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REVIEW ARTICLE

# Epigenetic therapy in hematological cancers

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The increasing depth of knowledge about cancer biology throughout the last decades, has underlined the importance of not only genetic aberrations, but also epigenetic abnormalities in cancer cells. The extensive exploration of the cancer epigenome has provided insights into key pathways involved in tumorigenesis, as well as has allowed the development of novel epigenetic therapies. In this review, we will present the important role of epigenetic alterations in hematological diseases, with special focus on epigenetically-based targeting of hematological malignancies.

Key words: Epigenetics; hematology; leukemia; lymphoma; myeloma.

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## EPIGENETIC MARKS IN HEMATOLOGICAL CANCERS

The term epigenetics was coined by Conrad Hal Waddington in 1942, in an effort "to understand how the genotypes of evolving organisms can respond to the environment in a more co-ordinated fashion" (1). The current definition of epigenetics includes heritable changes in gene expression that cannot be attributed to alterations in the DNA sequence. Thus, if DNA is imagined as a book, epigenetic marks are page markers, set in different pages of the book, defining which book pages are being read at a cell at any given time.

The major marks that outline the epigenetic status of a cell (epigenome) are DNA methylation and chromatin structure, the latter defined by covalent histone modifications and positioning of nucleosomes. DNA methylation in humans is exclusively found on the cytosine of CpG dinucleotides (cytosine followed by guanine) (2) and its presence can either inhibit or facilitate gene transcription, depending on the genomic location of the methylated CpG sites; methylation on promoter areas is typically associated with transcriptional silencing, while methylated gene bodies are found in transcriptionally active genes (3). Post-translational modifications of histones include a variety of chemical groups that are added on the protruding histone tails, affecting the local structure of chromatin. In this review, we will mainly focus on histone acetylation and histone methylation; however, other modifications, such as phosphorylation, sumoylation or ubiquitination of specific positions, have been found to play an important role in the epigenetic control of gene expression (4). Histone lysine acetylation is a major contributor to maintaining the structure of transcriptionally active chromatin, since the addition of acetyl groups neutralizes the positive charge of histones and subsequently their interaction with the negatively charged DNA, allowing for a looser chromatin form that permits the binding of transcription factors (5). Similarly, histone deacetylation results in a tighter chromatin structure and transcriptional inactivity. Histone methylation, on the other hand, is a more dynamic and complex mark, with diverse functions in different regulatory areas of the genome. For example, H3K4me3 is typically located in active promoters while H3K27me3 in transcriptionally inactive promoter areas, H3K4me1 is often found in enhancer regions, and H3K36me3 is found on the gene bodies of actively transcribed genes (6).

The epigenetic changes of cells are permanent only on rare occasions (such as in tissue-specific promoter DNA methylation or X chromosome

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inactivation), thus allowing for plasticity and adaptation of the epigenome in response to environmental changes. For that to be possible, an extensive network of enzymes, known as epigenetic regulators, can catalyze reactions that either add epigenetic marks ("writers"), remove epigenetic marks ("erasers"), or translate specific epigenetic marks ("readers"). Activating or deactivating mutations affecting at least one known epigenetic regulator such as *DNMT3A* or *TET2*, or histone modifiers, such as the *MLL* family or *EZH2*, are seen in almost all hematological cancers (7–10). An overview of the most well-known mutated epigenetic regulators involved in hematological malignancies is given in Table 1.

The presence of epigenetic modifiers does not only allow a finetuned intrinsic control of the epigenome, but also its therapeutic targeting with epigenetic drugs. Thus, in contrast to genetic aberrations, which are typically harder to target, epigenetic abnormalities can be specifically targeted through therapeutic inhibition of a specific epigenetic enzyme. A few of these epigenetic drugs, such as 5-azacytidine (that targets DNA methylation) or panobinostat (that targets histone acetylation), have already been approved for some hematological cancers, but additional, novel epidrugs are currently being tested in clinical trials. An overview of the most important epigenetic therapies in hematology will be presented in the next section.

## APPROVED EPIGENETIC THERAPIES IN HEMATOLOGICAL MALIGNANCIES

### DNA-methyltransferase inhibitors

The cytidine analogs, 5-azacytidine and 5-aza-2′-deoxycytidine (or decitabine), first appeared in the 1970s as novel chemotherapeutic agents against acute leukemia (11, 12). Early clinical trials showed some anti-leukemic efficacy at relatively high doses (varying from 150 to 750 mg/m$^2$), however with pronounced toxicity (12–14). In the meantime, a groundbreaking discovery was made; a low dose of 5-azacytidine induced a reduction of DNA methylation in cell culture and led to the development of cardiac muscle cells from embryonic mouse cells, suggesting that it was more than a simple cytostatic drug, since it could at lower, non-cytotoxic doses induce severe phenotypic changes (15, 16). It was later shown that 5-azacytidine exhibited this effect by reducing the levels of DNA methylation, making it an epigenetic drug (16). Thus, the administration of 5-azacytidine in higher doses, in order to achieve a direct cytotoxic effect, was abandoned and lower dosing regimens aiming for an epigenetic effect began to emerge (17). 5-azacytidine has since proven to be particularly efficient in patients with myelodysplastic syndrome (MDS) in several clinical trials, improving both the response rate and the overall survival (18, 19). Its consequent approval by the Food and Drug Administration (FDA) for the treatment of patients with MDS marked the first approval of an epigenetic drug used in cancer therapy (20). Decitabine has also been tested in MDS in several clinical trials, but was initially not approved by the FDA due to lack of an overall survival benefit; however is has now also been approved by the FDA for the same indications as 5-azacytidine (21–23); however, in Europe, the European Medicines Agency (EMA) has approved both drugs but for different indications (24).

On a molecular level, DNA-methyltransferase inhibitors (DNMTi) exhibit their mechanisms of action by incorporating into the DNA of proliferating cells (decitabine is a deoxycytidine analog, so it incorporates exclusively into the DNA, while 5-azacytidine incorporates mainly into the RNA), but a smaller fraction also gets metabolized to deoxycytidine derivatives that get incorporated into the DNA (11), where they covalently sequester DNMT1, targeting it for proteasomal degradation

**Table 1.** An overview of the most important epigenetic regulators that are mutated or translocated in one or more hematological malignancies

| Name | Epigenetic mark | Function | Disease |
|---|---|---|---|
| DNMT3A | DNA methylation | Writer | MDS, AML |
| TET2 | DNA methylation | Eraser | MDS, AML, B- and T-cell lymphomas |
| p300 | Histone acetylation | Writer | B-cell lymphomas |
| CBP (*CREBPP*) | Histone acetylation | Writer | MDS, AML, B-cell lymphomas |
| MLL1 (*KMT2B*) | Histone methylation (H3K4) | Writer | AML, ALL, MLL |
| MLL2 (*KMT2D*) | Histone methylation (H3K4) | Writer | Follicular lymphoma |
| EZH2 | Histone methylation (H3K27) | Writer | MDS, B-cell lymphomas |
| UTX (*KDM6A*) | Histone methylation (H3K27) | Eraser | ALL, multiple myeloma |
| MMSET | Histone methylation (H3K36) | Writer | Multiple myeloma |

MDS, myeloplastic dysplasia; AML, acute myeloblastic leukemia; CBP (CREBPP), CREB-binding protein (cAMP response element-binding protein); ALL, acute lymphocytic leukemia; MLL, mixed lineage leukemia; EZH2, enhancer of zeste homolog 2.

(25, 26). Since DNMT1 is mainly responsible for copying the methylation pattern to the newly synthesized DNA strand during replication (27), the original methylation pattern is successively lost during the next cell divisions. It is still unclear how the inhibition of DNMT1 could result in anti-tumor effects, but there are different proposed models. Traditionally, it has been thought that the main effect of DNMTi is the promoter demethylation and subsequent reactivation of aberrantly silenced tumor suppressor genes (28, 29). However, this effect has been shown to be both transient and not as pronounced as it was originally believed (30–32). In addition, apart from demethylating promoter areas, DNMTi can also demethylate gene bodies, resulting in the downregulation of cancer oncogenes (33, 34). Other suggested mechanisms of DNMTi involve activation of the immune system. It has for example been shown that treatment with DNMTi upregulates dormant antigens, such as cancer/testis antigens, in malignant cells, which then become immunogenic and trigger an anti-tumor immune response (35, 36). Shortly after the demethylating action of 5-azacytidine was unveiled, it was shown that it was able to induce transcriptional activation of endogenous retroviruses (ERVs) (37). This observation seemed to be of unknown significance, until recently, when it was shown that DNMTi can also activate and direct the immune system against malignant cells, through a viral mimicry mechanism, which involves the upregulation of endogenous retroviral transcripts (38, 39). A summary of the mechanisms of actions of DNMTi is given in Fig. 1.

The clinical efficacy of DNMTi in lymphoid malignancies and multiple myeloma (MM), is less prominent than in acute myeloid leukemia (AML) or MDS. Decitabine is currently being tested as monotherapy in relapsed/refractory diffuse large B-cell lymphoma (NCT03579082). A phase II, single-arm study evaluating the effect of 5-azacytidine in relapsed MM had to be terminated due to lack of efficacy (NCT00412919). Thus, the role of DNMTi as a monotherapy in lymphomas and myeloma is disputable. However, there are studies showing that DNMTi in combination with standard chemotherapy could result in improved clinical response in an lymphomas and/or resensitization to prior chemotherapy, with further ongoing studies (40–42). In myeloma, another pilot trial explored the efficacy of the combination of lenalidomide (Len) together with 5-azacytidine as an induction therapy, followed by an autologous stem cell support in 17 patients with a newly diagnosed MM (NCT01050790). Stem cell mobilization was not affected by the treatment, with 16/17 (94.1%) patients being able to mobilize stem cells and continue with high-dose therapy (HDT) and autologous stem cell transplantation (ASCT); however, the combination was relatively toxic, with approximately half of the patients experiencing serious adverse effects. This could probably be due to the unreduced dose of 5-azacytidine, which was given at 75 mg/m$^2$ for five days. Finally, another study used low-dose 5-azacytidine in combination with lenalidomide and low-dose dexamethasone (Dex) in 40 patients with relapsed or refractory MM (NCT01155583). 5-azacytidine was well tolerated up to 50 mg/m$^2$ twice a week in combination with Len-Dex, yielding a response rate of 22.9%, but with grade 3/4 toxicities seen in 23/40 (58%) patients. The study was initiated in 2010, with five patients remaining in the study in 2015, suggesting that a subset of patients might be more sensitive to epigenetic therapy. Finally, it has been shown that the immune-mediated effects of 5-azacytidine also include upregulation of the PD1-PDL1 axis, which might in fact inhibit the anti-tumor activity of the immune system (43, 44). Based on these results, the combination of 5-azacytidine with anti-PD1 or anti-PDL1 antibodies is currently being tested, with promising results (45, 46).

Apart from 5-azacytidine and decitabine, which are FDA-approved DNMTi, there are additional DNMTi that have shown promising results in preclinical and early clinical studies. Oral azacytidine (CC-486) has shown a favorable safety profile and clinical activity in patients with MDS and chronic myelomonocytic leukemia (CMML) (47). The positive results from oral azacytidine in lower-risk MDS patients are currently being confirmed in the phase III QUAZAR Lower-Risk MDS trial (AZA-MDS-003) (48). Guadecitabine (SGI-110) is a new-generation DNMTi, which is a dinucleotide that is resistant to degradation by cytidine deaminase (49) and has a much longer half-life and thus *in vivo* exposure and with more pronounced immunomodulatory effects than its predecessors (50). A phase I study showed that guadecitabine administrates at 60 mg/m$^2$ daily for 5 days subcutaneously was well-tolerated and biologically active in patients with MDS and AML (51). These results were later confirmed in a larger cohort of AML patients (52) and guadecitabine is currently being tested in a large, phase III trial (NCT02348489). The most recent breakthrough in DNMTi is oral decitabine (ASTX727), which is a combination of decitabine with a cytidine deaminase inhibitor (cedazuridine or E7727) to avoid the first-pass clearance and increase its bioavailability after oral ingestion. Preliminary results from a phase II study comparing ASTX727 with intravenous decitabine in patients

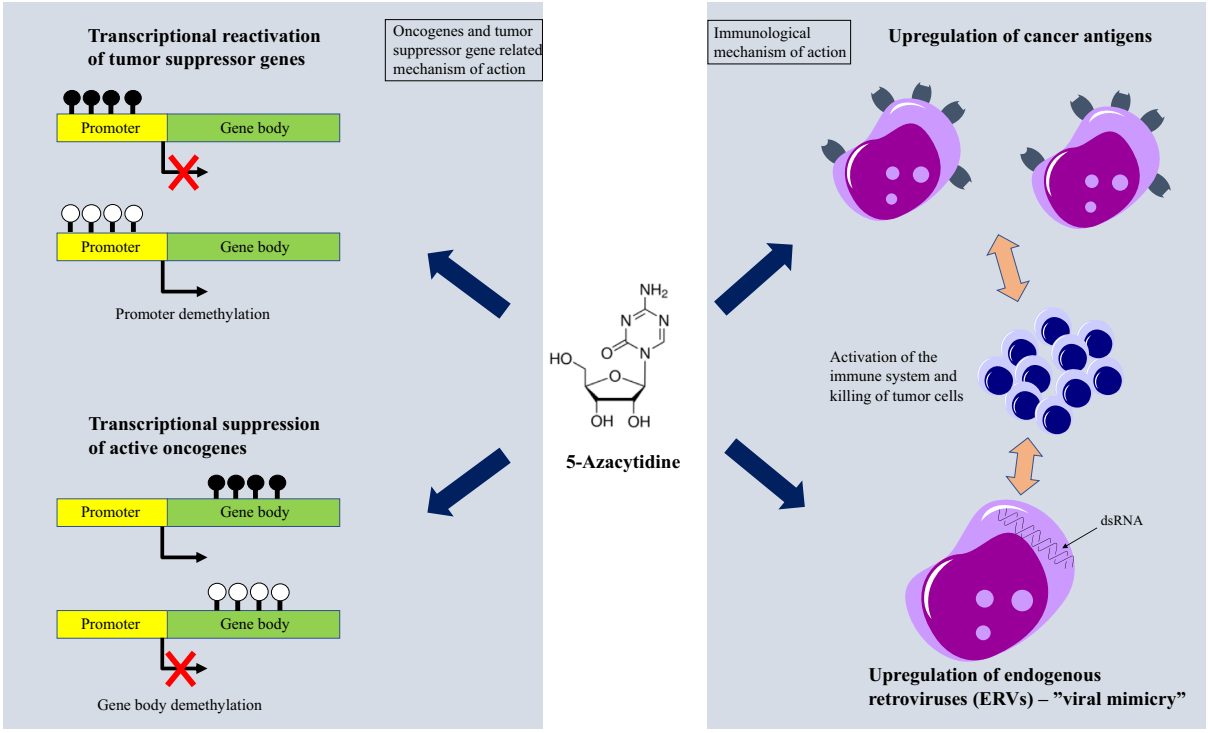


**Fig. 1.** The different mechanisms of actions of 5-azacytidine. Treatment with 5-azacytidine can reactivate silenced tumor suppressor genes by demethylating their promoter area and/or reducing the expression of oncogenes by demethylating their gene bodies. In addition, 5-azacytidine has some immunomodulatory effects and activates the immune system either by overexpression of silenced cancer antigens or by activation of endogenous retroviruses (ERVs) in the cancer cells.

with MDS, presented in American Society of Hematology (ASH) in 2017 (53), showed comparable pharmacokinetics and pharmacodynamics, safety profile, and response rates between the two therapies, awaiting further confirmation in bigger clinical trials.

## Histone deacetylase inhibitors

Acetylation of histones is an essential epigenetic mark, controlled by two different classes of enzymes: histone acetyl-transferases (HATs) and histone deacetylases (HDACs). HATs catalyze the acetylation of histone lysine residues, which neutralizes their positive charge and reduces their interaction with the negatively charged DNA strands, thus resulting in a more "open" chromatin structure, allowing transcriptional activity. HDACs belong to a family of enzymes that remove acetyl groups from histone lysine residues. So far, 18 HDACs have been discovered and they are divided into four distinct subclasses (54). Class I includes HDACs 1, 2, 3 and 8, with exclusively nuclear localization, class II includes HDACs 4, 5, 6, 7, 9 and 10, with both nuclear and cytoplasmic localization, class III includes a family of proteins known as sirtuins and finally class IV includes HDAC11, which is exclusively located in the cytoplasm (55). Since HDACs are also located in the cytoplasm, it is apparent that they do not only interact with histones, but also with other proteins. Indeed, it has been shown that HDACs directly interact with key proteins that are involved in carcinogenesis, such as p53, NF-kB, c-MYC, and STAT3 (56–59). Not only are HDACs non-specific to histones, but they also exhibit pleiotropic activity, being involved in a plethora of cellular functions, such as cell cycle regulation, stress response, protein degradation, cytokine signaling, and apoptosis (60). As such, HDAC inhibition appeared to be a rational epigenetic therapy in cancer and several HDAC inhibitors soon made their appearance as chemotherapeutic agents (61). An overview of all the known histone deacetylase inhibitors (HDACi) tested in clinical trials is given in Table 2.

The anti-tumor activity of HDACi quickly became apparent and preclinical data showed a specifically increased efficacy of vorinostat (suberoylanilide hydroxamic acid (SAHA)) and romidepsin against T-cell lymphomas (62, 63).

**Table 2.** Overview of the most important histone deacetylase inhibitors inhibitors (HDACi), their inhibitory ability, and their status in clinical trials involving hematological malignancies

| Chemical structure | Name | Specificity | Clinical status |
|---|---|---|---|
| Hydroxamic acid derivatives | LBH589 (panobinostat) | Classes I, II, and IV | Approved (multiple myeloma) |
| | SAHA (vorinostat) | Classes I, II, and IV | Approved (CTCL) |
| | PXD-101 (belinostat) | Classes I, II, and IV | Phase II (B-cell and T-cell lymphomas) |
| | ITF2357 (givinostat) | Classes I, II, and IV | Phase II (polycythemia vera) |
| | 4SC-201 (resminostat) | Classes I, II, and IV | Phase II (Hodgkin lymphoma) |
| | LAQ824 (dacinostat) | Classes I, II, and IV | Phase I (solid tumors) |
| | PCI24781 (abexinostat) | Classes I and II | Phase II (B-cell lymphomas) |
| | ACY-1215 (ricolinostat) | HDAC6 | Phase II (multiple myeloma) |
| | SB939 (pracinostat) | Classes I, II, and IV | Phase II (AML, myelofibrosis) |
| Benzamide derivatives | MGCD0103 (mocetinostat) | Class I | Phase II (B-cell lymphomas) |
| | MS-275 (entinostat) | Class I | Phase II (B-cell lymphomas) |
| Cyclic peptides | Depsipeptide (romidepsin) | Class I | Approved (CTCL) |
| Short chain fatty acids | Valproate | Classes I and IIa | Phase II (MDS, AML) |
| | Butyrate | Classes I and IIa | Phase I (CLL, AML) |

CTCL, cutaneous T-cell lymphoma; SAHA, suberoylanilide hydroxamic acid; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia.

These findings were further investigated by two clinical trials, confirming the safety and clinical activity of vorinostat in the treatment of cutaneous T-cell lymphoma (CTCL) (64, 65). This led to the approval of vorinostat for the treatment of CTCL, making it the second epigenetic drug to be approved for the treatment of a hematological malignancy (66). Similarly, two other clinical trials confimed the efficacy of romidepsin in CTCL and romidepsin was also approved by the FDA for the treatment of relapsed/refractory CTCL (67, 68). As of today, according to clinicaltrials.gov, romidepsin is currently being tested in approximately 50 studies, either as monotherapy or in combination with other drugs, mainly for the treatment of T-cell lymphomas.

Apart from T-cell lymphomas, numerous early preclinical studies also showed that several HDACi exhibit high anti-myeloma activity *in vitro*, even at very low doses (69–73). However, early clinical studies with HDACi as monotherapy for the treatment of MM showed minimal efficacy, with only panobinostat and vorinostat showing minimal response rates (74–77). Nevertheless, HDACi were not entirely abandoned for the treatment of myeloma, since additional preclinical studies showed that HDACi possibly enhance the toxicity of other agents, strengthening the rationale for a combinatorial approach, especially together with proteasome inhibitors (78–81). This led to two large randomized, double-blinded, and placebo-controlled phase III trials with vorinostat (VANTAGE-008 trial) and panobinostat (PANORAMA 1 study) together with bortezomib and dexamethasone, recruiting 637 and 768 patients, respectively (82, 83). Even though the progression-free survival was significantly higher for the arm including an HDACi in both studies, the survival benefit was under a month for vorinostat and approximately four months for panobinostat. However, the data were enough for the FDA to approve panobinostat for the treatment of relapsed MM, together with bortezomib and dexamethasone and panobinostat thus became the last epigenetic drug that got approval by the FDA for the treatment of a hematological cancer.

Despite the efficacy of HDACi in lymphomas and myeloma, they seem to be clinically inactive in myeloid malignancies. Panobinostat, as well as vorinostat and belinostat have been tested in AML as monotherapy, without any evidence of efficacy (84–86). Even though this might as well be due to biological causes specific to the disease, it could also be due to the lack of specificity of HDACi and their uncontrolled, off-target effects. Therefore, a combinatorial approach with lower doses to enhance other chemotherapeutic drugs, while at the same time minimizing toxicity, might result in better efficacy. Alternatively, the use of more specific HDACi may also maximize the anti-tumor effect with a more favorable toxicity profile. An interesting example is HDAC6, a cytoplasmic HDAC (and thus not a true epigenetic target) that plays a pivotal role in protein degradation especially of misfolded proteins, by facilitating the formation of the aggresome, a secondary mechanism to proteasome degradation (60). As a result, simultaneous inhibition of the proteasome and HDAC6 will lead to the accumulation of misfolded proteins and induction of cell death, and synergy between bortezomib and ACY-1215 (ricolinostat), a specific HDAC6-inhibitor has been confirmed in a preclinical setting (87). There are ongoing trials evaluating the efficacy of ricolinostat in MM, in combination with bortezomib (NCT01323751), lenalidomide (NCT0158

3283), and pomalidomide (NCT01997840), while it is also being tested in B-cell lymphomas (NCT0209 1063 and NCT02787369).

## HISTONE METHYLATION

Histone methylation can occur as mono-, di-, or trimethylation on specific lysine residues on histone tails. There are six different known residues that can be methylated on histones 3 and 4: H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20 (88). Since several histone methyltransferases (KMTs) and histone demethylases (KDMs) are involved in the pathogenesis of hematological malignancies (see Table 1), they are of special interest as epigenetic targets. The last decade has witnessed the development of a plethora of novel epigenetic drugs targeting specific KMTs or KDMs that have been or are currently being tested in clinical trials. In this review, we will mainly focus on two different well-characterized approaches: the targeting of enhancer of zeste homolog 2 (EZH2) and the targeting of the mixed lineage leukemia (MLL) family of proteins and/or disruptor of telomeric silencing 1-like (DOT1L).

### EZH2

Enhancer of zeste homolog 2 is the enzymatically active part of a protein complex known as polycomb repressive complex 2 (PRC2) and catalyzes the formation of H3K27me3 (89). PRC2-mediated gene silencing is a major, DNA methylation-independent mechanism of transcriptional repression, often utilized by cancer cells (90). Mutations of *EZH2* have been found in both myeloid and lymphoid malignancies, albeit with opposing effects. *EZH2* has been shown to be mutated in myelodysplastic (MDS) syndromes with loss-of-function mutations (91), while mutations of *EZH2* in follicular lymphoma and germinal-center type of diffuse large B-cell lymphoma result in increased enzymatic activity, allowing therapeutic targeting (92, 93). In addition, mutations of *UTX* (or *KMD6A*), which has an opposing action to EZH2, catalyzing the demethylation of H3K27me3, have also been described in some hematological malignancies, such as acute lymphoblastic leukemia (94) or myeloma (95, 96), possibly leading to increased responsiveness to therapeutic inhibition of EZH2.

Since the discovery of the importance of EZH2 for the pathogenesis of lymphoid malignancies, numerous small molecules that inhibit EZH2 have emerged (97, 98). GSK-126 was one of the first EZH2 inhibitors to be tested in a clinical trial, recruiting patients with lymphoid malignancies and myeloma (NCT02082977). However, GSK-126 has the disadvantage of intravenous administration and potential off-target effects, as the study had to be terminated due to insufficient evidence of clinical activity, even after the maximal dose and schedule were attained. Tazemetostat (EPZ-6438) is another, orally administered EZH2 inhibitor with promising results in clinical trials. Early results from a phase I/II trial with tazemetostat alone or combined with prednisone in patients with B-cell lymphomas showed clinical activity in both wild-type and EZH2-mutated patients, while at the same time exhibiting minimal toxicity (NCT01897571). The activity of tazemetostat is also being investigated exclusively in patients with B-cell lymphomas bearing an EZH2 mutation (NCT03456726). Following these promising results in a relapsed setting, tazemetostat is now being tested in combination with R-CHOP (called "Epi-RCHOP) in patients with newly diagnosed diffuse large B-cell lymphoma (NCT02889523). However, this study is currently suspended due to the development of a secondary T-cell lymphoma in a pediatric patient receiving tazemetostat in a different trial. Other novel EZH2 inhibitors such as CPI-1205 or SHR2554, are currently being tested in phase I studies including patients with relapsed/refractory lymphoid malignancies (NCT02395601, NCT03603951). Thus, the potential of EZH2 inhibition in the treatment of lymphoid malignancies remains unknown but is currently thoroughly investigated.

### The MLL family and DOT1L

The mixed lineage leukemia (MLL) family of proteins includes five different members that all methylate H3K4, thus regulating the active gene transcription (99). In humans, the *MLL1* (also known as *ALL1* or *KMT2A*) gene is frequently involved in chromosomal translocations in acute leukemias that can be both of lymphoid (ALL) and myeloid (AML) lineages, as well as biphenotypic leukemias (mixed lineage leukemias – MLL), offering a particularly poor prognosis in infant ALL, but not in *de novo* AML in adults (100, 101). The most common translocations involving the *MLL1* gene are t(4,11)(q21;q23) or *MLL–AF4*; t(9;11)(p22;q23) or *MLL–AF9*; t(11;19)(q23;p13.3) or *MLL–ENL*; t(10;11)(p12;q23) or *MLL–AF10*; and t(6;11)(q27;q23) or *MLL–AF6*, all resulting in chimeric fusion proteins (102). Interestingly, the aforementioned fusion partners for MLL have been shown to interact directly with DOT1L, which is currently

the only known H3K79 methyltransferase (103) and it seems that the oncogenic activity of MLL-fusion proteins is based on the recruitment of DOT1L and methylation of H3K79 (104–106) (Fig. 2). In fact, DOT1L seems to play a central role in the genome-wide transcriptional changes caused by the chimeric MLLs, as the inhibition of DOT1L has exhibited strong anti-leukemic activity in preclinical models of MLL- translocated leukemias (107–109).

Since there are to date no known direct inhibitors of MLL1 or its fusion alternatives in MLL-translocated leukemias, DOT1L seems to be a very promising target for the treatment of this specific subtype of leukemias. To this day, only a single inhibitor of DOT1L, pinometostat (EPZ-5676), has been tested in two different clinical trials. In the first trial (NCT02141828), pinometostat was tested in pediatric patients with ALL bearing MLL translocations. Preliminary data from this study (presented at ASH in 2016) showed that a dose of 70 mg/m$^2$ given as a continuous intravenous infusion daily until disease progression had an acceptable safety profile and led to transient reductions in peripheral or bone marrow blasts in approximately 40% of patients, however with no objective clinical responses (110). Another study tested pinometostat in adults with relapsed/refractory leukemias (AML, ALL, or MLL) with MLL translocations (NCT01684150). Again, preliminary results from this study presented at ASH in 2015 showed a favorable toxicity profile and a clinical response in 6 out of the 49 patients recruited (111). It will be interesting to investigate not only the relationship of clinical response with pharmacodynamics and pharmacokinetics of pinometostat, but also its clinical efficacy in MLL-translocated leukemias when given in combination with other chemotherapeutic agents.

## PERSPECTIVES

Epigenetic therapy in hematological malignancies is a rapidly advancing field with a massive potential. With already four approved epigenetic therapies and a plethora of novel drugs under development, it will be interesting to observe the evolution of epitherapeutics in hematology. An interesting example of novel epidrugs is a class of drugs that target the 'readers' of epigenetic marks, some of which are currently being tested in clinical trials. For example, JQ1, an inhibitor of the bromodomain protein BRD4, has exhibited high anti-myeloma activity *in vitro*, by downregulating genes that are critical for the development of multiple myeloma (MM),

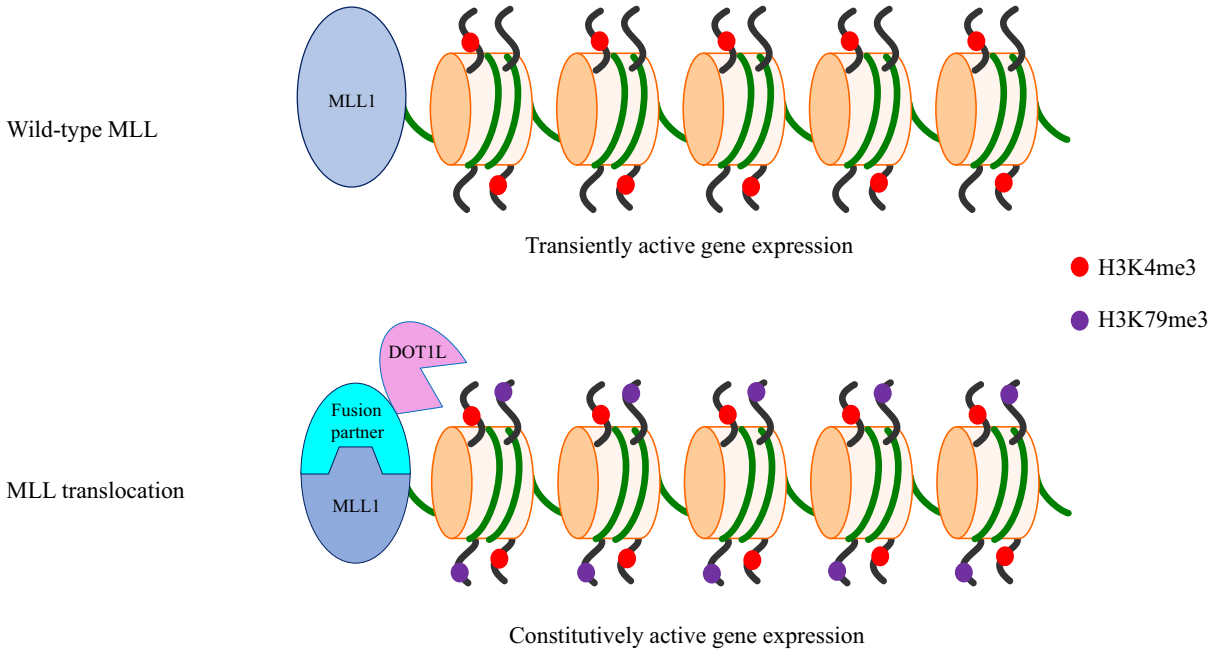


**Fig. 2.** MLL1 is a member of the mixed lineage leukemia (MLL) family, the members of which are H3K4 methyltransferases. In the case of an MLL translocation, a chimeric MLL protein is formed, with a fusion partner such as AF9 or AF10, which directly binds the H3K79 methyltransferase disruptor of telomeric silencing 1-like (DOT1L), allowing for a much more potent and constitutive activation of gene expression.

including MYC (112). *MYC* is one of the key genes in the pathogenesis of MM, is found upregulated in up to 50% of cases, and has been associated with the transition from monoclonal gammopathy of undetermined significance (MGUS) to symptomatic MM, as well as late disease progression (113). JQ1 selectively inhibits the binding of BRD4 on super-enhancers, thus directly inhibiting the MYC transcription with depletion of the c-Myc oncoprotein, as well as selective downregulation of the c-Myc transcriptional program (112, 114). Apart from myeloma, JQ1 has also shown activity in diffuse large B-cell lymphoma (115) and high-risk myeloid leukemia (116). So far, no MYC-specific inhibitors have been developed, and given the fundamental role of MYC upregulation in MM but also in other tumors, JQ1 might thus be one of the many important future epigenetically based cancer therapies.

Finally, it is extremely important to precisely characterize the mechanisms of action of epigenetic drugs, in order to increase efficacy while at the same time minimizing side effects or off-target effects. Based on most of the clinical trials, monotherapy with an epigenetic drug is rarely sufficient to achieve disease control; however, it seems that epigenetic therapy might be able to enhance the cytotoxic activity of other chemotherapeutic agents (79, 81, 117, 118). Even more interestingly, epigenetic therapy has been shown to restore sensitivity to chemotherapy in both myeloid (119) and lymphoid cancers (40–42, 120), as well as myeloma (121, 122). Lastly, there might also be synergy between different epigenetic therapies. Recent data have shown that the upregulation of ERVs following decitabine treatment is even more pronounced when G9a, a H3K9 methyltransferase, is concurrently inhibited and the potential of the combination of decitabine and a G9a inhibitor requires further investigation (123). In addition, it has been shown that the inhibition of either DNA methyltransferases (DNMTs) or EZH2 might enhance the cytotoxicity of panobinostat in different hematological malignancies (124–126). Thus, a more targeted approach, where epigenetic therapy is part of a multidrug regime, can be used to employ synergy and maximize the efficacy of standard chemotherapy, or epigenetic therapy can be given prior to chemotherapy, to "prime" the epigenome and eventually (re)sensitize the malignant cells to a given therapy. Future preclinical and clinical studies are thus needed to evaluate the best possible use of epidrugs in cancer.

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