Protein methyltransferase inhibitors as personalized cancer therapeutics

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The protein methyltransferases (PMTs) have emerged as a novel target class, especially for oncology indications where specific genetic alterations, affecting PMT activity, drive cancer tumorigenesis. This target class has proved quite druggable; small molecule inhibitors (PMTis) of several PMT enzymes have been reported, that display a diversity of chemical structures and target binding modalities. Here we review recent progress in identifying, characterizing and optimizing PMTis for eventual use as personally targeted cancer therapeutics.

Introduction

A confluence of molecular mechanisms work in concert to effect strict control of gene transcription in eukaryotic cells. Paramount among these mechanisms is a collection of post-translational modifications of chromatin that facilitate conformational transitions between transcriptionally permissive and suppressive chromatin structures [1,2]. Each of these modifications is catalyzed by a specific set of enzymes, as summarized in Table 1. Not surprisingly, dysregulation of chromatin modification leads to pathologic alterations in gene transcription, hence to human disease. Among chromatin modifying enzymes (CMEs), the protein methyltransferases (PMTs) stand out because of the large size and druggability of the class, and the disease association of many of these enzymes.

In 2009 we reviewed the PMT class with respect to target validation and disease association [1]. In the present review we focus attention on recent developments in the search for small molecule inhibitors of these enzymes as a starting point toward novel therapeutic agents.

PMTs in human cancers

With respect to regulation of gene transcription, methylation of specific histone locations (i.e. amino acids) is clearly the most relevant function of this target class. Hence, many refer to these enzymes as histone methyltransferases (HMTs). Yet, it is clear that at least some of these enzymes catalyze physiologically important methylation of proteins other than histones; in some cases dysregulation of these non-histone methylation reactions may contribute to pathogenesis [1]. For this reason, the target class is more correctly referred to as PMTs [1], and this is the nomenclature that we will use throughout this review.

The PMT target class is composed of two distinct enzyme families: the protein arginine methyltransferases (PRMTs) and protein lysine methyltransferases (PKMTs) [1,2]. The two families are distinguished not only by the amino acid side chain nitrogen atoms that are methylated but also by the architecture of the enzyme active sites for the two families, both at the amino acid sequence level and at the three-dimensional tertiary structure level. Recently, Richon et al. [3] have performed a systematic survey of the human genome for proteins that share structural relatedness to the active sites
<table>
<thead>
<tr>
<th>Enzyme class</th>
<th>Number of putative human class members</th>
<th>Pros as drug targets</th>
<th>Cons as drug targets</th>
<th>Marketed drugs</th>
<th>Key reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone acetyltransferases</td>
<td>28</td>
<td>o Some alterations associated with disease</td>
<td>o No drug-like inhibitors reported</td>
<td>None</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Examples of small molecule inhibitors in clinical use</td>
<td>o Challenging medicinal chemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histone deacetylases</td>
<td>18</td>
<td>o Precedent for potent small molecule inhibitors in clinical use</td>
<td>o Small target class with overlapping biological functions</td>
<td>Vorinostat</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Validated target class for specific cancers</td>
<td>o All known inhibitors act through metal chelation, making selectivity challenging</td>
<td>Romidepsin</td>
<td></td>
</tr>
<tr>
<td>Protein methyltransferases</td>
<td>96</td>
<td>o Large target class</td>
<td>o No clinical proof of concept to date</td>
<td>None</td>
<td>[1,19]</td>
</tr>
<tr>
<td>PKMTs</td>
<td>52</td>
<td>o Multiple examples of genetic alterations associated with cancer</td>
<td>o Few examples of selective SAM competitive inhibitors, especially for PKMTs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRMTs</td>
<td>44</td>
<td>o Multiple examples of drug-like inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine demethylases</td>
<td>26</td>
<td>o Examples of genetic alteration in disease</td>
<td>o Iron or flavin dependence of catalysis; medicinal chemistry of active-site inhibitors therefore challenging</td>
<td>None</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Small molecule inhibitors reported</td>
<td>o Unclear how much overlap of biological function exists among class members and with other iron-dependent proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine deiminases</td>
<td>5</td>
<td>o Disease association reported for some enzymes</td>
<td>o Small target class</td>
<td>None</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Small molecule inhibitors reported</td>
<td>o Overlapping biological function of class members</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinases</td>
<td>17</td>
<td>o Well established target class</td>
<td>o No enzymes specific to chromatin modification</td>
<td>None (for chromatin modifying kinases)</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Many examples of drugs in clinical use</td>
<td>o Unclear association with pathogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA methyltransferases</td>
<td>5</td>
<td>o Precedent for potent small molecule inhibitors in clinical use</td>
<td>o Small target class</td>
<td>Dacogen</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Validated target class for specific cancers</td>
<td>o To date efficacy seen in only small group of cancers</td>
<td>Vidaza</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Representative genetic alterations in human cancers affecting the enzymatic function of protein methyltransferases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cancer</th>
<th>Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PKMTs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOT1L</td>
<td>Mixed lineage leukemia</td>
<td>Recruited to ectopic sites by MLL translocation</td>
</tr>
<tr>
<td>EHMT2</td>
<td>Lung, prostate and hepatocellular carcinoma</td>
<td>Increased expression</td>
</tr>
<tr>
<td>EZH2</td>
<td>Non-Hodgkin lymphoma, breast, prostate, colon, gastric, bladder, liver and melanoma</td>
<td>Somatic mutations; amplification</td>
</tr>
<tr>
<td>MLL</td>
<td>Leukemia</td>
<td>Translocated</td>
</tr>
<tr>
<td>MLL4</td>
<td>Pancreatic, glioblastoma</td>
<td>Amplified</td>
</tr>
<tr>
<td>NSD1</td>
<td>Acute myeloid leukemia</td>
<td>Translocated</td>
</tr>
<tr>
<td>PRDM14</td>
<td>Breast cancer</td>
<td>Amplified; highly expressed</td>
</tr>
<tr>
<td>SMYD3</td>
<td>Breast, liver, colon and gastric cancers</td>
<td>Increased expression</td>
</tr>
<tr>
<td>SUV39H1</td>
<td>Colon cancer</td>
<td>Increased expression</td>
</tr>
<tr>
<td>WHSC1</td>
<td>Myeloma</td>
<td>Translocated; highly expressed</td>
</tr>
<tr>
<td>WHSC1L1</td>
<td>Lung, breast cancer</td>
<td>Amplified</td>
</tr>
<tr>
<td><strong>PRMTs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRMT5</td>
<td>Lymphoma</td>
<td>Increased expression</td>
</tr>
<tr>
<td>CARM1</td>
<td>Breast and prostate cancers</td>
<td>Increased expression</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTX&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Myeloma</td>
<td>Loss-of-function mutation leading to increased H3K27me3</td>
</tr>
</tbody>
</table>

Data compiled and updated from [1]

<sup>a</sup> UTX is actually a protein demethylase, rather than a protein methylase. As described in the text, however, the loss-of-function mutation of this demethylase is enzymatically equivalent to a gain-of-function for the corresponding methylase, EZH2.

of PKMTs or PRMTs of known biochemical activity. The results of this survey suggest that the PMT class may contain as many as 96 members, including groups of proteins that have heretofore not been identified as PMTs.

Among this putatively large target class are several enzymes for which genetic alterations have been identified in association with specific forms of human cancer, as summarized in Table 2. Two enzymes are presented below that exemplify the diversity of genetic alterations that may impact PMT activity in a pathogenic manner, leading to human cancers: DOT1L and EZH2.

**DOT1L**

MLL rearranged leukemia (MLL) is a genetically distinct form of acute leukemia that affects people at all stages of life, from infancy to late adulthood; patients with MLL have a relatively poor prognosis and are not well treated by currently available therapies. A universal hallmark of MLL disease is a chromosomal translocation affecting the MLL gene on chromosome 11q23 [4]. Normally, the MLL gene encodes for a PKMT that catalyzes the methylation of lysine 4 of histone H3 (H3K4) at specific gene loci [4]. Gene localization is conferred by specific recognition elements within MLL, external to the catalytic domain [4]. In the disease-linked translocations, the catalytic domain (SET domain) is lost and the remaining MLL protein is fused to a variety of partners, including members of the AF and ENL family of proteins, such as AF4, AF9, AF10 and ENL [4]. Thus, the MLL gene translocation is a common feature of MLL disease and has been thought to play a causal role in this malignancy. The mechanistic basis for how this gene translocation might confer a pathogenic phenotype was unclear until recently. The loss of the MLL catalytic domain, resulting from the chromosomal translocation, might suggest a loss-of-function with respect to histone methylation. In fact, however, the chromosomal translocation results in a change-of-function with respect to histone methylation by the ectopic recruitment of another PKMT, DOT1L.

DOT1L catalyzes the methylation of histone H3 on lysine 79 (H3K79); H3K79 methylation is an activating mark with respect to gene transcription. DOT1L forms high-affinity complexes with the various MLL-fusion proteins via the fusion partner domains (i.e. AF4, AF9, AF10, ENL). As a consequence of the localization elements within the MLL portion of the fusion protein, the fusion-protein-DOT1L complex results in ectopic gene localization of the DOT1L enzyme, where it catalyzes H3K79 methylation and consequent gene activation. Armstrong and colleagues have shown
that ectopic H3K79 methylation, resulting from MLL fusion protein recruitment of DOT1L, leads to enhanced expression of several leukemogenic genes, including \textit{HOXA9} and \textit{MEIS1} [5]. Hence, while DOT1L is not genetically altered in the disease \textit{per se}, its mislocated enzymatic activity is a direct consequence of the chromosomal translocation affecting MLL patients; thus, DOT1L has been proposed to be a catalytic driver of leukemogenesis in this disease [6].

\section*{EZH2}

EZH2 is the catalytic subunit of a multi-protein PKMT complex known as Polycomb Repressive Complex 2 (PRC2). PRC2 catalyzes the mono-, di- and tri-methylation of H3K27. Tri-methylation of H3K27 is a transcriptionally repressive mark that silences affected genes. Elevated levels of H3K7me3 (hypertrimethylation) are often seen at tumor suppressor gene loci in a variety of human cancers [7]. Several mechanisms for effecting H3K27 hypertrimethylation have been documented in different human cancers where they are thought to be tumorigenic (Table 2). For example, amplification of PRC2 genes, including EZH2, is found in breast, prostate and other solid cancers. In myeloma, loss-of-function mutations have been identified in the histone demethylase UTX, which removes methyl groups from the H3K27 site. The loss of demethylase activity at H3K27 should result in a hypertrimethylated phenotype and consequent transcriptional repression of tumor suppressor genes, in an equivalent manner to elevation of the PRC2 methyltransferase. Yet another mechanism for elevating tumorigenic H3K27me3 is by overexpression of the auxiliary protein PHF19/PCL3, a protein that enhances the ability of PRC2 to trimethylate H3K27 [8].

Most recently, Sneeringer \textit{et al.} [9] and subsequently Yap \textit{et al.} [10] have presented an unusual molecular mechanism of H3K27 hypertrimethylation associated with a specific human cancer. Subsets of patients with non-Hodgkin lymphoma had been found to be heterozygous for point mutations at tyrosine 641 within the catalytic domain of EZH2 (Y641F, Y641N, Y641S and Y641H); these patients were found to express both the wild type and mutant enzymes. In the original description of these mutants [11], the authors concluded that they represented loss-of-function mutations as the mutant EZH2 constructs, reconstituted into recombinant PRC2 complexes, showed no ability to methylate a simple peptide bracketing the H3K27 site. Sneeringer \textit{et al.} [9] reexamined the enzymology of these mutants and found that they actually represent change-of-function mutations. While wild type EZH2 is most efficient at performing the first, mono-methylation reaction – and its catalytic efficiency wanes with each subsequent methylation reaction – all of the mutant enzymes display the exact opposite pattern of substrate utilization; the mutants are essentially unable to perform the first methylation reaction, but once presented with the mono-methylated H3K27 precursor, they are much more efficient than wild type enzyme in performing the di- and especially tri-methylation reactions. Hence, the hypertrimethylated H3K27 phenotype, associated with patient heterozygosity for mutant EZH2, is the result of a uniquely pathogenic coupling of enzymatic activity between the wild type and mutant enzymes: the wild type enzyme performs the first methylation reaction and the mutants then accelerate the subsequent transformations to the tumorigenic H3K27me3 species.

What is most critically exemplified by the above descriptions of DOT1L and EZH2 is that specific patient populations, most probably to benefit from PMTs-based therapeutics, may be identified prospectively (i.e. before treatment) on the basis of unique genetic alterations in particular PMTs. As we shall see below, these genetic alterations can lead to unique dependencies on PMT activity in cancer, thus forming a basis for selective killing of cancer cells (and hence a large therapeutic index) by treatment with a potent and selective PMTi.

\section*{Small molecule PMTis}

The past 5 years have seen significant effort toward design and discovery of small molecule ligands that function as direct inhibitors of specific PMTs. Table 3 provides representative examples of such PMTs. The data presented in Table 3 illustrate several important points with respect to the overall druggability of the PMTs as a target class. First, it is clear that nanomolar affinity is achievable for multiple PMTs with compounds that are reasonable starting points for medicinal chemistry efforts. Second, the compounds that have been reported display a reasonable degree of diversity, both in terms of their chemical structures and their target binding modality. The latter point distinguishes the PMTs from other large enzyme classes, such as the protein kinases, where substrate (i.e. ATP) competitive inhibitors dominate the chemical biology space. Rather, for the PMTs there appear to be multiple sites of intervention on these proteins. Compounds have been reported that are SAM (S-adenosylmethionine) competitive, noncompetitive, uncompetitive and designed to be bisubstrate inhibitors. This diversity of binding modalities offers multiple opportunities to achieve potent and selective inhibition of specific target enzymes. Indeed, target selectivity has proved not to be a significant challenge for inhibitors of PMTs, even in the case of SAM competitive inhibitors. For example, the DOT1L inhibitor EP2004777 is a picomolar, SAM competitive inhibitor of its target enzyme. This compound was tested against a panel of 9 other PMTs at concentrations up to 50 \textmu M. No inhibition was observed for 8 of the 9 PMTs tested; for PRMT5 an IC$_{50}$ of 521 nM was obtained. Hence, the minimum selectivity of EP2004777 for DOT1L over other PMTs is >1200-fold [12]. Similarly high levels of selectivity have been reported for other PMTs against other target enzymes.
Table 3. Selected examples of small molecule PRMTis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Potency and mode of binding</th>
<th>Cellular activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PKMT inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPZ004777</td>
<td></td>
<td>DOT1L inhibitor, IC50 = 0.4 nM. Competitive with SAM</td>
<td>Compound selectively inhibits H3K79 methylation in various cells with IC50 &lt; 1 μM and shows selective antiproliferative effects in MLL translocated cells with IC50 values between 0.17 and 6.47 μM</td>
<td>[12]</td>
</tr>
<tr>
<td>Tokyo Medical and Dental University compound 1c of Ref. [23]</td>
<td></td>
<td>SET7/9 inhibitor, IC50 = 10 μM Presumed to be competitive with SAM</td>
<td>No cellular activity reported</td>
<td>[23]</td>
</tr>
<tr>
<td>BIX-01294</td>
<td></td>
<td>Dual EHMT1/2 inhibitor with IC50s of 34 and 133 nM, respectively. Uncompetitive with SAM</td>
<td>Reduced H3K9 me2 levels in MDA-MB231 cells with an IC50 of 500 nM. The EC50 for cellular toxicity was 2.8 μM</td>
<td>[24]</td>
</tr>
<tr>
<td>UNC-0224</td>
<td></td>
<td>Dual EHMT1/2 inhibitor with IC50s of 50 and 43 nM, respectively. Noncompetitive with SAM</td>
<td>No cellular activity reported</td>
<td>[25]</td>
</tr>
<tr>
<td>UNC-0638</td>
<td></td>
<td>Dual EHMT1/2 inhibitor with IC50s of 19 and &lt;15 nM, respectively. Noncompetitive with SAM</td>
<td>Reduced H3K9 me2 levels in MDA-MB231 cells with an IC50 of 81 nM. The EC50 for cellular toxicity was 11.2 μM</td>
<td>[26]</td>
</tr>
<tr>
<td>AZ505</td>
<td></td>
<td>SMYD2 inhibitor, IC50 = 0.12 μM. Uncompetitive with SAM</td>
<td>No cellular activity reported</td>
<td>[27]</td>
</tr>
<tr>
<td><strong>PRMT inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 5 of Ref. [28]</td>
<td></td>
<td>PRMT1 inhibitor, IC50 = 55.4 μM. Presumed to be a bisubstrate inhibitor</td>
<td>IC50 for inhibition of cellular methylation in HepG2 cells = 150 μM</td>
<td>[28]</td>
</tr>
</tbody>
</table>
Table 3 (Continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Potency and mode of binding</th>
<th>Cellular activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylgene compound 7a of Ref. [29]</td>
<td><img src="image" alt="Structure" /></td>
<td>CARM1 inhibitor, IC50 = 60 nM. Noncompetitive with SAM</td>
<td>No cellular activity reported</td>
<td>[29]</td>
</tr>
<tr>
<td>Bristol-Myers Squibb compound 7f of Ref. [30]</td>
<td><img src="image" alt="Structure" /></td>
<td>CARM1 inhibitor, IC50 = 40 nM. Noncompetitive with SAM</td>
<td>No cellular activity reported</td>
<td>[30,31]</td>
</tr>
</tbody>
</table>

A final point illustrated by the data in Table 3 is that there are now multiple examples of PMTis that demonstrate inhibition of specific intracellular methylation events. In the case of the DOT1L inhibitor EPZ004777, not only were intracellular methylation effects observed, but these translated into cell specific antiproliferative effects both in cell culture and in vivo, as described below.

One PMT that has garnered great attention within the cancer biology and drug discovery communities is EZH2. As described above, EZH2 is a well-validated target for several solid and hematologic cancers. Several groups have reported potent and selective inhibitors of EZH2 in oral presentations at scientific meetings. To date, however, no chemical structures for direct EZH2 inhibitors have been publicly disclosed.

**Cellular and in vivo efficacy**

Much progress has been made in the discovery and design of small molecule PMTis that have demonstrated potent inhibition of target enzymes in biochemical assays. In a few cases, the ability to demonstrate intracellular inhibition of substrate methylation has also been reported. Yet, until recently the concept of PMTis as a basis for cancer therapy has been

![Figure 1](image)

**Figure 1.** (a) Selective cell killing of cultured leukemia cells harboring MLL translocations by the potent, selective DOT1L inhibitor EPZ004777. The antiproliferation IC50 of EPZ004777 is plotted for leukemia cells containing MLL translocations and for other leukemia cells without such translocations. (b) Effect of varying doses (50, 100 and 150 mg/mL) of EPZ004777 on survival in a disseminated mouse model of mixed lineage leukemia. A statistically significant (P < 0.05) survival advantage is realized at all doses of compound relative to the vehicle treated control animals. Panels (a) and (b) were redrawn from data in Daigle et al. [12].
viewed as unproven in that no demonstration of selective cell killing or \textit{in vivo} efficacy had been reported.

In 2011, Daigle \textit{et al.} [12] reported the first clear evidence that inhibition of a specific PMT could lead to selective cancer cell killing and resultant \textit{in vivo} efficacy in a cancer animal model. These workers reported studies of EPZ004777, a potent ($K_i$ 300 pM) and selective ($\geq1200$-fold over other PMTs) DOT1L inhibitor. The compound blocked global H3K79 methylation in leukemia cells regardless of whether or not the cells harbored MLL gene translocations. However, despite the ability to affect intracellular H3K79 methylation in all cell types, the compound was selectively cytotoxic for leukemia cells containing the MLL gene translocations, with minimal effects on non-translocated cells (Fig. 1a). Daigle \textit{et al.} [12] went on to test the effects of EPZ004777 in a highly aggressive, disseminated mouse model of mixed lineage leukemia. The compound was administered by continuous i.v. administration at three concentrations of 50, 100 and 150 mg/mL for 14 days, and survival was monitored over the course of 30 days. At all doses administered, statistically significant survival advantages over the vehicle-dosed animals were achieved (Fig. 1b). As illustrated in Fig. 1b, the extent of survival increased with increasing dose of compound. These data represent the first \textit{in vivo} evidence that selective PMT inhibition can lead to anti-tumor efficacy.

\textbf{Conclusions}

The field of cancer therapeutics has undergone a continuous evolution of focus from early days of indiscriminant cytotoxicities, such as mustard gas derivatives, to molecularly targeted agents, such as the various kinase inhibitors in clinical use today. Beginning, perhaps, with the introduction of Gleevec to target the chromosomal translocation product of chronic myelogenous leukemia, cancer therapy has entered a new epoch: that of personally targeted therapeutics. The concept of personally targeted therapeutics resonates well in this current era of advanced genetic, proteomic, biochemical and biological understanding of cancer cells. Personally targeted therapeutics can be operationally defined by three stages: identify the genetic alteration(s) that drive specific cancers; develop targeted therapeutics that selectively affect those genetic alterations; define patient populations most probably to benefit from the therapy, based on the presence of the specific genetic alteration(s).

Increasingly, oncologists are moving away from descriptions of patient’s disease based solely on tissue of origin or site of lesion (\textit{e.g.} breast cancer, prostate cancer, and the like), and moving instead toward more genetic descriptors based on increased understanding of the alterations that drive individual cancers. Contemporary examples of how this sea change is affecting therapeutic strategies include the development of Vemurafenib to specifically treat melanoma patients carrying the B-Raf V600E mutation [13,14] and of Crizotinib to specifically treat lung cancer patients harboring a chromosomal translocation of the protein kinase ALK [15]. As described in this review, the PMTs seem exquisitely well poised to become the next wave of clinical targets for personally targeted therapeutics in cancer and perhaps in other diseases as well.

In this brief article, we have reviewed the PMTs as a target class for cancer drug discovery and the progress made toward small molecule PMTs as cancer therapeutics. Specific genetic alterations affecting PMT function are known in certain human cancers, where they appear to be drivers of tumorigenesis. Hence, targeting of these specific PMTs is likely to be a cogent approach toward personalized cancer therapeutics. We discussed as examples of this approach, the genetic alterations affecting DOT1L in mixed lineage leukemia and EZH2 in non-Hodgkin lymphoma. As efforts toward more complete cancer genomic analysis continue, additional examples of genetic alterations in PMT function are likely to emerge.

Progress toward small molecule inhibitors of the PMTs has accelerated over the past 5 years, with many examples of potent, small molecule PMTs now disclosed in the scientific literature and within patent applications. Most recently, \textit{in vivo} efficacy has been demonstrated with a PMT that selectively targets DOT1L for applications in mixed lineage leukemia. This result strengthens the hypothesis that targeting of PMTs will be an effective approach to cancer therapy. Through selective inhibition of genetically altered PMTs, there is great promise of a large therapeutic index for killing of targeted cancer cells while sparing normal cells; this has proved to be the case in cell culture for MLL translocated leukemia cells treated with the selective DOT1L inhibitor EPZ004777.

Despite these encouraging developments, the field is still young and no clinical demonstration of safety or efficacy for a PMT approach in human disease has yet been presented. The progress to date portends that PMTs are likely to enter human clinical trials within the next few years. It will thus be interesting to see whether the promise shown in preclinical studies translates into meaningful safety and efficacy in cancer patients, and whether the concept of targeting specific genetic alterations in PMT function will translate into sufficient therapeutic indices. Given the importance of PMT function in development, there may be limitations on the use of PMTs for pediatric applications and in women of child-bearing years. These issues will need to be studied and resolved empirically to define fully the clinical utility for PMT-based cancer therapies.

While the focus of this review has been on the application of PMTs as cancer therapeutics, it should be noted that emerging biology is suggesting potential roles for PMTs as therapeutic agents in other human diseases as well [1,16]. PMT function has been implicated in neurodegenerative
diseases, inflammatory diseases and viral infections. Hence, the full spectrum of clinical uses for small molecule inhibitors of PMTs remains to be elucidated.

Acknowledgements
I wish to thank all my colleagues at Epizyme for their help and support. In particular, I wish to thank Robert Gould, Jason Rhodes, Mikel Moyer, Victoria Richon, Richard Chesworth, Edward Olhava and Roy Pollock for helpful comments and suggestions during the writing of this review. I also thank Derek Young for her help in preparing the manuscript.

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