A, H2B, H3, and H4, and associated isoforms) assemble into an octamer forming a core about which nuclear DNA is wound in ~150 base pair loops, forming the nucleosome. The dynamic association between DNA and this histone core determines the degree of accessibility of transcriptional machinery to DNA and accounts for fine-tuned regulation of gene expression. Chromatin structure ranges from tightly packed, condensed heterochromatin to a more relaxed, open, euchromatin state. The restricted availability of DNA in heterochromatin generally correlates with more repressed transcriptional activity, whereas a more loosely packed euchromatin state allows for the binding of polymerase machinery, resulting in active transcription. Covalent modifications of specific nucleotides and histone amino acid residues are controlled by a wide array of epigenetic enzymes [2].

Specific marks modified on DNA that are embedded in the genetic code confer gene silencing. Induced by DNA methyltransferases (DNMTs), the methylation of the 5′ carbon of the cytosine nucleotide largely takes place at CpG dinucleotides and is associated with transcriptional repression [3]. Five-hydroxymethyl cytosine (5hmc) has been identified as an additional mark of gene silencing [4]. Believed to be an intermediate step in cytosine demethylation, 5hmc is thought to play a role in somatic reprogramming, hematopoiesis and neuronal development [4–10]. Scientists continue to identify novel epigenetic modifiers that read, write, and erase histone post-translational marks (PTMs), thereby contributing to covalent alterations collectively known as histone code. PTMs of

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Article highlights

- Here we provide a context for understanding the function of PRMT enzymes as post-translational modifiers that play a role in target protein function and epigenetic transcriptional regulation
- We describe members of the PRMT family and review structure/function work that has led to development of novel pharmacologic inhibitors.
- We provide an overview of the roles of arginine methylation in driving cancer.
- We provide an overview of PRMT inhibitors with anti-cancer potential that are being investigated in pre-clinical and emerging clinical studies.
- Finally, we provide an expert opinion on PRMT targeting as a promising experimental therapeutic strategy for solid and blood cancers.

This box summarizes key points contained in the article.

histones include methylation or demethylation of lysine and/or arginine within histone tails; acetylation or deacetylation of lysine residues; ubiquitination of lysine molecules; and, finally, phosphorylation of serine residues on histones [2]. As work continues to identify the mechanism and nature of these enzymes, their role in both normal cellular function and pathologic disease states is becoming more clearly defined. Small molecule inhibitors have successfully been designed to target acetyl transferases, deacetylases, methylases and demethylases, kinases, ubiquitin and SUMO ligases, as well as ATP-dependent nucleosome remodeling enzymes.

Interest in developing novel classes of pharmacological inhibitors continues to grow [11–13]. Protein arginine methyltransferases (PRMTs) have more recently surfaced as a family of highly conserved gene products that act as major players in normal development and disease, positioning this class of enzymes as potentially intriguing therapeutic targets [14,15]. In this review, we will address the mechanism of action of these enzymes and the numerous roles they play in normal cellular function and disease pathophysiology. This review will also discuss current efforts in the field of experimental therapeutics to inhibit the function of several classes of enzymes in this family. Finally, we will conclude with an expert opinion on the relevance of PRMTs as therapeutic targets in cancer.

2. Protein arginine methyltransferases

Arginine contains a guanidine group that provides five potential hydrogen bond donors, promoting a high propensity to engage in bonding with hydrogen bond acceptors. At physiological pH, the guanidine group is protonated; however, it can also be methylated at one or both of its terminal nitrogen atoms [16]. Three types of arginine methylation exist in the form of ω-N^G-monomethylarginine (MMA), ω-N^G,N^G–asymmetric dimethylarginine (ADMA), and ω-N^G,N'^G–symmetric dimethylarginine (SDMA) [17]. Unlike other PTMs, the covalent addition of a methyl group does not change the overall charge of arginine, but rather its addition is absorbed by resonance throughout the guanidinium group. With little change in charge and pKa of the amino acid, the main consequence of this modification is a change in shape. Arginine dimethylation results in a bulkier side chain that eliminates hydrogen bond donor sites and increases overall hydrophobicity [18–20].

Each of the aforementioned histone modifications is catalyzed by a family of nine PRMTs that can methylate both histone and non-histone proteins. An overview of the types of mammalian PRMTs and their associated functions is provided in Table 1. This family is classified into three subtypes, based on the nature of the methylation reaction that is catalyzed. Domain architecture of the PRMTs is summarized in Figure 1. PRMTs type I, II, and III are able to generate ADMA, SDMA, or MMA, respectively, as summarized in Figure 1. PRMT1, the major type I enzyme, is responsible for driving the majority of ADMA modifications. PRMT5, a type II enzyme, drives the formation of SDMA marks (Figure 2) [17]. Furthermore, all PRMTs catalyze the production of MMA.

2.1 Type I PRMTs

Type I classification includes PRMT 1–4, 6, and 8 (Table 1, Figure 1). They initiate reactions utilizing *S*-adenosyl-L-methionine (SAM) as a cofactor that provides the methyl group and catalyze the formation of a monomethylated arginine intermediate. All members of this class go on to utilize a second SAM molecule to subsequently add an additional methyl group, finally forming the characteristic asymmetric mark, ADMA.

Table 1. Overview of mammalian PRMTs.

	Primary marks	Function	Knockout *in vivo*
PRMT1	H4R3me2a, H2AR3me2a	Transcriptional activation	Embryonic lethal
PRMT2	H3R8me2a, H4 site unknown	Transcriptional regulation	Knockout mice are viable
PRMT3	Non-histone RPS2	Ribosomal homeostasis	Knockout mice are viable, but embryos are smaller than wildtype
PRMT4/CARM1	H3R2me2a, H3R17me2a, H3R26me2a, non-histone proteins	Transcriptional activation, mRNA splicing	Knockout mice die shortly after birth Embryos have defects in cellular development
PRMT5	H3R8me2s, H4R3me2s, H2AR3me2s		Embryonic lethal
PRMT6	H3R2, H2AR92, H4R3me2a	Transcriptional activation and repression	Null mice are viable
PRMT7	H4R3, H2AR3, H3R2- monomethyl	Male germline imprinting, DNA repair	
PRMT8	Unknown	Brain specific function	Embryonic and neuronal developmental defects in zebrafish
PRMT9	Non-histone SAP145/49	RNA splicing	

Main marks associated with each PRMT and their functional consequences as well as *in vivo* phenotypes.
CARM1: co-activator-associated arginine methyltransferase 1; PRMT: protein arginine methyltransferases.

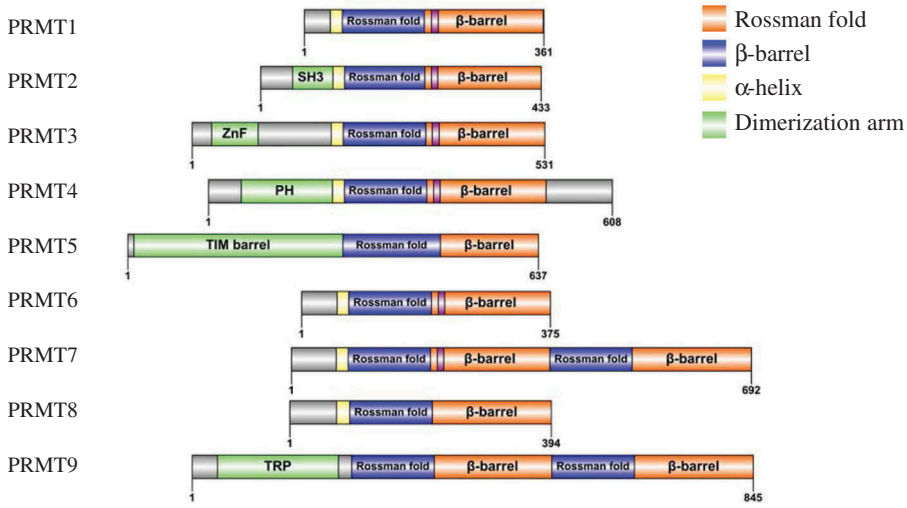


Figure 1. Domain architecture of nine human PRMTs. Abbreviated: SH3, SH3 domain; ZnF, zinc finger motif; PH, Pleckstrin homology domain; TPR, tetratricopeptide repeat.

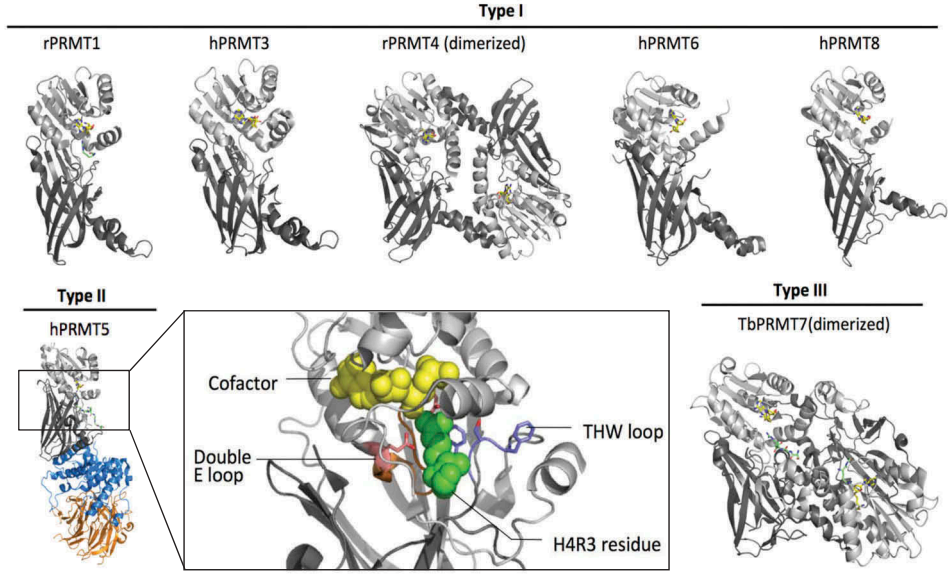


Figure 2. Crystal structures of PRMTs. Conserved catalytic core are shown in grey (Rossman fold in light grey while β-barrel in dark grey). Cofactor or its analogs are indicated in yellow, peptide is in green. TIM barrel domain of PRMT5 is colored in blue, the additional protein binding partner MEP50 is colored in orange. 'Double E' loop is colored in pink and 'THW' loop is in purple. PDB ID: PRMT1, 1OR8; PRMT3, 2FYT; PRMT4, 3B3M; PRMT5, 4GQB; PRMT6, 4HC4; PRMT7, 4M38; PRMT8, 4 × 41.

2.1.1 PRMT1

PRMT1 was the first mammalian PRMT identified. PRTM1 is responsible for over 80% of the steady-state levels of ADMA in the proteome [21]. Substrate specificity of PRMT1 has been shown to favor methylation of arginine residues with neighboring glycines in the +1 or +2 position in a glycine- and arginine-rich (GAR) motif [22,23]. Three-dimensional structure has shown that PRMT1 exists as a homodimer containing two active sites that allow for semi-processive catalysis, in which the substrate remains bound to the enzyme through monomethylation and subsequent dimethylation prior to its release in an ADMA state [24,25]. PRMT1 exerts its epigenetic function by methylating histone 4 at arginine 3, generating the H4R3me2a mark. Interestingly, the first five residues of H2A and H4 are identical, and it is likely that most of the activities at H4R3 hold true for H2AR3 as well [26]. Physiological duplication of this motif provides an efficient means of amplifying signals at each of these sites. H4R3me2a has been characterized as a mark associated with transcriptional activation and is necessary for subsequent acetylation of H3 and H4 [27]. Additionally, PRMT1 has been shown to function as a coactivator and facilitate the recruitment of tumor suppressor proteins Yin Yang-1, p53, and runt-related transcription factor 1 to target gene promoters [28]. Inhibition of PRMT1 leads to cell death and furthermore, PRMT1 null mice are embryonic lethal [27]. Inducible PRMT1 knockout in mouse embryonic fibroblasts leads to induction of genomic instability and cell cycle arrest, emphasizing the importance of PRMT1 in the orchestration of controlled cell division [29].

2.1.2 PRMT2

Initially, because of its weak methyltransferase activity, PRMT2 was not believed to have enzymatic capabilities. However, it was discovered to catalyze asymmetric demethylation of H3R8 and H4, although the exact nature of methylation of this histone protein remains poorly characterized [30]. PRMT2 contains an N-terminal Src homology 3 (SH3) domain that recognizes proline-rich protein motifs and allows it to bind to the N-terminal domain of PRMT8, in order to guide this enzyme to substrate proteins [31,32]. The activity of PRMT2 has been associated with enhanced gene transcription as it regulates the androgen receptor and the estrogen receptor (ER) alpha, the importance of which will be discussed below [33,34]. While it has also been shown to inhibit the function of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and promote cellular apoptosis, PRMT2 knockout mice are viable and exhibit normal development, suggesting that its function is, to some extent, redundant [35,36].

2.1.3 PRMT3

Unlike other methyltransferases in the PRMT family, PRMT3 is located exclusively in the cytosol and does not appear to exhibit any known direct epigenetic functions. Rather, PRMT3 modifies a variety of non-histone substrates that control protein synthesis and the spliceosome. PRMT3 contains a zinc-finger domain at its N-terminus (Figure 1), which allows it to target the 40S ribosomal protein S2 (rpS2) and maintain ribosomal homeostasis within the cell (Figure 1) [37]. Mouse embryos expressing a targeted mutant PRMT3 are smaller than wildtype embryos. Despite this initial developmental abnormality, all survive after birth and reach a normal size during adulthood. Mice that are deficient in PRMT3 activity also exhibit hypomethylated rpS2, confirming this is an *in vivo* substrate for PRMT3 [38].

2.1.4 CARM1 (PRMT4)

PRMT4, also referred to as co-activator-associated arginine methyltransferase 1 (CARM1), was the first PRMT shown to coordinate transcriptional regulation [39]. By generating the H3R17me2a and H3R26me2a marks, CARM1 works with several other transcriptional factors including p53, NF-κB, peroxisome proliferator-activated receptor gamma, and c-Fos to regulate target gene expression [17]. Numerous instances of histone crosstalk have been linked to CARM1-associated marks. CBP/P300-driven acetylation of H3K18 converts H3 to an improved substrate for CARM1 and increases the rate of the methyltransferase reaction [40]. It is hypothesized that by neutralizing the positive charge of K18 the nucleophilic attack on the sulfur-methyl bond of SAM becomes more favorable [41]. It is also hypothesized that the H3R26me2a mark antagonizes methylation of H3K27 by the polycomb repressive complex-2 by preventing enzymatic activity, but not binding of the complex [42]. In addition to epigenetic regulation, CARM1 also methylates transcription factors to coordinate gene expression, splicing factors to couple transcription and splicing processes, as well as RNA polymerase II [43]. Mice with a CARM1 deletion illustrate the *in vivo* importance of this enzyme. Mouse embryos show defects in development of T lymphocytes, adipose tissue, chondrocytes, muscles, and lungs [44–47]. Newborn mice are smaller than wildtype counterparts and die shortly after birth [48].

2.1.5 PRMT6

PRMT6 has been shown to act as both a transcriptional activator and repressor. PRMT6 is located exclusively in the nucleus and targets GAR sequences, asymmetrically methylating H3R2 as well as H4R3/H2AR3 [49]. The H3R2me2a mark antagonizes the activating H3K4me3 mark, alluding to its role as a transcriptional repressor [50]. Conversely, additional reports demonstrate that PRMT6 has the capacity to act as a co-activator of nuclear receptors, although the exact role of this interaction is unclear [51]. While work is ongoing to confirm the true nature of PRMT6, *in vivo* experiments have provided some insight. PRMT6 knockout mice are viable, but embryonic fibroblasts from these mice undergo cell cycle arrest and premature senescence [52]. Transgenic mice that bear PRMT6 fused to the hormone-binding portion of the ER that is inducible by tamoxifen exhibit a dysregulated pro-inflammatory response and die within a 3-week period [53]. *Ex vivo* experiments demonstrate direct binding of PRMT6 with the RelA subunit of NF-κB, facilitating recruitment of NF-κB to selected target promoters and NF-κB-regulated gene expression [53].

2.1.6 PRMT8

PRMT8 is a unique member of this protein family as it is the sole PRMT enzyme to be distributed in a tissue specific manner, largely restricted to neurons. Despite displaying 80% sequence homology and substrate preference with PRMT1, PRMT8 contains a unique N-terminal myristoylation motif that directs its location to the plasma membrane [54,55]. Interestingly, removal of this N-terminal region results in increased enzymatic activity and mono- or asymmetric dimethylation of H2A, H4, and myelin basic protein. In addition to a unique PTM, PRMT8 also possesses two proline-rich regions, allowing it to bind SH3 domains, including that of PRMT2 *in vitro* [32]. Deep sequencing of cancer genomes from a variety of tissues shows that of all the PRMTs PRMT8 is most frequently mutated, with over 100 mutations in the coding region, with over 25,000 samples tested [56,57]. Recently, Lin *et al*. demonstrated a critical role for PRMT8 for both embryonic and neuronal development in a zebrafish model, highlighting its relevance in developmental biology in vertebrate organisms [58].

2.2 Type II PRMT

2.2.1 PRMT5

PRMT5 is the sole type II PRMT enzyme and is largely responsible for catalyzing the SDMA mark on a wide array of proteins and histones within chromatin [59]. The tertiary structure of PRMT5 assembles into a two-domain structure, forming a triose-phosphate isomerase (TIM) barrel at the N-terminus. Ultimately, PRMT5 forms a hetero-octomeric complex with the methylosome protein 50 (MEP50), with PRMT5 tetramer in the center, surrounded by four MEP50 molecules interacting with the TIM barrel domains [60]. PRMT5 in complex with

MEP50 shows enhanced capacity to drive its characteristic type II SDMA marks on histones H2A, H3, and H4 (H2AR3me2s, H3R2me2s, H3R8me2s, and H4R3me2s) [60–63].

Interestingly, the PRMT5-MEP50 complex is capable of methylating hypoacetylated histones more efficiently than those that are hyperacetylated and of driving target gene transcriptional activation via differential ratios of H4R3me2s/H3R8me2s [64,65]. PRMT5 has been shown to play a pro-survival role by methylating non-histone proteins as well. The post-transcriptional methylation of P53 and the NF-κB P65 subunit causes altered recruitment to target genes, leading to cell cycle progression, increased production of inflammatory cytokines, and cellular transformation [66–69].

PRMT5 is capable of methylating histones prior to incorporation into chromatin during embryogenesis and contributes toward maintaining the undifferentiated and pluripotent state of embryonic stem cells (ESCs) [70,71]. This role is essential for ESCs as PRMT5 null mice die before birth [71]. PRMT5 conditional knockout mice demonstrate aberrant erythroid differentiation and thymocyte development as well as a loss of lineage committed progenitor stem cells. Furthermore, PRMT5 null mice exhibit stem cell exhaustion and ultimately succumb to bone marrow failure. These effects appear to directly result from the loss of PRMT5 methyltransferase activity, impaired cytokine signaling, and increased p53 activity [72–74].

In addition to the role in cellular development and differentiation, PRMT5 assembles into larger macromolecular complexes including SWItch/sucrose non-Fermentable (SWI/SNF) chromatin remodelers to silence numerous regulatory and tumor suppressor genes via chromatin hypermethylation at gene promoters [62]. PRMT5-mediated marks facilitate global repression of genes that coordinate a variety of cellular functions including RNA processing, transcriptional regulation, and signal transduction pathways, all of which are relevant to embryonic development and, when dysregulated, contribute to oncogenesis and maintenance of the malignant phenotype of a variety of solid tumor and blood cancers [75].

2.2.2 PRMT9

While initial studies have revealed the structure of PRMT9, the enzyme has not been fully characterized, and multiple aspects of the mechanisms that regulate its expression and cellular function remain unknown. Like PRMT7, PRMT9 contains two SAM binding domains; unlike other PRMTs, it possesses three tetratricopeptide repeat motifs that facilitate protein–protein interactions [54,56]. Mutant PRMT9 devoid of any of these domains does not exhibit functional methyltransferase activity [76]. Recent work by Yang *et al*. demonstrated that PRMT9 has type II PRMT activity and symmetrically dimethylates spliceosome-associated proteins-SAP145 and SAP49 [77]. Methylation of SAP145 creates a binding site for the Tudor domain containing Survival of Motor Neuron protein. This modification allows for the assembly of ribonucleoproteins and ultimately facilitates mRNA splicing [77]. Work from the same group showed that PRMT9 demonstrates relatively weak methylation activity compared to common PRMT substrates and appears to be relatively specific for the splicing factor [76]. It is interesting that both type II PRMTs (PRMT5 and 9) are involved in the post-translational methylation of RNA splicing machinery.

2.3 Type III PRMTs

2.3.1 PRMT7

Multiple recent reports have shown PRMT7 to catalyze both a monomethyl and a symmetric dimethyl arginine mark in a substrate-dependent manner, categorizing it as either a type II or type III PRMT [78,79]. However, Zurita-Lopez and colleagues have shown that PRMT7 exclusively produces the MMA mark on both histone and non-histone substrates *in vitro* [80,81]. Like PRMT9, PRMT7 uniquely contains two SAM-binding motifs. Mouse PRMT7 has been implicated in histone methylation, as was identified by Feng *et al*. via top-down mass spectroscopy experiments that demonstrated its ability to preferentially methylate-specific arginine residues surrounded by lysines within RXR motifs of H2B, and support formation of MMA [82]. This study identified specific substrate motifs that PRMT7 recognizes. Authors speculate that methylation of H2B by PRMT7 may play a role in transcriptional regulation since the N-terminal region of H2B that contains the motif recognized by PRMT7 plays a role in transcriptional repression and also mediates histone methylation by the methyltransferase Dot1 and facilitates telomeric silencing [82,83].

PRMT7 has been implicated in a variety of functions. Reports have shown that PRMT7 has a role in male germline imprinted gene methylation through its interaction with CTCF like/brother of the regulator of imprinted sites (CTCFL), a regulator of imprinted gene expression [84]. In addition, PRMT7 has been shown to negatively regulate the expression of genes involved in DNA repair, with conflicting publications showing a loss of PRMT7 to promote both resistance and sensitivity to DNA damaging agents [85,86]. Several reports have demonstrated a role for PRMT7 in the stemness of both ESCs and satellite cells. In ESCs, PRMT7 indirectly regulates the expression of pluripotent genes through a microRNA controlled double-negative feedback loop [87]. Depletion of PRMT7 in satellite cells results in premature senescence, delayed activation of myogenic differentiation, and loss of stem cell properties, resulting in the inability to engage in tissue repair and defects in muscle regeneration [88]. This regulation is mediated through PRMT7-dependent methylation of both the *DNMT3b* and *Cdnk1a* promoters [88]. Finally, two separate studies have identified patients with autosomal recessive developmental disorders related to PRMT7 mutations. These genetic studies support the role of PRMT7 in human intellectual and physical development [89,90].

2.4 Demethylation

Multiple experiments that investigated the turnover of arginine methylation have shown it to be a particularly stable mark [27,56,91]. However, dynamic changes in the appearance and disappearance of the methylarginine mark raises the question of whether it is in fact a reversible modification [92,93] . While it is still unclear as to how exactly this mark is removed, several mechanisms have arisen as potential candidates for arginine demethylation: direct demethylation by jumonji C domain-containing histone demethylases (JmjC KDMs), or deimination by peptidyl arginine deiminases

(PADIs) [94–96]. Ultimately, further investigation into the exact mechanism of arginine demethylation is essential to understanding the functionality of this modification.

2.4.1 Peptidyl arginine deiminase activity

Peptidyl arginine deiminases (PADIs) are known to convert arginine to citrulline through hydrolysis of the guanidinium group [97]. It was hypothesized that PADIs also have the capacity to convert methylated arginine in the same manner to citrulline; however, structural analysis of the PADI active site and *in vitro* assays with mono- and dimethylated peptides suggest otherwise. Studies have shown that the demethylimination reaction occurs 100–10,000-fold slower for mono- or dimethylated arginine residues than it does for unmethylated arginine [19,97]. Thus, it appears that rather than a conversion of methylated arginine to citrulline, citrullination works to directly antagonize arginine methylation and that these two modifications are mutually exclusive. As work continues to investigate this family of enzymes, the kinetics of the modification itself will continue to be explored and the stability of the mark will be clarified. While citrullination does remove the methyl-arginine mark, it is demethylimination and not true demethylation,

2.4.2 JmjC oxygenases

The identity of a true arginine demethylase has been the topic of controversy for several years. JMJD6 is a JmjC domain-containing protein reported to demethylate both H3R2me2 and H4R3me2, both symmetric and asymmetric dimethylation [98]. While JMJD6 is considered by some to be a true demethylase, contradictory reports have surfaced since its initial characterization. Structural studies by Hong *et al*. and Mantri *et al*. have shown that the active site of this enzyme is not conducive to arginine demethylation [27,99,100]. Other studies by Webby *et al*. have shown that JMJD6 functions rather as a lysine hydroxylase and they were unable to detect any demethylation on H3R2me2 or H4R3me2 peptides *in vitro* [101,102]. Ultimately, there are conflicting reports about the true demethylase nature of JMJD6 [103]. The crossover between lysine demethylation and arginine demethylation led to continued work within the JmjC oxygenase family. Recently it was shown by Walport *et al*. that lysine demethylases KDM3A, KDM4E, and KDM5C also exert similar functions for mono as well as asymmetrically and symmetrically dimethylated peptides [104]. The dual functionality of these enzymes alludes to a unique interplay between lysine and arginine methylation and supports the role of these marks in histone crosstalk. As these JmjC enzymes favor methylated lysine substrates, further exploration into their role *in vivo* and the identification of a sole arginine demethylase is imperative [91].

2.5 Consequences of arginine methylation

Before specific instances of arginine methylation are highlighted in a later section, it is important to give a general overview of the broad consequences of arginine methylation.

One significant outcome of arginine methylation is its effect on histone crosstalk. Methylation of arginine residues by PRMTs directly prevents the binding of other epigenetic writers. For example, methylation of H3R2me2a by PRMT6 directly prevents methylation of H3K4me3 by the mixed-lineage leukemia 1 (MLL1) complex, thus preventing further transcription of MLL1 target genes [50,105,106]. As previously discussed, arginine methylation can be mutually exclusive to other PTMs other than lysine methylation, including lysine acetylation as well as arginine citrullination. Additionally, binding of epigenetic readers containing PHD, Chromo, Tudor, and WD40 domains is also affected [27,105,106].

Conversely, arginine dimethylation can facilitate differential recruitment of effector molecules depending on the symmetry. For example, the PHD domain of the *de novo* DNA methyltransferase DNMT3A has been shown to bind to the PRMT5-specific symmetric H4R3me2s mark. Binding of this enzyme results in DNA methylation, gene silencing, and further illustrates the fact that H4R3me2s is mark that promotes transcriptional silencing and is thus epigenetically repressive [107]. With a few exceptions, asymmetric marks, on the other hand, generally lead to gene activation. These marks represent the majority of those made by the PRMT family and result in the recruitment of complexes that open chromatin and facilitate transcription of target genes. It is through this differential recruitment of activating or repressive complexes that PRMTs are able to directly alter gene transcription.

Finally, arginine methylation is a PTM that extends to non-histone proteins as well. Like other PTMs, changes in methylation can alter interactions with binding partners, targeted substrates, and overall protein stability. Under normal cell physiological conditions, PRMTs operate in a tightly controlled fashion to direct essential cellular processes. However, under malignant conditions, these enzymes may become dysregulated and alter the epigenetic landscape and proteome to drive cell growth and survival.

3. Arginine methylation and cancer

Dysregulated PRMT expression has been documented in a wide variety of solid and hematological malignancies. Most often, PRMT expression is upregulated in cancer and research has shown that a number of the enzymes in this family contribute to oncogenesis. Here, we highlight key members of this family, both type I and type II, that have been shown to play an integral role in oncogenesis and maintenance of the malignant phenotype of both mature and stem-cell components of tumors.

3.1 PRMT1

The overexpression or aberrant splicing of PRMT1 has been found in breast, prostate, lung, colon, and bladder cancers as well as leukemia. The transcriptionally activating epigenetic mark catalyzed by PRMT1 has been correlated with clinical features including tumor grade and overall prognosis in primary and refractory solid tumors [108–111]. Recently, PRMT1 was shown to promote transcriptional activation of zinc finger E-box-binding homeobox 1, a key regulator of epithelial–mesenchymal transition in breast cancer cells [112]. Methylation of this promoter induces migration and invasion of these cells as well as triggering a stem-cell-like state [112].

The role of PRMT1 in cancer is not limited to epigenetic regulation. It has been shown that the interaction with non-histone substrates plays an important role as well. The ability of PRMT1 to regulate signaling pathways such as the ER and promote cell proliferation is potentially an important way that this methyltransferase promotes tumorigenesis. It was reported that the PRMT1 directly methylates ER within the DNA binding domain in MCF7 breast cancer cells, localizing it exclusively to the cytoplasm, and facilitating assembly of the focal adhesion kinase and tyrosine kinase Src. This association leads to activation of serine/threonine-protein kinase AKT and ultimately results in enhanced cell proliferation and survival [113,114]. Additionally, PRMT1 can act directly on substrates of AKT such as forkhead box protein O1 and BCL-2 antagonist of cell death. By methylating residues within an RxRxxS/T motif, PRMT1 acts to directly prevent phosphorylation by AKT on both substrates, suggesting an inhibitory role of PRMT1 methylation against AKT phosphorylation [115,116].

In other work, PRMT1 has been implicated as a driver of acute myeloid leukemia (AML) development. By direct interaction with the MLL-EEN fusion protein, PRMT1 catalyzes aberrant methylation at target loci and enhances genes associated with hematopoietic potential and promotes survival of leukemia cells [117]. PRMT1 has also been shown to directly interact with and promote transcriptional activation of the AML1-ETO fusion gene to promote cell growth and survival [118]. Finally, PRMT1 has been shown to bind lysine demethylase KDM4C and facilitate epigenetic reprogramming to drive transformation of cells [119].

Methylation of DNA repair proteins 53BP1 and MRE11 by PRMT1 helps facilitate localization and regulate exonuclease activity, respectively, and is required for DNA damage checkpoint control [120,121]. In addition, PRMT1 has been shown to associate with telomeric repeat-binding factor 2 or TRF2, part of a larger shelterin complex that maintains telomere length, helps to recruit telomerase, promote the formation of the t-loop, and protects telomeres from being recognized as DNA damage [122–125]. Mitchell *et al*. have shown that TRF2 interacts with and is methylated by PRMT1 [122]. Furthermore, knockdown of PRMT1 promotes telomerase shortening in cancer cells [122]. It is possible that this is a result of the negative feedback loop existing to stabilize telomere length in cancer cells[126]. Alternatively, given its role in telomere protection and regulation of the DNA repair pathway, inhibition of this enzyme may promote the accumulation of genomic damage within the cell [56,122]. Despite this, mounting evidence of the role of PRMT1 in cancer suggest that it could be a promising target for inhibition in experimental treatment of both solid and hematological malignancies.

3.2 CARM1, also known as PRMT4

In addition to controlling a number of normal cellular processes, CARM1 expression is elevated in androgen-dependent and -independent prostate cancers as well as aggressive breast cancer [127–130]. Several studies have shown the ability of CARM1 to regulate the retinoblastoma protein (Rb)/E2F1 axis in breast cancer. CARM1 has been shown to enhance activity and stability of the steroid receptor coactivator AIB1 through asymmetric methylation of arginine residues in the C-terminal affecting both stability and activity [131]. Notably, AIB1 is amplified in ovarian and overexpressed in ovarian and breast cancer cells [132]. Additionally, the AIB1/CARM1 interaction is required for estrogen-induced CARM1 recruitment to ER-promoters [131,133]. Furthermore, this interaction is necessary for driving the production of E2F1 and its target genes, driving cellular proliferation [134]. In addition to directly affecting E2F, CARM1 can negatively regulate by methylating at R7878, which affects its ability to be phosphorylated, bind to E2F1, ultimately impairing cell cycle regulation and ablating the tumor suppressive activity of Rb [135]. Recently, CARM1 was shown to methylate the RNA polymerase mediator complex subunit MED12. Building on previous research, it was shown that methylation of MED12 enhances sensitivity of cancer cells to chemoresistance in both solid and hematological malignancies [136,137].

Furthermore, CARM1 was shown to specifically methylate BAF155, a core member of the SWI/SNF nucleosome remodeling complex that has been identified as a tumor suppressor gene in multiple solid tumor cell lines [138,139]. BAF155 methylation by CARM1 in breast cancer cell lines was shown to affect the distribution of BAF155 binding to chromatin sites as identified by a chromatin immunoprecipitation sequencing approach and to correlate with enriched association of BAF155 with promoters of c-Myc pathway genes [138]. Expression of wildtype BAF155 in a mouse breast cancer model was associated with downregulation of metastasis repressor genes CDH11 and KISS1R and downregulation of metastasis-promoting genes CCL7, CDH6, COL4A2, CXCL12, MMP13, and MYCL, compared with expression of a mutated version of BAF155 R1064K that is unable to be methylated. Interestingly, immunohistochemistry analyses showed that methylated BAF155 levels correlated with elevation of CARM1 protein levels in breast cancer tissues. Notably, long-term follow-up and multivariate analyses performed using the Cox proportional hazards model showed that tumors containing methylated BAF155 were more likely to recur with (recurrence hazard 1.789) compared with tumors that did not show methylated BAF155 [138].

Finally, CARM1 demonstrated additional implications of PRMTs in histone crosstalk. It has been shown that CARM1 can directly methylate histone acetyltransferases CREB-binding protein (CBP) and p300, affecting both auto-acetylation and activity [140]. For example, CARM1 methylation of CBP has been shown to facilitate its interaction with glucocorticoid receptor interacting protein 1 (GRIP1), and steroid hormone-dependent transcriptional activation [141]. Methylation of p300, on the other hand, prevents binding to GRIP1 [142]. Interestingly, it has been shown that CBP is differentially recruited to estrogen-regulated genes dependent upon its methylation status [140].

3.3 PRMT5

PRMT5 is a type II PRMT believed to largely function as transcriptional repressor. Overexpression of PRMT5 has been described in numerous aggressive malignancies including glioblastoma, melanoma, ovarian, lung, colon, gastric, bladder,

germ cell, and several lymphomas [14,70,143–153]. This impaired regulation has been linked to loss of control of the miR92b and miR96 axis, as well as transcriptionally through nuclear transcription factor Y and transforming growth factor beta (TGF-β) stimulation [152–154].

While studies continue to elucidate the mechanism behind PRMT5 upregulation in cancer, additional work strives to describe modifications of PRMT5 and associated proteins that affect its function. The interaction between PRMT5 and JAK2 has recently been further defined. Patients with myeloproliferative disorders express the constitutively active JAK2 mutant JAK2 V617F and demonstrate an increased interaction and subsequent phosphorylation of PRMT5 [74]. This PTM of PRMT5 decreases its methyltransferase activity and prevents its interaction with cofactor ME50, resulting in reduced enzymatic activity of PRMT5. Ultimately, PRMT5 knockdown in human CD34+ cells leads to cellular proliferation and expansion as well as erythroid progenitor differentiation, which in turn suggests that PRMT5 phosphorylation by JAK2 V617F contributes to myeloproliferation induced by mutant JAK2 [74].

Additional modifications affect PRMT5 activity. Aggarwal *et al*. show that cyclin D1-CDK4 phosphorylates MEP50, resulting in the increased activity of PRMT5 [67]. This same study demonstrated that increased PRMT5 activity results in silencing of the expression of *CUL4A/CUL4B* ubiquitin ligases, preventing the degradation of DNA replication factor CDT1 and allowing for initiation of DNA replication, activation of DNA damage checkpoints, and potential for malignant transformation [67].

The physiological consequences of PRMT5 expression in cancer have been explored in a number of malignancies. Overexpression of PRMT5 results in the silencing of a wide variety of tumor suppressor genes including *ST7* and NM23 and regulatory genes resulting in cellular hyperproliferation [62]. Additionally, PRMT5 has been shown to directly methylate several transcription factors including p53, E2F1, HOXA9, as well as NF-κB [66,155–157]. Most notably, the repression of *TP53* and *E2F1* further perpetuates the role of PRMT5 in cancer by allowing this methyltransferase to control growth inhibition, cell cycle arrest, and cell death. Specifically in glioblastoma, PRMT5 knockdown results in arrested cell growth, decreased cell migration, restoration of tumor suppressor genes, and caspase-dependent apoptosis in fully differentiated glioblastoma cells, while in undifferentiated neurospheres it leads to hypophosphorylation of retinoblastoma, resulting in cellular senescence [143,158]. The ability of PRMT5 to regulate both mature and immature cell types is not unique to glioblastoma. In chronic myeloid leukemia, imatinib-insensitive leukemia stem cells rely on PRMT5 for self-renewal and are sensitive to PRMT5 knockdown or treatment with a PRMT5 inhibitor [159]. Finally, PRMT5 was shown to work in concert with key oncogenic drivers including cyclin D1, c-Myc, NOTCH1, and MLL-AF9 in several lymphoma/leukemias, acting as a key point of convergence during lymphomagenesis [69].

Recently, investigations have started to explore the dynamics of the PRMT5-driven methylation mark. Initially this characterized as a repressive mark, but several groups are now showing that PRMT5 activates distinct subsets of genes. Tarighat *et al*. demonstrated that PRMT5 activated SP-1 transcription factor and as a result increases the expression of FLT3 in AML cells both *in vitro* and *in vivo* [65]. Additionally, in lung adenocarcinoma, PRMT5 was shown to activate genes controlling TGF-β-induced epithelial-to-mesenchymal transition [153].

4. Development of selective drugs to inhibit PRMT enzyme activity

Because of the crucial roles that PRMTs play in cancer and other disease states, selective inhibitors are in high demand, not only for enzyme activity inhibition but also for usage as chemical probes to facilitate basic research exploring biological regulatory mechanisms. Advances in X-ray crystallography have expedited exploration of protein structures at the atomic level, providing vast amounts of information to aid in development of selective pharmacological inhibitors.

4.1 PRMT structures and the active site

The first PRMT crystal structure (rPRMT3 conserved core, protein data bank (PDB) ID: 1F3L) was published in 2000 by Cheng *et al*. [160]. Over the past decade, crystal structures of many PRMTs (Table 2) have been solved alone or together with cofactor and substrates, providing insights into their methyltransferase activity. Most of the structures were obtained by co-crystallization of PRMTs with one or more ligands (i.e. cofactors, cofactor analogs, histone peptide

Table 2. PRMT crystal structures.

Isoform	PDB ID
PRMT1	1ORI[a], 1OR8[a,b], 1ORH[a], 3Q7E[a]
PRMT2	N/A
PRMT3	1FL3[a], 1WIR[c,d], 2FYT[a], 3SMQ[c], 4HSG[c], 4QQN[c], 4RYL[c]
PRMT4 (CARM1)	2OQB, 3B3F[a], 3B3G, 3B3J[c], 4IKP[a], 5U4X[c]
PRMT5	3UA3[a], 3UA4, 4G56[a], 4GQB[a,b,c], 4X60[a,c], 4X61[a,c], 4X63[a,c], 5EMJ[a,c], 5EMK[a,c], 5EML[a,c], 5EMM[a,c], 5FA5[b,c], 5C9Z[a]
PRMT6	4HC4[a], 4LWO, 4LWP[a], 4QQK[a], 4CO3[a], 4CO4[a], 4CO5[a], 4CO6, 4CO7, 4CO8, 4QPP[a], 4Y2H[a,c], 4Y30[a,c], 5E8R[a], 5EGS[a,c], 5HZM[a,c], 5WCF[c]
PRMT7	3WST[a], 4C4A[a,c], 3X0D[a], 4M36, 4W37[a], 4M38[a,b]
PRMT8	4X41[a], 5DST[a]
PRMT9	N/A

[a]Structure contains either SAH or cofactor analog.
[b]Structure contains a peptide substrate.
[c]Structure contains an inhibitor or other interacting partners.
[d]Structure solved by solution.
PRMT: protein arginine methyltransferases; CARM1: co-activator-associated arginine methyltransferase 1; NMR: Nuclear Magnetic Resonance.

substrates, or small molecule inhibitors), suggesting that ligand binding stabilizes the protein conformation [161]. Visual representations of the PRMT crystal structures and the conserved catalytic core are shown in Figure 2.

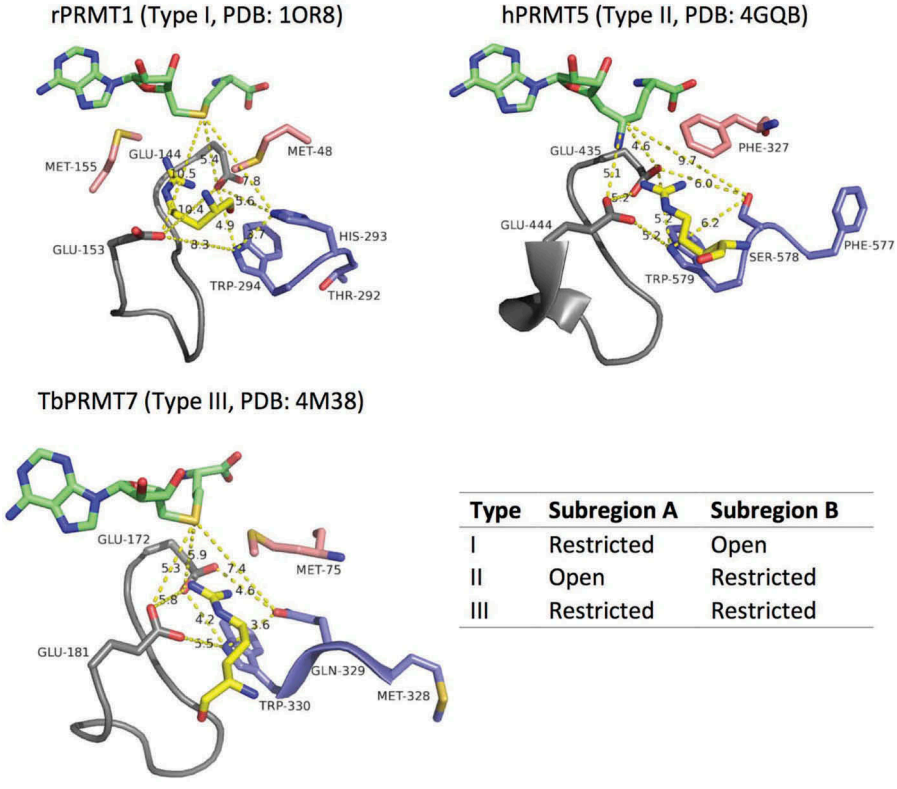
The nine human PRMTs are proposed to share a conserved core, which includes a Rossman fold for cofactor binding and a β-barrel for substrate binding, with the active site located at the fold/barrel interface [162]. Some PRMTs contain domains that facilitate protein–protein interactions, as shown in Figure 1. Evidence shows that these unique domains are responsible for recruiting other proteins, forming oligomers or taking part in substrate binding, regulating the PRMT enzyme activity levels [163,164].

4.2 Catalytic activity of PRMTs

Examples of select crystal structures of PRMTs and a detailed view of the human PRMT5 active site are shown in Figure 2. The positively charged methyl-accepting arginine residue side chain is inserted into a negatively charged pocket generated by two conserved glutamate residues, referred to as a 'double E' hairpin loop (Glu-X8-Glu; known as E144-E153 in PRMT1, E435-E444 in PRMT5, and E172-E181 in PRMT7), with the help of 'THW' loop (known as Thr–His–Trp in PRMT1, Phe–Ser–Trp in PRMT5, and Met–Gln–Trp in PRMT7). The interaction of substrate arginine residues with the 'double E' loop redistributes the positive charge on the guanidino group toward one amino group, while leaving a lone pair of electrons on the other amino group to attack the cationic methyl-sulfonium moiety of SAM in an nucleophilic substitution (bi-molecular) or S_N2-like mechanism [162,165]. The addition of methyl groups to an arginine residue changes its shape and bulkiness; however, methylation does not neutralize the cationic charge of an arginine residue within a substrate protein [17,20].

Structural alignments of known type I, II, and III PRMTs show that the geometries of the active sites are highly conserved within each PRMT enzyme type. However, the shape of the pocket that binds the guanidino group varies in some of the isoforms and is believed to play a role in product specificity of each isoform [166–168]. It has been demonstrated that both 'double E' loop that is assigned to subregion A and 'THW' loop assigned to subregion B are involved in generating product specificity [168]. A structural model proposed by Jain *et al*. highlighted residues that are critical to the methylation reaction and could potentially account for differential enzymatic activity of specific PRMT type I, II, or III enzymes (Figure 3) [168] . Subregions A and B seem to possess varying degrees of special restriction. For example, type III enzyme PRMT7 contains two restricted subregions A and B, which might be the key feature for limiting activity to monomethylation [168].

Additional crucial residues have been identified within the active site of PRMTs through site-directed mutagenesis. These residues are believed to play a key role in the methylation process itself as well as help determine product specificity. For example, alteration of Met48 in rat PRMT1, a residue conserved in the αY helix of many PRMTs, to Phe led to the production of SDMA along with ADMA and MMA [168]. Site-directed



Type	Subregion A	Subregion B
I	Restricted	Open
II	Open	Restricted
III	Restricted	Restricted

Figure 3. PRMT active sites display distinct spatial architectures. The active site of any PRMT can be roughly divided into two subregions: left (subregion A, colored in orange) or right (subregion B, colored in blue) side of the substrate arginine residue. Specifically, type I PRMTs contain a spatially restricted subregion A and an open subregion B, while type II PRMTs contain an open subregion A and a restricted subregion B. Type III contains two restrained subregions.

mutagenesis studies have also shown Phe379 in *Caenorhabditis elegans* and Phe327 in human PRMT5 to be critical residues specific for generating SDMA [60,169].

Recently, Morales *et al*. proposed a catalytic mechanism for PRMT enzymes [166]. A suggested catalytic mechanism for type II PRMT dimethylation is proposed. Kinetic studies have shown that PRMT5 exhibits a distributive methylation mechanism where MMA is released before dimethylation occurs [170,171]. When the MMA substrate returns to the active site, it is reoriented by Phe327 through a cation–pi interaction between the aromatic ring of Phe327 and the positively charged monomethylated guanidyl group, thus promoting orientation of arginine to promote generation of SDMA. Note that the final product is also restrained by the distinct spatial architecture of type II PRMTs in producing SDMA rather than ADMA.

4.3 PRMT inhibitors

Based on the knowledge accumulated by the structure and function of PRMT enzymes, significant attention has been directed at designing potent and selective pharmacological inhibitors of this enzyme family [172]. Thus far, specific inhibitors for PRMT1, 3, 4, 5, and 6 have been developed and several micromolar or nanomolar inhibitors have been obtained for key cancer-related PRMT enzymes such as PRMT1, CARM1, and PRMT5. A comprehensive and detailed review on PRMT-specific small molecule inhibitors was published recently by Hu *et al*. [172]. Furthermore, a summary of PRMT inhibitors used in preclinical testing is provided in Table 3, and chemical structures of inhibitor compounds are shown in Figure 4.

The earliest PRMT inhibitors reported are cofactor (SAM) mimics, such as compounds **1** (SAH) and compound **2** (sinefungin) [173,174] (Figure 4). These small molecules can inhibit the activity of SAM-dependent methyltransferases by competing with SAM binding [163]. In 2004, Cheng *et al*. identified the first set of small molecule inhibitors targeting PRMTs, represented by compound **3** (AMI-1), via a high-throughput screening of a diverse 9000-compound library [175]. However, many of these early-stage molecules are promiscuous PRMT inhibitors and lack both specificity and potency. An important goal in the campaign of PRMT drug discovery is to develop effective lead compounds that are highly specific for particular isoforms of each enzyme [172]. Structural studies show that active sites of methyltransferases bear different degrees of plasticity and structural uniqueness; thus, it is crucial to incorporate the protein structural information when developing specific PRMT inhibitors [164].

Computation-based methods are widely used in modern drug discovery. Progress has been made in PRMT enzyme preliminary hit identification, including structure-based, ligand/pharmacophore-based, and fragment-based virtual screening techniques [163]. These approaches are favorable, particularly at early stages of development due to improved efficiency at all levels (time, labor, cost). Traditional medicinal chemistry strategies, such as the analysis of structure–activity relationship (SAR), are often applied during the lead optimization phase as well. With the help of co-crystallization methods, homology modeling, molecular docking (MD), and MD simulation, further lead optimization or rational design has become an attractive way to develop inhibitors with desired properties. It is worthwhile to note that a cutting-edge computational method called multiple ligand simultaneous docking has been used to develop the first-in-class PRMT5 inhibitor HLCL-61 [65]. Chemical structures of several significant PRMT inhibitors are shown in Figure 4 and reviewed elsewhere [163,172].

4.3.1 PRMT1 inhibitors

Compounds **4**–**6** depicted in Figure 4 are examples of PRMT1-specific inhibitors. Compound **4** is an early-stage inhibitor found from a virtual screening on the NCI diversity library of 1990 compounds based on a homology-modeled PRMT1 structure exhibiting an IC_{50} of 1.7 μM in enzyme assays [176]. Yan *et al*. successfully described carbocyanine compound (**5**) series and diamidine compound (**6**) series with adequate potency and much higher specificity toward other PRMT

Table 3. Preclinical development of PRMT inhibitors.

PRMT target	Drug	Preclinical testing
PRMT1	Allantodapsone	Breast cancer
	E84	Erythrocytic leukemia, chronic myeloid leukemia, acute myeloid leukemia
	DB75	Chronic myeloid leukemia, acute promyelocytic leukemia, acute myeloid leukemia, T cell leukemia, erythrocytic leukemia
PRMT3	7	Adenocarcinoma
	SGC707	
PRMT4/CARM1	CMPD-1	Non-small cell lung carcinoma
	CMPD-2	
	SGC2085	
	TP-064	
	MS049	
	TBBD	
PRMT5	CMP5	Glioblastoma
	EPZ015666	Epstein-Barr virus-positive non-Hodgkin lymphoma
	LLY283	Mantle cell lymphoma
PRMT6	EPZ020411	Skin melanoma
	GMS	Breast cancer
	MS049	
	MS023	
PRMT7	DS-437	Breast cancer

PRMT: protein arginine methyltransferases.

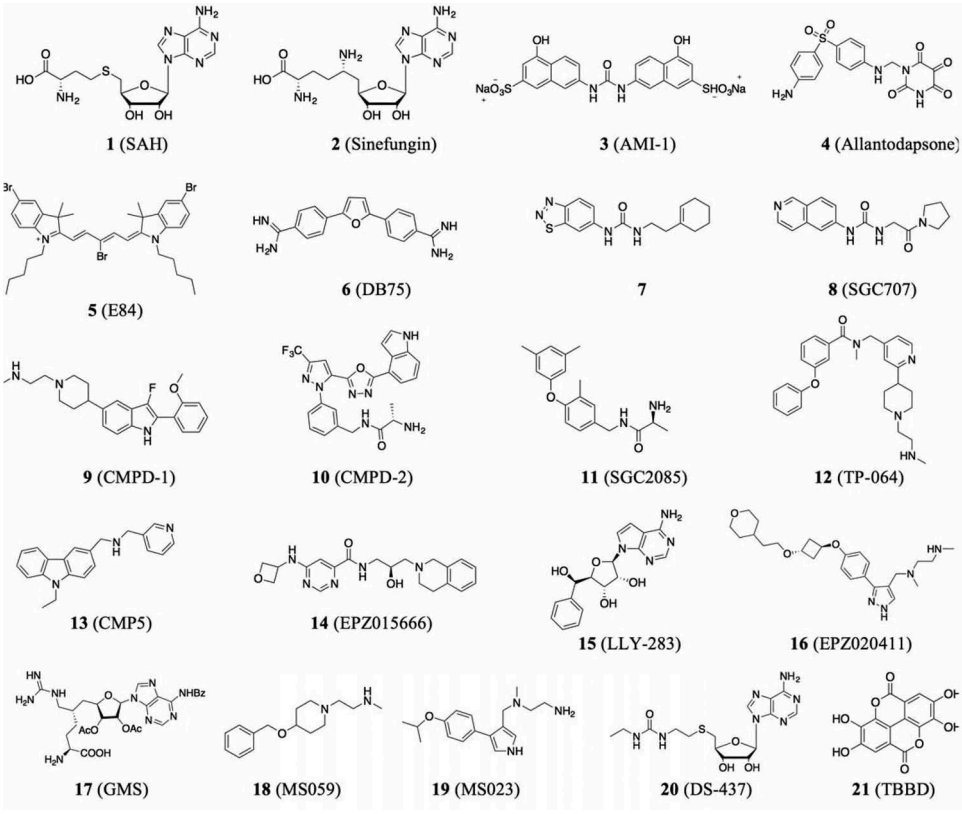


Figure 4. Examples of reported PRMT inhibitors.

isoforms [177,178]. However, the absence of co-crystal structures for generating PRMT1-specific inhibitors will make validation and further development more of a challenge.

4.3.2 PRMT3 inhibitors

Compound **7** (Figure 4), the first described specific inhibitor for PRMT3, was discovered by screening a 16,000-compound diverse library and is demonstrated to be an allosteric inhibitor bound into the dimer interface [179]. Compound **8** (Figure 4) was developed based on compound **7** by the same research group and found to exhibit improved cellular activity, potency (IC_{50} 0.03 μM) and outstanding selectivity [180]. Co-crystal structure of PRMT3-compound **8** complex is presented in Figure 5(a).

4.3.3 CARM1 inhibitors

CARM1 inhibitor compounds **9** and **10** are screened out as pyrazole (**9**) and benzo[d]imidazole (**10**) inhibitors, respectively, with high potency (IC_{50} = 0.03 μM) and satisfactory selectivity (Figure 4) [181]. According to the co-crystal structures obtained, they both bind to the substrate arginine binding pocket; in addition, cofactor binding is a prerequisite for the binding of inhibitor [172]. Ternary structure of CARM1-SAH-compound **9** complex is shown in Figure 5(b). In 2016, a potent inhibitor compound, SGC2085 (compound **11**), was identified by virtual screening and two rounds of structure-based optimization (Figure 4) [182]. This CARM1 inhibitor showed substantially improved potency and selectivity over other PRMTs with an IC_{50} of 50 nM. Compound **12** or TP-064 was developed as the first potent, selective and cell active chemical probe for CARM1 with IC_{50} < 10 nM for methylation of H3 (1–25) [183]. The reported crystal structure (PDB: 5U4X) of CARM1 in complex with SAH and TP-064 shows this molecule to be a substrate-competitive inhibitor.

4.3.4 PRMT5 inhibitors

PRMT5 is the major type II enzyme and also an important therapeutic cancer target. However, very few inhibitors have been reported for PRMT5 so far. In 2015, compound **13** (CMP5) (Figure 4), the first PRMT5 selective inhibitor described, was discovered during a virtual screen utilizing a computational PRMT5 homology model [14]. In the same year, the research group at Epizyme, Inc. reported a screened-out inhibitor called EPZ015666 (compound **14**) (Figure 4) [15]. This molecule shows outstanding selectivity to PRMT5 compared to the other PRMTs and a much higher potency (IC_{50} = 0.022 μM) than compound **13**. Enzymatic and biochemical studies show that compound **14** acts as a substrate competitor, which is further confirmed by co-crystal structure of PRMT5:MEP50-SAM-compound **14**. Detailed active site structure is illustrated in Figure 5(c). Note that the cation–pi interaction between the ligand and enzyme is critical in its enzyme selective inhibition. Similar to the CARM1-compound **9** complex, the binding of the cofactor SAM is a prerequisite for the inhibition activity.

Recent studies indicated an enhanced dependency of methylthioadenosine phosphorylase (MTAP) deletion cancer cells on PRMT5 [184–186]. MTAP-deleted cells have reduced PRMT5 methylation activity and increased sensitivity to PRMT5 depletion because of the accumulation of the metabolite methylthioadenosine (MTA), found to be a PRMT5 self-inhibitor as a cofactor analog

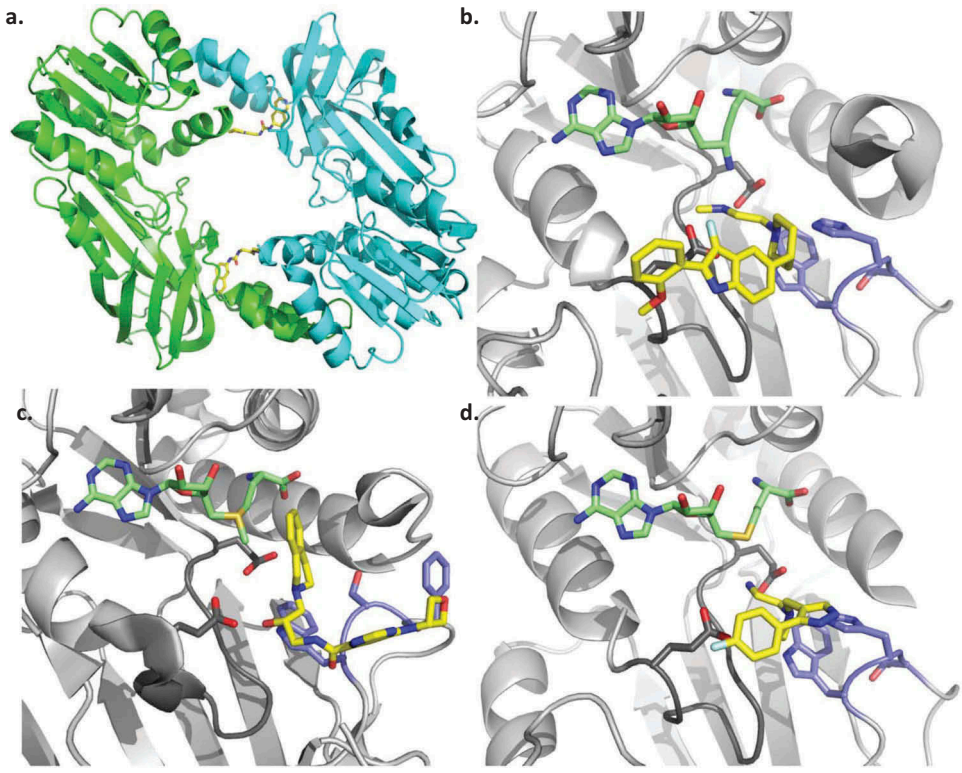


Figure 5. Co-crystal structures of PRMTs and their inhibitors. (**a**). Allosterically bound dimer of PRMT3-compound **8** (PDB: 4RYL); (**b**). Active site of ternary complex CARM1-SAH-compound **9** (PDB: 2Y1W); (**c**). Active site of quaternary complex PRMT5:MEP50-SAH-compound **14** (PDB: 4X61); (**d**). Active site of ternary complex PRMT6-SAH-compound **16** (PDB: 4Y2H).

[184]. It should be noted that excess MTA is reported to increase the IC_{50} of EPZ015666 by an order of magnitude in assays of PRMT5 activity [185]. Based on mechanistic insights, it is possible that the reported SAM-cooperative mechanism of action of EPZ015666 limits its inhibition of PRMT5 in the setting of excess MTA, and the reduced SAM binding further tilts the equilibrium between the active PRMT5:SAM and the inactive PRMT5:MTA complexes toward the inactive form [184,185]. Thus, inhibitors that mimic the mode of action of cofactor (e.g. SAM/MTA-competitors) or MTA-cooperators will be useful to pharmacological exploitation of PRMT5-targeted therapeutics [185]. Using this strategy, the first potent and selective SAM-competitive chemical probe for PRMT5, LLY-283 (compound **15**, Figure 4) was identified. It is a nucleoside analog with IC_{50} = 20 nM for methylation of an H4R3-derived peptide substrate that exhibits potent inhibition in cellular methylation assays [187].

4.3.5 PRMT6 inhibitors

Based on the work on type I inhibitors, structure optimization was performed to obtain inhibitors (e.g. compound **16**) with high potency (IC_{50} = 10 nM) as well as moderate selectivity (~12-fold) for PRMT6 (Figure 4) [188]. The co-crystal structure of PRMT6-SAH-compound **16** shown in Figure 5(d) reveals that the inhibitor occupies the arginine binding site. A bisubstrate inhibitor called 6′-methyleneamine sinefungin (GMS or compound **17**) was discovered by Wu *et al*. in 2016 (Figure 4) [189]. GMS is an analog of sinefungin (compound **2**) with significantly increased inhibition activity than other cofactor-competitive inhibitors. This compound can occupy both the substrate arginine binding site (PDB: 4QQK) and cofactor binding pockets. In addition, a dual inhibitor of both CARM1 and PRMT6 called MS049 (compound **18**) was developed through SAR studies based on their previously designed potent, selective inhibitor of type I PRMTs inhibitor MS023 (compound **19**, Figure 4) [190,191].

4.3.6 PRMT7 inhibitors

Compound **20** is a rationally designed molecule based on the structure of PRMT5 that was ultimately found to be a potent PRMT5/PRMT7 dual inhibitor (IC_{50} = 6 nM for both enzymes) while inactive on other PRMTs (Figure 4) [192]. Members of this set of compounds are considered to be bi-substrate inhibitors that combine structural features from both substrate and cofactor.

Some PRMT inhibitors are found to possess capability of binding to the histone tail peptides [172]. Compound **21** (known as allagic acid or TBBD) was found to be a CARM1 inhibitor (IC_{50} = 25 μM), which specifically inhibited methylation of H3R17 (Figure 4) [193]. Isothermal titration colorimetry assays showed direct binding between histone substrate and TBBD. There are also peptide inhibitors reported [194]. However, the limitation of peptide inhibition is its low stability *in vivo* and poor cell membrane penetration [163].

Small molecule inhibitors remain the priority for PRMT drug discovery. Despite continued efforts in inhibitor development, potency and attractive PK profiles remain challenges that are currently the focus of multiple groups. Pharmacological inhibitors of PRMT2, 8, 7, 9 enzymes have

yet to be described [172]. Since the majority of the reported PRMT inhibitors lack co-crystal structures, effort needs to be directed at solving the structures of PRMT-inhibitor complexes to validate proposed protein–ligand (inhibitor) interactions and facilitate lead drug optimization efforts.

5. Expert opinion: arginine methylation as an ideal target for cancer therapy

The diverse role of arginine methyltransferases in a wide variety of physiologic processes clearly illustrates the importance of this class of enzyme in coordinating orderly cellular development and homeostasis. A growing number of reports over the past decade have pointed toward dysregulation of select PRMTs in driving both benign and malignant diseases. As work in this field continues to evolve, a developing body of evidence supports the need to design selective inhibitors to target PRMTs.

Small molecule inhibitors against other epigenetic modifiers have shown promising results and have laid important groundwork in drug discovery for this class of enzymes. Continued work on individual PRMT structures and the methyltransferase enzymology itself aids in the drug development process. As drug design advances, newer generations of arginine methyltransferase inhibitors allow for greater specificity and potency by targeting multiple regions within active sites and domains in PRMT enzymes or partner proteins. Generation of more sophisticated compounds through drug design aid the production of highly specific tool compounds, and more recently, clinical drug candidates. While early work targeting epigenetic modifying enzymes began with inhibiting a broad spectrum of isoenzymes, work in the PRMT field has focused on activities of select enzymes identified as critical drivers of disease activity.

PRMT5 has been found to play a role in numerous key pathways that contribute toward several driver activities in multiple malignancies including MYC, Notch, and CYCLIN D1 [69]. With this knowledge, the first clinical trial delivering targeted therapy against an enzyme in the PRMT family chose to focus on PRMT5 (https://clinicaltrials.gov/ct2/show/NCT02783300) [14,67,69,143,195]. New developments in PRMT5 biology have pointed toward specific molecular markers, like MTAP deficiency, that identify particular cancers as ideal candidates for PRMT5 inhibitor therapy. Accumulating evidence is pointing toward PRMT5 as an ideal therapeutic candidate for benign (sickle cell anemia, autoimmune disease) and malignant (glioblastoma, AML, aggressive lymphomas, squamous cell carcinoma, and carcinomas of lung, colon, and breast) diseases.

As we learn from preclinical and clinical studies, it will be critical to anticipate potential toxicities when developing PRMT-targeted strategies to treat patients with cancer. For example, work by Liu *et al*. has pointed toward the relevance of PRMT5 activity in supporting normal hematopoiesis and the potential for myelosuppression [72–74]. The association of PRMT enzymes with a broad array of multimeric chromatin remodeling complexes, regulatory proteins, and the spliceosome highlights the need for more scientific exploration to better define the biology and anticipate potential problems that may be encountered with targeting these enzymes therapeutically [62,66,67,195,196]. Careful pharmacokinetic and pharmacodynamic evaluation is essential to maximize the therapeutic impact while minimizing toxicity.

It is clear that PRMTs control numerous cellular processes and exhibit a number of pleiotropic effects. The consequences of inhibiting these enzymes extend beyond direct targets of PRMTs and carries over to other modifications. The indirect effects of PRMT inhibition become apparent as the dynamic relationships of histone crosstalk are further explored. For example, PRMT5 inhibition can alter PI3K/AKT, cell cycle, B cell receptor activity, and WNT/β CATENIN pathways. Several of these pathways have been identified as being critical to maintenance of cancer stemness and thus hold potential for targeting both mature tumor and cancer stem cell compartments, a strategy that could lead to breakthrough advances for malignancies such as leukemia and malignant gliomas. The widespread effects of PRMT inhibition also provide the opportunity to use these inhibitors as part of a combination therapy in order to achieve a greater synergistic effect while reducing toxicity due to lower concentrations required in synergistic interactions with other classes of drug. While this gives potency to PRMT inhibitors, it poses a potential problem when addressing specificity. Ultimately, the prevalence of PRMTs in cancer and widespread influence on controlling important cancer hallmarks points toward this class of enzymes as attractive therapeutic targets for cancer. In conclusion, it is our hope that the work summarized here not only outlines PRMT form and function but also provides the rational for targeting this class of enzymes in specific disease settings and gives insight into the design strategy for the next wave of epigenetic inhibitors.

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Declaration of interest

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