Chapter

Unique Therapeutic Approaches for Targeting Epigenetic Machinery in T-cell Lymphoma

Jacob Cogan and Jennifer E. Amengual

Abstract

Growing knowledge on T-cell lymphoma (TCL) biology has led to the understanding that TCLs harbor derangements in proteins modulating epigenetic control. Some such derangements include mutations in TET2, IDH2, DNMT3A, EP300, and CBP. In addition, overexpression of epigenetic modifiers such as EZH2 also exists in the absence of mutations. HDAC inhibitors are approved for use in relapsed T-cell lymphoma. There may be unique methods to targeting epigenetic derangements using new agents such as DNMT, EZH2, IDH, and BET inhibitors to name a few. In this chapter, we will review and explore unique methods for therapeutic targeting of epigenetic machinery for TCL.

Keywords: T-cell lymphoma, epigenetics, EZH2, DNMT3A, IDH2, TET2, BET, EP300, CBP, DOT1L

1. Introduction

Peripheral T-cell lymphomas (TCL) are a diverse group of non-Hodgkin lymphomas that tend to have aggressive courses and poor prognoses. TCLs account for 10–15% of all non-Hodgkin lymphomas. Common subtypes are anaplastic large cell lymphoma (ALCL), angioimmunoblastic T-cell lymphoma (AITL), cutaneous T-cell lymphoma (CTCL), and peripheral T-cell lymphoma NOS (PTCL-NOS). Even more rare subtypes include adult T-cell leukemia/lymphoma (ATLL) and natural killer/T-cell lymphoma (NKTCL). The diagnosis of these conditions can be challenging, as the largest group of these conditions – PTCL-NOS – cannot currently be further categorized beyond that point [1]. Similarly, the approach to treating TCLs has generally been empiric and homogenous, rather than specific and targeted to the particular subtype.

TCL patients frequently do not respond well to chemotherapy. The median time to relapse or progression after primary therapy was 6.7 months in a sample of 153 patients with PTCL-NOS, AITL, and ALCL, three of the most common TCL subtypes [2]. Further, median overall survival and progression free survival after relapse or progression in this sample was 5.5 and 3.1 months, respectively. Given this poor response to chemotherapy, the subsequent lack of candidacy for stem cell transplantation in most TCL patients given this poor response, and the tendency of TCL patients to develop resistance to chemotherapy, optimal therapies for this population remain undefined. Clinical trials are thus often the recommended first-line treatment for these patients.

Limitations in the specificity of treatment of TCL may be related to prior lack of insight into the genetics and molecular underpinnings of these malignancies. However, as understanding of the genetics of these diseases advances, therapy targeting epigenetic modifiers and transcriptional dysregulation have emerged as a target for TCL therapy [1, 3]. Epigenetic modulators have been found to be widely mutated in TCLs, and are thought to play a central role in these diseases. The heritability of epigenetic profiles across cell lines, and the consistency with which this process is aberrant in TCLs makes targeting epigenetic modifiers an area of promise in treating these particularly resistant conditions [4].

Epigenetics refers to aspects of chromatin biology that affect gene expression without altering DNA sequence. Chromatin is comprised of DNA wrapped around a core of four types of histone proteins, forming nucleosomes. The ability of the transcriptional machinery to access chromatin determines gene expression. This accessibility is determined by posttranslational modifications of the components of the nucleosome complex, such as methylation and acetylation of DNA and histones. Given the inherently plastic and reversible nature of epigenetic modifications, they have emerged as an appealing therapeutic target, in contrast to the more fixed nature of genetic alterations. Further, epigenetic regulators often have enzymatic activities or binding domains that lend themselves well to small molecule inhibition [3].

Epigenetic abnormalities targeted in TCLs tend to be related to methylation and acetylation of histones and DNA. Histone deacetylase (HDAC) inhibitors were the first approved epigenetic therapy for TCL. HDACs tend to be recruited by oncoproteins to support repressive malignant gene expression [3]. The U.S. FDA has approved three HDAC inhibitors for use in relapsed or refractory TCLs. Vorinostat, an oral agent, was approved for relapsed/refractory CTCL in 2006, with romidepsin—an IV agent—following shortly after in 2009 [5]. Romidepsin and belinostat were also approved for the treatment of relapsed/refractory peripheral TCLs 2011 and 2014, respectively. Adverse effects include cytopenias and gastrointestinal symptoms. In addition, romidepsin can lead to EKG changes that may be clinically significant in patients with pre-existing cardiac disease. Interestingly, the clinical benefit derived from HDAC inhibitors in TCLs have not shown the same efficacy

Epigenetic Target	Drug	Cell Line	Reference
EZH2	GSK126	Non-specific TCL	Kumar et al. [25]
	DZNep	ATLL	Daisuke et al. [23]
	DZNep	NKTL	Yan et al. [24]
CBP/EP300	A-485	NHL	Lasko et al. [28]
DOT1L	EPZ-5676	Non-specific TCL	Kumar et al. [30]
DNMT3A	Azacitadine or Decitabine + HDAC inhibitor	CTCL	Marchi et al. [8]
	Decitabine + Chidamide	PTCL-NOS	Ji et al. [26]
	Azacitadine + Romidepsin	Sezary Syndrome	Rozati et al. [7]
	JQ1	CTCL	Kim et al. [40]

Table 1.
Pre-clinical studies of novel epigenetic inhibitors in T-cell lymphoma.

in B-cell lymphomas, further underscoring the importance of pursuing this treatment paradigm for TCLs. Romidepsin, belinostat and vorinostat are currently being studied in combination with conventional chemotherapies [6], such as CHOP [7, 8], gemcitabine [9] and ifosfamide-containing regimens [10].

Given the success of HDAC inhibitors in TCLs, much research has been dedicated towards elucidating other epigenetic targets for TCL therapy (**Tables 1** and **2**). Candidates have included DNA methyltransferases (DNMTs), the ten-eleven

Target	Drug	Phase	Population	Results	Toxicities	Reference
EZH2	DS-3201b	I	5 TCL patients (2 ATLL, 2 AITL, 1 PTCL-NOS)	ORR 80% one CR, three PRs	Hematologic, dysgeusia, diarrhea nasopharyngitis, alopecia, rash, decreased appetite, dry skin	Maruyama et al., [21]
DNMT3A	Azacitadine	I	19 relapsed/ refractory TCL patients (12 AITL)	ORR 53%; 75% ORR for AITL patients, 5 CR	Hematologic, diarrhea	Delarue et al. [31]
	Azacitadine + Romidepsin	I/II	10 TCL patients	ORR 83%, 50% CR	Hematologic, febrile neutropenia	Falchi et al. [32]
IDH2	AG-221	I/II	AITL patients	In progress		NCT02273739

Table 2.
Clinical trials of novel epigenetic inhibitors in T-cell lymphoma.

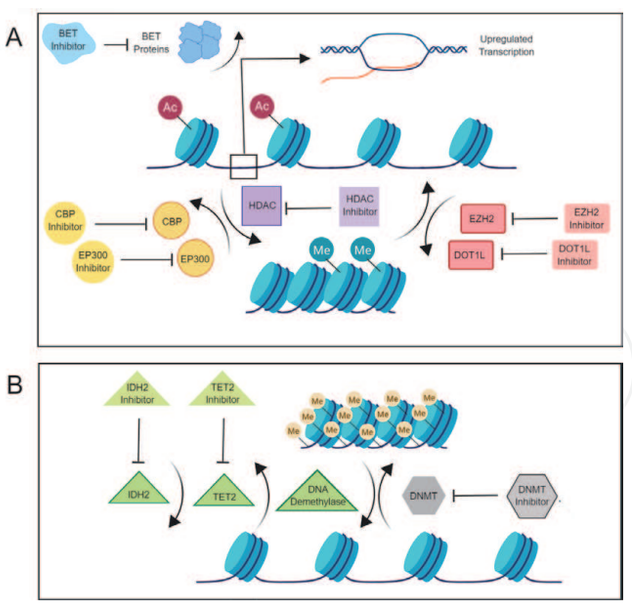

Figure 1.
(A) Histone acetylation makes chromatin more accessible for transcription, while histone deacetylation makes chromatin more compact and less transcriptionally active. Histone acetylation is mediated by histone acetyltransferases, such as CBP and EP300; histone deacetylation is mediated by histone deacetylases (HDACs). Inhibitors of CBP, EP300, and HDACs have all shown promise in treating TCLs. BET proteins recognize acetylated histones and promote transcription at related DNA regions, and can be targeted by BET inhibitors. By contrast, methylation of histones renders DNA transcriptionally inactive, while demethylation of histones increases DNA receptiveness to transcription. EZH2 and DOT1L are histone methylators, and are targets in TCL therapy as well. (B) DNA methylation is associated with transcriptional repression, while de-methylated DNA is more transcriptionally active. TET2 promotes DNA demethylase activity, and IDH2 regulates TET2. TET2 and IDH2 can both be targeted in TCL treatment. Additionally, a major category of TCL therapy involves inhibitor of DNA methyltransferases (DNMTs), which render chromatin more transcriptionally inactive.

translocation (TET) family of proteins, isocitrate dehydrogenase (IDH) enzymes, bromodomain and extra-terminal (BET) proteins, enhancer of zeste homolog 2 (EZH2), cyclic AMP-response element binding, binding protein (CBP) and E1A binding protein p300 (EP300), and disruptor of telomeric silencing 1-like (DOT1L) (**Figure 1**). Further, it has been postulated that combination therapy of different epigenetic modulators may have added utility in TCL treatment. Tumor suppressors are often down regulated by abnormal histone deacetylation and/or DNA methylation. By combining HDAC inhibitors and DNMT inhibitors, synergy may be achieved to relieve this abnormal transcriptional repression [11]. Thus, epigenetic combination therapy may represent a new paradigm for the treatment of TCLs [5, 12]. In this manuscript, we will review the TCL subtypes and relevant documented mutations, survey the literature regarding novel epigenetic therapies for TCL, and discuss future directions for this therapeutic strategy.

2. T-cell lymphoma subtypes

2.1 Anaplastic large cell lymphoma (ALCL)

Anaplastic large cell lymphoma (ALCL) represents up to 32% of TCLs in North America, and about 2.4% of NHLs [5]. It is more common in men (2:1 male: female ratio), and displays a bimodal age distribution, with peaks at age 25 and 60. ALCL frequently involves the lymph nodes or skin, but can also affect the gastrointestinal tract, lung, and bone. Translocations involving the Anaplastic Lymphoma Kinase (ALK) are common in ALCL, with t(2,5) translocations occurring in 50% of cases, and leading to a Nucleophosmin-ALK (NPM-ALK) fusion protein. This protein induces a number of oncogenic signaling pathways, leading to malignant transformation of T-cells [13]. ALK status is important for prognosis, with ALK+ positive displaying a 70% 5-year survival, while ALK- patients have a 49% survival rate [5]. Chemotherapy is the first line treatment for this disease, and crizotinib, an ALK tyrosine kinase inhibitor, can be used in chemotherapy-resistant disease ALK+ ALCL. However, resistance to crizotinib frequently develops, motivating pursuit of other treatment strategies. DNA methylation profiling of five ALCL patients displayed similar patterns of methylation in all samples, regardless of ALK status. This abnormal methylation was noted in genes involved in T-cell differentiation and immune response [14], suggesting a role of epigenetic derangements in driving ALCL development. Inhibitors of BET proteins, which recognize acetylated histones and recruit transcription factors to these regions, have been used in pre-clinical models to treat ALCL, described later in this review.

2.2 Angioimmunoblastic T-cell lymphoma (AITL)

Angioimmunoblastic T-cell lymphoma (AITL) comprises 20% of TCLs and 1% of all NHLs [15]. Patients tend to be diagnosed with advanced stage disease, and have a median survival of less than 3 years. AITLs are unique in their gene expression pattern, with mutations in genes responsible for epigenetic modifications – particularly DNA methylation – emerging as particularly commonplace. In a study of 85 AITL patients, 65 (76%) had TET2 mutations, 43 of which displayed two or three TET2 mutations, indicating strong selective pressure for TET2 abnormalities in AITL. Twenty-eight patients (33%) had DNMT3 mutations, and 17 (20%) had mutations in IDH2. Of note, all but two of the patients in this sample with DNMT3 and IDH2 mutations also had TET2 mutations. Mutations in these specific types of

epigenetic modulators are more commonly seen in myeloid malignancies than in other lymphomas. This could help explain the poor outcomes AITL patients have after treatment with chemotherapy regimens developed against BCLs. The multi-step tumorigenesis model for AITL hypothesizes that early mutations in epigenetic modifiers interact with late cooperative mutations to enable malignant transformation [5]. As such, TET2, DNMT3, and IDH2, among other epigenetic modulators, have emerged as therapeutic targets in AITL.

2.3 Cutaneous T-cell lymphoma (CTCL)

Cutaneous T-cell lymphoma (CTCL) includes mycosis fungoides (MF), Sezary syndrome (SS), and other related lymphoproliferative disorders that originate in the skin. CTCL is a male-predominant disease (1.7:1.0 M:F ratio), has a median age of diagnosis in the mid-1950s, and is more common in African Americans [5]. MF tends to present with patches, plaques, tumors, and ulcers, while SS presents with exfoliative erythroderma, lymphadenopathy, and circulating Sezary cells. The prognosis of these patients generally correlates with the extent of cutaneous and systemic involvement, with MF displaying an 88% 5-year survival and SS patients demonstrating a 5-year survival of 24%. Currently available therapies have a success rate of 30–50%, and relapses are common [5, 11]. Epigenetics have been important in improving diagnoses of these conditions, with detection of promoter hypermethylation of chemokine-like CMTM2 proving sufficient to distinguish SS from erythrodermic inflammatory dermatosis [16]. As mentioned earlier, CTCL was the first malignancy for which HDAC inhibitors were approved. One such agent, romidepsin, showed an overall response rate (ORR) of 35% and complete response in 6% when used as monotherapy in relapsed/refractory CTCL patients [11]. Currently, a topical HDAC inhibitor for use in early stage CTCL is underway with promising results [17]. There is thought that CTCLs may be susceptible to additional epigenetic modifiers. SS has also been shown to have a high prevalence of methylation abnormalities [4]. TET2 mutations are one of the early genetic abnormalities in SS, and mutations in isocitrate dehydrogenase (IDH) have been described as well [18].

2.4 Adult T-cell leukemia/lymphoma (ATLL)

ATLL is associated with the human T-cell lymphotropic virus-1 (HTLV-1). The virus—endemic to southwestern Japan, the Caribbean, and Central Africa—is transmitted by blood transfusions, needle sharing, sexual intercourse, and breastfeeding. HTLV-1 immortalizes human T-cells, and was the first retrovirus found to be directly associated with malignancy. ATLL occurs in 2.5% of carriers in endemic areas, with growing rates of prevalence in non-endemic areas. Subtypes include smoldering, chronic, lymphoma, and acute ATLL, with 4-year survival rates ranging from 63% for smoldering to 5% for acute. Chemotherapy plus central nervous system prophylaxis is the current first-line treatments for ATLL, with some electing to add antiviral therapy, though virus eradication does not necessarily lead to improved survival [5]. As a result, attention has turned towards understanding the genetic and epigenetic factors involved in causing and maintaining malignant disease in HTLV-1 infected T-cells. ATLL cells display H3K27me3 hypermethylation, a feature mediated by EZH2. EZH2 overexpression leads to this histone hypermethylation, leading to silencing of anti-apoptotic pathways [19]. It is thought that this reprogramming occurs at an early stage of ATLL T-cell development, potentially as a result of the HTLV-1 protein Tax enhancing EZH2 promoter activity. Further, 22% of a 27-patient ATLL cohort were found to

have EP300 mutations, with smaller numbers demonstrating mutations in TET2 and DNMT3A [20].

2.5 Natural killer/T-cell lymphoma (NKTL)

Natural killer/T-cell lymphoma (NKTL) constitutes about 11% of TCLs. Prior to the 1990s, the rare disease was known as lethal midline granuloma, in which destructive midline facial lesions would develop, progressing rapidly to cause patient death. Advances in pathology led to recognition that this condition was a neoplasm of lymphoid origin [21]. The disease is predominantly seen in males (2:1 M:F), and the median age of diagnosis is 50 years old. They can be classified as nodal and extranodal, and further as nasal (nose, upper aerodigestive tract; 80% of cases) and non-nasal (skin, GI tract, testes, salivary glands; 20% of cases). Additionally, peripheral blood involvement is common, and further labels the disease as a lymphoma/leukemia subtype. Interestingly, all NKTL lymphoma cells are infected with EBV. Detection of the virus is required for diagnosis, and prognosis can be correlated with EBV levels as time of diagnosis and variation of levels in response to therapy. NKTL is a particularly aggressive condition, with median survival ranging from 12 months for the nasal subtype to 2 months for the lymphoma/leukemia subtype. Concurrent chemoradiation is the mainstay of treatment for NKTL [5], with non-anthracycline based regimens (such as those containing L-asparaginase) proving more effective. While epigenetic therapies have not to this point been utilized in treating NKTL, EP300 and EZH2 mutations have been documented, indicating a possible role for epigenetic therapy in this disease [22].

2.6 Peripheral T-cell lymphoma not otherwise specified (PTCL-NOS)

Peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) constitutes both the largest group of TCLs and the least characterized. PTCL-NOS accounts for 25% of TCLs, and is a diagnosis of exclusion, when the disease does not fit any of the other WHO classifications mentioned above. It tends to affect older men, with a median age of presentation of 60. Nodal disease is common, though any organ can be affected, and about 70% of cases present at advanced stages. PTCL-NOS patients tend to be treated with anthracycline-based chemotherapy regimens. Recurrent mutations in TET2, IDH2, and DNMT3A have been found in the T-follicular helper phenotype of PTCL-NOS. This disease has since been designated as its own entity, distinct from the larger group of PTCL-NOS. Overall, PTCL-NOS patients display an overall survival rate of 32–45%. The HDAC-inhibitors romidepsin and belinostat are approved for relapsed/refractory PTCL-NOS [6].

3. Epigenetic agents targeting histone methylation and acetylation

Epigenetic modification of histones has both documented and emerging therapeutic importance in TCL treatment. Histone acetylation is a dynamic process comprised of acetylation with lysine acetyltransferases and deacetylation with histone deacetylases (HDACs). Acetylation allows for chromatin configurations more accessible to transcription, whereas deacetylation generates more compact, less transcriptionally active chromatin [3]. The importance of HDAC inhibitors have been well documented in TCLs such as CTCL and PTCL-NOS. Transcriptional co-activators such as CBP and EP300 are involved in promoting histone acetylation, and have been targeted in the treatment of ATLL. Histone methylation, by contrast,

alters the ability of DNA-reading proteins to bind to methylated residues, while demethylation of histones render associated chromatin more transcriptionally active. EZH2 and DOT1L are clinically important histone methylators, relevant to the treatment of ATLL and NKTL, among other subtypes.

3.1 Enhancer of zeste homolog 2 (EZH2)

Enhancer of zeste homolog 2 (EZH2) is the enzymatic component of polycomb repressor complex 2 (PRC2). PRC2 has documented involvement in cell fate decisions by establishing and maintaining transcriptional repression through post-translational modifications of histones via EZH1 and 2 [23]. EZH2 trimethylates lysine residue 27 of histone H3 (H3K27me3), causing down-regulation of genes involved in tumor suppression and cell differentiation [24]. Elevated levels of H3K27me3 are thought to correlate with the aggressiveness of malignancies such as lymphoma, in addition to brain, breast, kidney, lung, and prostate [25]. While EZH2 is thought to be dispensible for normal hematopoiesis, given it is redundancy with EZH1, EZH2 is the PRC2 component most often implicated in the development of hematological malignancies [3]. EZH2 has emerged as a target for T cell lymphoma therapy. Overexpression of EZH2 has been noted in ATLL [26] and NKTL [27], in particular.

Several pre-clinical studies have demonstrated utility of EZH2 inhibition in T cell lymphoma. GSK126, a commercially available EZH2 inhibitor, was shown to decrease cell viability in an in vitro TCL line [28]. In one study, PTEN-inactivated cells derived from a TCL mouse model were utilized. High concentrations of GSK126 were required to achieve in vitro biological activity, attributed to the high levels of L-amino acid transporter 1 (LAT1) in these cells. As opposed to LAT2, which is expressed in normal cells, LAT1 is predominantly expressed in malignant cells. Such cells with high levels of LAT1 tend to be more resistant to therapeutic treatment, as opposed to cells with lower levels. The authors felt that the high LAT1 levels likely gave the in vitro TCL cells an enhanced ability to combat chemical attacks, via mechanisms such as enhanced essential amino acid sequestering potential. This observation supports their notion that combination therapy will be the most efficacious method to achieving results when using therapies such as EZH2 inhibitors. This point highlights the importance of utilizing combination epigenetic therapies in treating TCLs.

Work performed in our laboratory sought to combine EZH2 and HDAC inhibitors as a means of dual targeting of histone methylation and acetylation. GSK128 was combined with romidepsin across a large panel of lymphoma cells (N-21). Synergy was observed in cell lines with known EZH2 dysregulation including ATLL. We found that the combination led to decreased histone methylation and increased acetylation as compared to treatment with either drug alone. This in turn induced p21 expression leading to caspase 3 and PARP cleavage. The combination is safe and effective in mouse models of lymphoma. Synergy could be predicted in cell lines, which were enriched in chromatin silencing, gene silencing, epigenetic regulation of gene expression and protein acetylation pathways as measured by gene set enrichment analysis (GSEA) [29]. Dual targeting of histone modifications could be a rational approach for diseases driven by epigenetic derangements.

DZNep, an EZH2 inhibitor, has demonstrated pre-clinical efficacy in ATLL and NKTL. In ATLL cell lines, DZNep been shown to deplete levels of both EZH2 and BCL2 [26]. The authors demonstrated that, while untreated ATLL cells demonstrate decreased levels of micro RNA 181a (miR-181a), ATLL cells treated with DZNep showed elevated levels. miR-181a production is modulated by EZH2, and acts as a negative regulator of BCL2 expression. Thus, the

inhibitory effects of DZNep on EZH2 results in increased production of miR-181a, decreased levels of BCL2, and consequently apoptosis. Further, in [27], DZNep inhibited growth of NKTL cell lines in an unexpected fashion. These authors noted an oncogenic role of EZH2 independent of its methyltransferase activity. Cells with mutations in EZH2 that depleted their histone methyltransferase activity still had oncogenic potential, likely through increased production of cyclin D1. DZNep was still able to inhibit growth of these cells as well. This indicates that the oncogenicity of EZH2 mutation is more complicated than simply arising through overactive histone methylation, and that therapies should not simply focus on targeting the enzymatic activity of EZH2, but perhaps on the production of EZH2 itself.

In humans, DS-3201b, an oral EZH1/2 dual inhibitor, was utilized in a phase 1 study which included five TCL patients (two ATLL, two AITL, and one PTCL-NOS) [24]. Overall response rate (ORR) in TCL patients was 80%, with one complete response (CR) and three partial responses (PR). ORR in the entire cohort (15 total patients, 10 with other BCLs) was 55%. Adverse events were mainly transient hematologic toxicities.

3.2 Histone acetyltransferases (HATs): CBP/EP300

Cyclic AMP-response element binding, binding protein (CBP) and E1A binding protein p300 (EP300) are highly related transcriptional coactivators, with 75% similarity across their entire length and 63% homology at the amino acid level [19]. They are known to enhance transcription through linking sequence-specific transcription factors to RNA polymerase II. They also facilitate histone acetylation, which promotes transcription. [13]. CBP and EP300 are two of the most frequently mutated histone acetyl transferases in hematologic malignancies [3]. Mutations have been documented in CTCL, ATLL, NKTCL, and PTCL-NOS [1, 3, 20, 30]. They have also been implicated in the actions of viral oncoproteins such as HTLV-1 Tax protein [20]. Acetylation of p53 by CBP has been shown to enhance the DNA binding ability of p53, and loss of CBP has been shown to cause T-cell lymphomagenesis in vitro [31]. In [20], Shah et al. showed that EP300 mutations were present in 22% of a North American cohort of 27 ATLL patients. Five EP300-mutant ATLL cell lines derived from these patients were treated with decitabine, a DNMT-inhibitor. All five lines were sensitive to this treatment, and the addition of doxorubicin demonstrated synergy.

Drug development has focused on small molecule inhibitors of both CBP and EP300. A-485, a combination CBP/EP300 catalytic inhibitor, was evaluated preclinically across a variety of malignant cell lineages. The broadest sensitivity was seen in hematological malignancies, including non-Hodgkin's lymphoma cell lines, with markedly less sensitivity observed in cells derived from solid malignancies [32]. Histone acetyltransferase inhibitors may be of use in treating TCLs via this pathway.

Histone acetyltransferase activators are also in early development. Inactivating mutations in HAT enzymes are monoallelic creating a haploinsufficient state of acetylation. Lymphomas harboring a multitude of epigenetic abnormalities, such as TCLs are poised to capitalize on this relative deficiency. A library of HAT activating compounds have demonstrated efficacy across a panel of lymphoma cell lines and xenograft mouse model [33]. The compounds demonstrated acetylation of histone and p53. In addition, HAT activators demonstrated synergistic cytotoxicity when combined with the HDAC inhibitor, romidepsin. Activating HAT enzymes could therefore be thought of as activating a tumor suppressor and offers a direct method of targeting pathology that drives TCL.

3.3 Disruptor of telomeric silencing 1-like (DOT1L)

Disruptor of telomeric silencing 1-like (DOT1L) is the only known H3 lysine 79 (H3K79) methyltransferase in mammals. Genetic silencing of DOT1L has been shown to impact mitotic spindle formation and cell cycle progression. Preclinical models have shown utility of DOT1L inhibition in AML. Mutations in Mixed-lineage leukemia (MLL) genes, which encode for a family of histone methyltransferases, have been linked to AML (MML1 mutations) and BCLs (MLL2 mutations). The DOT1L containing complex is a frequent translocation partner of MLL fusions in MLL-associated leukemias. Consequently these leukemias have been shown to have atypical H3K79 methylation patterns. Incidentally, AML models carrying recurrent mutations in IDH 1/2 and DNMT3A have also been shown to be susceptible to DOT1L inhibitors. Pinometostat (EPZ-5676), a recently developed DOT1L inhibitor, is currently in phase 1 trials of advanced hematologic malignancies. Given the promising findings of DOT1L inhibition in MLL-associated leukemias, and the efficacy of DOT1L inhibitors in AML models with mutations in epigenetic regulators typical of TCLs, there may be promise in using DOT1L inhibitors in TCLs as well. Thus far, pinometostat has been shown to decrease cell viability in PTEN-inactivated TCL mouse model cells, which have also been utilized in pre-clinical studies of EZH2 inhibitors [3, 34, 35].

4. Epigenetic agents targeting DNA methylation

The regulation and maintenance of DNA methylation plays a key role in cellular differentiation and genome stability. A dynamic process of DNA methylation and demethylation at the carbon-5 position of cytosine nucleotides contributes to the epigenetic milieu of any given cell. DNA methyltransferases such as DNMT3A and DNMT3B are responsible for de novo DNA methylation, while DNMT1 handles maintenance methylation. Conversely, the TET family of proteins handles cytosine demethylation. In contrast to histone acetylation, where an acetylated state leads to more transcriptionally active chromatin, methylated cytosine residues tend to be associated with transcriptional repression. On the other hand, chromatin without cytosine methylation is more transcriptionally active [3]. Abnormalities in DNA methylation have been found to be important in AITL, CTCL, PTCL-NOS, and ALCL.

4.1 DNMT3

DNMT3A, responsible for de novo DNA methylation, is known to be mutated in many hematological malignancies. Azacitidine (5-AZA) and decitabine, two DNMT3A inhibitors, are approved for use in myelodysplastic syndromes and AML. They are nucleoside analogues which incorporate into newly synthesize DNA and RNA strands and subsequently inhibit DNMTs irreversibly. This results in decreased DNA methylation, and consequently, daughter cells likely do not inherit the aberrant methylation pattern. Given the prevalence of DNMT3A mutations in AITL, utilizing DNMT3A inhibitors in this disease has been a focus of much recent research. In a study conducted in France by Delarue [36], 19 patients with relapsed/refractory peripheral TCL were treated with subcutaneously administered 5-AZA. The overall response rate for the entire study population was 55% (10/19). Twelve of the 19 patients had AITL and the ORR for these patients was 75% (9/12). Five of the AITL patients achieved CR, and only 2 of the 9 total responders had experienced progression at the time of analysis. Notably, 8 of the AITL patients had documented TET2 mutations, and all of these patients responded to therapy. 5-AZA treatment elicited no response in 6 of the remaining

7 patients in the study with other TCL subtypes, with the only responder relapsing after cycle 2 of treatment.

PTCL-NOS commonly displays mutations in genes involved in histone methylation and acetylation. The presence of histone modifier gene mutations was associated with decreased progression-free survival in a cohort of 125 PTCL-NOS patients. PTCL-NOS cells were shown to experience growth inhibition when treated with a HDAC inhibitor, chidamide, and decitabine, both in vitro and in vivo [30]. Dual therapy enhanced the interaction of KMT2D with the transcription factor PU.1, consequently inactivating the MAPK, which tends to be constitutively activated in TCL. PU.1 interact with DNMTs to control hematopoiesis and suppress leukemia, and KMT2D is a histone modifier.

Combination therapy involving DMNT3A inhibitors has also shown promise. Transformed CTCL lines were exposed to combination of a DNMT-inhibitor (decitabine or 5-AZA) and an HDAC inhibitor (belinostat, panobinostat, romidepsin, or vorinostat), with induction of apoptosis in all lines treated [12]. Combination therapy with decitabine and belinostat also induced significant growth delay in an in vivo mouse model, compared to mice treated with single agent therapy. Gene expression analysis showed that, as opposed to the 138 genes modulated by romidepsin monotherapy, combination therapy led to modulation of an additional 390 genes, with many involved in apoptosis and cell cycle arrest. These findings further elucidate the molecular basis of the synergism elicited by combination therapy.

Another study utilized a combination of romidepsin and 5-AZA in cell lines from SS patients. Synergistic antiproliferative effects and induction of apoptosis were observed with combination therapy. The concentration of each agent in the combination was 50% less than the IC50 of each when used individually, which may result in improved tolerability when these agents are used clinically [11].

Building off these results, in [37], Falchi et al. presented a phase 1/2 trial with combination therapy of romidepsin and 5-AZA in the treatment of lymphoma patients. 10/30 patients in the study had TCLs. While the ORR for all 25 cohort patients evaluable for efficacy was 28% (7/25), with a 16% CRR (4/25), the 6 evaluable TCL patients demonstrated an 83% ORR (5/6) and a 50% CRR (3/6). Combination therapy was generally well tolerated: 5 dose limiting toxicities were recorded, including neutropenia (2), thrombocytopenia (1), pleural effusion (1), and a missed dose (1). Five total patients experienced thrombocytopenia, with three experiencing febrile neutropenia.

4.2 TET2

The ten-eleven translocation (TET) family of proteins are dioxygenases which catalyze DNA demethylation [38]. Aberrant demethylation has been linked to dysregulation of molecules such as BCL6, the transcription factor involved in the differentiation of T helper cells [39]. Abnormal differentiation of these cells provides opportunities for them to obtain late cooperative mutations rendering them malignant. TET2 mutations are frequently seen in mature TCLs [3]. In a study evaluating 190 TCL patients for TET2 mutations, 40/86 (47%) AITL patients carried the mutation, as did 22/58 (38%) PTCL-NOS patients. No other forms of TCL carried the mutation in this sample (18 ALCL patients and 12 NKTL patients, among others). TET2 mutations have been shown to occur in up to 80% of AITL patients [23]. TET2 mutations are also associated with advanced disease and shorter progression free survival.

The development of TET2 inhibitors for TCLs is currently in pre-clinical stages. TCL patients with TET2 mutations frequently also display mutations in RHOA, a GTP-ase important in stem cell differentiation, cell migration and cell shape. The cooperation of these two mutations in TCL progression, and whether small molecule inhibitors can halt or reverse disease progression, is under investigation [40].

4.3 IDH2

Changes in DNA methylation can occur indirectly, as a consequence of mutation of isocitrate dehydrogenase (IDH) enzymes. IDH2 is a regulator of TET2, and mutations in IDH2 have been described in up to 45% of patients with AITL [3]. Biochemically, IDH2 catalyzes the conversion of isocitrate to 2-oxoglutarate (2OG). In AITL, mutant IDH2 generates (R)-2-hydroxyglutarate (2HG), an oxymetabolite potentially contributing to malignant transformation through inhibition of 2OG-dependent enzymes. These enzymes are involved in functions ranging from DNA and histone modification to cellular differentiation, thus primed to produce malignancy when abnormally regulated. AG-221, a, orally available small molecule inhibitor of IDH2, has been tested in phase 1 and 2 trials of patients with AITL and AML (NCT02273739) [3, 39]. Given the frequent co-occurrence of IHD2 and TET2 mutations, combination therapy with inhibitors of each may have promise [41].

4.4 Bromodomain and extra-terminal (BET)

Bromodomain and extra-terminal (BET) proteins play a role in epigenetic memory and regulation of growth-promoting gene transcription [42]. BRD2, BRD3, BRD4, and testis-specific BRDT recognize acetylated lysine residues on histone tails and recruit transcriptional regulatory complexes to facilitate transcription of genes involved in cell cycle progression and apoptosis [43]. For instance, BRD4 regulates MYC transcription. OTX015, an oral BRD2/3/4 inhibitor, induced cell cycle arrest in 5/8 ALCL cell lines. ALK status had no impact on likelihood of response. OTX015 was found to suppress the transcription of the MYC gene in 4/4 cell lines [44].

Amplifications of the MYC oncogene are common in CTCL, occurring in 42.5% of leukemic CTCLs. JQ1, a selective small molecule inhibitor of BET proteins, dose-dependently decreased the cell number of CTCL cells through G1 cell cycle arrest and down-regulation of c-MYC expression. It also inhibited tumor growth of CTCL cells in vivo [45]. Several other types of BET inhibitors have been shown to induce dose-dependent decreases in viability of nine CTCL cell lines, and combining BET inhibitors with HDAC inhibitors potentiated this effect. Importantly, combination therapy was effective in cell lines from patients previously treated with other single therapies, including prior romidepsin monotherapy with relapse in one cell line. Significant reduction of BCL2 and MYC expression was seen in cells treated with combination therapy. Given that there is a 25% overlap in genes induced by BET inhibitors and HDAC inhibitors separately, it is theorized that the synergy of this combination therapy lies in the induction of HDAC-silenced genes, enabled by BET inhibition. Initial MYC amplification status was not found to be predictive of sensitivity to BET inhibition or the synergy of therapies used in combination. Finally, a functional interdependence between BRD4 and DOT1L in specific types of transcriptional regulation has been noted, suggesting a possible role for combination therapy involving inhibitors of these two molecules [3].

4.5 Anti-fols

The vitamin folate is essential as a source of the one carbon group required to methylate DNA. The folate pathway, and thus the 1-carbon pathway and DNA methylation is complex, built within it many checks and balances to maintain normal DNA methylation. Low folate status induced by diet or drugs can have "destabilizing consequences," resulting in impaired production of thymidine leading to uracil insertion in the DNA sequence, global DNA hypomethylation and ultimately chromosome instability and breakage.

There are two forms of folate that can enter the body depending on the mode of consumption. Folate comes from natural food sources, and folic acid comes from supplements or fortified foods. When folate enters the cell, it is metabolized to 5-methyltetrahydrofoate (5-methyl THF). Folic acid however is first reduced to dihydrofolate by dihydrofolate reductase and then to tetrahydrofolate (THF). Polyglutamination of THF allows for cellular retention. After a series of steps, THF is converted to 5-methyl-THF [46]. 5-Methyl THF is then converted to methionine and back to THF by methionine synthase which acts as the 1-carbon donor for S-adenosylmethionine (SAM). SAM donates methyl groups to DNA via DNMT1, 3a, and 3b. We have discussed the role of DNMT inhibitors as effective therapy for TCLs and will now focus on inhibition of other pathways as a means of decreasing DNA methylation (**Figure 2**).

Methotrexate is a widely used antimetabolite utilized as an antineoplastic agent since the 1950s [47, 48]. It has been used for the treatment of CTCL and other T-cell lymphoproliferative disorders such as lymphomatoid papulosis. Patients are often treated with low doses in a metronomic fashion. Methotrexate is a folic acid analogue and one of its actions is to block dihydrofolate reductase which disrupts the folic acid pathway. Evaluation in CTCL cell lines treated with methotrexate led to a decrease in SAM and subsequently decreased promoter region methylation leading to increase FAS protein expression. The addition of SAM was able to overcome the effects induced by methotrexate. Methotrexate, however, has not had the same success in the treatment of the more aggressive PTCL.

Pralatrexate is not merely a second-generation methotrexate. Although, similar to methotrexate, pralatrexate exerts its effects by inhibition of DHFR, it has higher affinity for the reduced folate carrier-1 (RFC-1) and has greater cellular retention through high a rate of forming polyglutamylated conjugates [49]. RFC-1 is an oncofetal protein expressed mostly on the membranes of fetal and tumor cells. This allows some selectivity of pralatrexate for malignant cells over normal cells. Interestingly, pralatrexate has enhanced activity in TCL and to date across several clinical studies, the only B-cell lymphoma that has demonstrated sensitivity to pralatrexate is follicular lymphoma. TCLs and follicular lymphoma biology's share the common feature of increased methylation of DNA and histone. One potential mechanism of action of pralatrexate, may be through modulation of methylation. Though not well

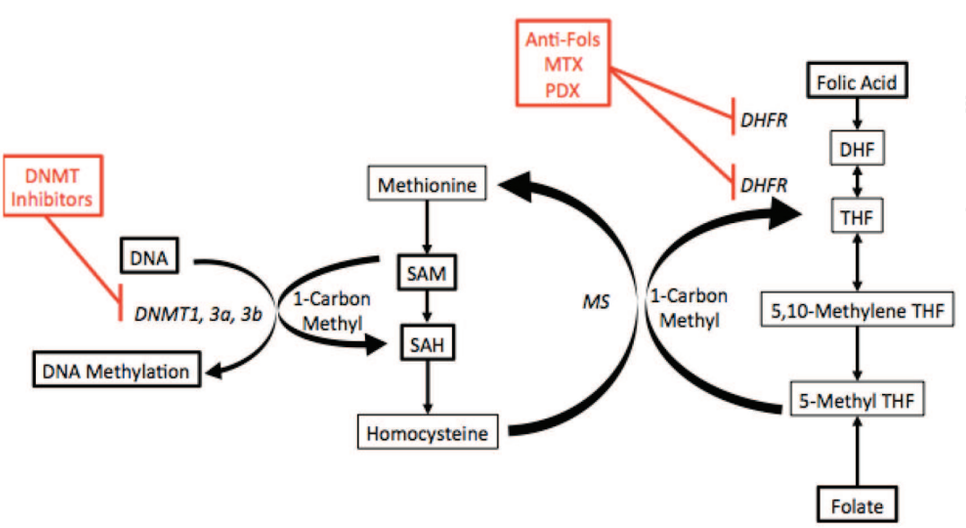

Figure 2.
Direct and indirect inhibition of DNA methylation. DNA methylation can be inhibited directly by inhibiting the DNMT enzymes with DNMT inhibitors azacitidine and decitabine. DNA methylation may be inhibited indirectly by decreasing the 1-carbon methyl donor pool. This can be accomplished via treatment with methotrexate or pralatrexate. Key: MTX, methotrexate; PDX, pralatrexate; DHF, dihydrofolate; THF, tetrahydrofolate; DHFR, dihydrofolate reductase; MS, methionine synthase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; DNMT, DNA methyltransferase.

understood, by inhibiting DHFR, the 1-carbon pathway may be disrupted. We have observed in our laboratory that treatment of TCL cell lines with pralatrexate does in fact lead to decreased SAM and increased SAH depleting the pool of 1-carbon methyl donors (unpublished data). These initial studies merit further investigation to validate whether this translates into modulation of DNA methylation.

Clinically, the combination of pralatrexate and romidepsin demonstrated an early signal of response for patient with TCL treated on a phase I clinical study [50]. Of the 14 patients with TCL that were evaluable for response, 10 (71%) achieved a response with a complete response rate achieved in 4/10 and a partial response in 6/10 patients. Of the B-cell lymphoma patients only those with follicular lymphoma responded, with 3/4 follicular lymphomas achieving a partial remission. This combination may be acting as a dual epigenetic therapy of sorts, with pralatrexate serving as the hypomethylating agent and romidepsin as the HDAC inhibitor. Thinking about anti-fols in this way, we may come to a new understanding of how to better utilize these old drugs (such as methotrexate) for new purposes.

5. Conclusion

As our understanding of the pathologic drivers of distinct subtypes of TCL grows, we are learning that epigenetic derangements play an important role in lymphomagenesis. Some such derangements include mutations in TET2, IDH2, DNMT3A, EP300, and CBP. Collectively these mutations contribute to a chromatin silenced and chemo-resistant state. In the 10 years since the approval of HDAC inhibitors for TCL, there has been a burst in the creation of novel agents and the repurposing of others to target such biology in TCL. Many of these agents are already being studied in the clinical setting and the clinical application of these agents in TCL is beginning to be realized. The next steps will involve finding the most safe and effective combinations that will best induce durable complete responses. These agents might be best utilized for discrete TCL subtypes, for example DNMT inhibitors for AITL and EZH2 inhibitors for ATLL. Furthermore, as we now have an understanding that epigenetics is crucial to the development of these lymphomas, we need to understand how this intersects with immune function and the microenvironment as well as the metabolic disposition of these malignant cells. Answering some of these questions will enable finding the right partners for these drugs whether it be PI3K inhibitors, PD1 inhibitors or BCL2 mimetics. As the field of targeted therapy for TCL grows, we now have the opportunity to supplant the ineffective chemotherapy based treatment for TCL.

Author details

Jacob Cogan and Jennifer E. Amengual*
Center for Lymphoid Malignancies, Columbia University Medical Center, New York, United States

*Address all correspondence to: jea2149@columbia.edu

References

[1] Moffitt AB, Dave SS. Clinical applicaations of the genomic landscape of aggressive non-Hodgkin lymphoma. Journal of Clinical Oncology. 2017;**35**(9):955-963. DOI: 10.1200/JCO.2016.71.7603

[2] Mak V, Hamm J, Chhanabhai M, Shenkier T, Klasa R, Sehn LH, et al. Survival of patients with peripheral T-cell lymphoma after first relapse or progression: Spectrum fo disease and rare long-term survivors. Journal of Clinical Oncology. 2013;**31**:1970-1976. DOI: 10.1200/JCO.2012.44.7524

[3] Gallipoli P. Seminars in Cancer Biology. 2017. DOI: 10.1016/j.semcancer.2017.07.005

[4] Whittaker S. Global patterns of methylation in Sezary syndrome provide insight into the role of epigenetics in cutaneous T-cell lymphoma. Journal of Investigative Dermatology. 2016;**136**:1753-1754. DOI: 10.1016/j.jid.2016.05.114

[5] Ma H, Abdul-Hay M. T-cell lymphomas, a challenging disease: Types, treatments, and future. International Journal of Clinical Oncology. 2017;**22**:18-51. DOI: 10.1007/s10147-016-1045-2

[6] Broccoli A, Zinzani PL. Review article: Peripheral T-cell lymphoma, not otherwise specified. Blood. 2017;**129**:1103-1112. DOI: 10.1182/blood-2016-08-692566

[7] Dupuis J, Morschhauser F, Ghesquieres H, Tilly H, Casanovas O, Thieblemont C, et al. Combination of romidepsin with cyclophosphamide, doxorubicin, vincristine, and prednisone in previously untreated patients with peripheral T-cell lymphoma: A non-radomised, phase 1b/2 study. Lancet Haematology. 2015;**2**(4):e160-e165. DOI: 10.1016/S2352-3026(15)00023-X

[8] Johnston PB, Cashen AF, Nikolinakos PG, Beaven AW, Barta SK, Bhat G, et al. Safe and effective treatment of patients with peripheral T-cell lymphoma with the novel HDAC inhibitor, belinostat, in combination with CHOP: Results of the Bel-CHOP phase 1 trial. Blood. 2015;**126**:253

[9] Pelligrini C, Dodero A, Chiappella A, Monaco F, Deg'Innocenti D, Salvi F, et al. Italian Lymphoma Foundation: A phase II study on the role of gemcitabine plus romidepsin (GEMRO regimen) in the treatment of relapsed/refractory peripheral T-cell lymphoma patients. Journal of Hematology and Oncology. 2016;**9**:38. DOI: 10.1186/s13045-016-0266-1

[10] Strati P, Chihara D, Oki Y, Fayad LE, Fowler N, Nastoupil L, et al. A phase I study of romidepsin and ifosfamide, carboplatin, etoposide for the treatment of patients with relapsed of refractory peripheral T-cell lymphoma. Haematologica. 2018;**103**(9):e416-e418. DOI: 10.3324/haematol.2018.187617

[11] Rozati S, Kim YH. Experimental treatment strategies in primary cutaneous T-cell lymphomas. Current Opinion Oncology. 2016;**28**(2):166-171. DOI: 10.1097/CCO.0000000000000272

[12] Marchi E, Zullo KM, Amengual JE, Kalac M, Bongero D, McIntosh CM, et al. The combination of hypomethylating agents and histone deacetylase inhibitors produce marked synergy in preclinical models of T-cell lymphoma. British Journal of Haematology. 2015;**171**:215-226. DOI: 10.1111/bjh.13566

[13] Scheifer AI, Vesely P, Hassler MR, Egger G, Kenner L. The role of AP-1

and epigenetics in ALCL. Frontiers in Bioscience. 2015;**7**:226-235

[14] Hassler MR, Pulverer W, Lakshminarasimhan R, Weinhaeusel A, Turner SD, Egger G. Insights into the pathogenesis of anaplastic large-cell lymphoma through genome-wide DNA methylation profiling. Cell Reports. 2016;**17**(2):596-608. DOI: 10.1016/j.celrep.2016.09.018

[15] Odejide O, Weigert O, Lane AA, Toscano D, Lunning MA, Kopp N, et al. A targeted mutational landscape of angioimmunoblastic T-cell lymphoma. Blood. 2014;**123**(9):1293-1296. DOI: 10.1182/blood-2013-10-531509

[16] van Doorn R, Slieker RC, Boonk SE, Zoutman WH, Goeman JJ, Bagot M, et al. Epigenetic analysis of Sezary syndrome define patterns of aberrant DNA methylation and identifies diagnostic markers. Journal of Investigative Dermatology. 2016;**136**:1876-1884. DOI: 10.1016/j.jid.2016.03.042

[17] Duvic M, Kim YH, LeBoeuf NR, Porcu P, Hastings J, Bassuner J, et al. A phase 2 randomized study of SHAPE gel (SHP-141) in patients with early-stage cutaneous T-cell lymphoma: Interim results. Journal of Clinical Oncology. 2016;**34**(15):7562. DOI: 10.1200/JCO.2016.34.15_suppl.7562

[18] Cyrenne BM, Lewis J, Weed J, Carlson K, Mirza FN, Foss F, et al. Synergy of BCL2 and histone deacetylase inhibition against leukemic cells from cutaneous T-cell lymphoma patients. Blood. 2017;**130**(19):2073-2083. DOI: 10.1182/blood-2017-06-792150

[19] Fujikawa D, Nakagawa S, Hori M, Kurokawa N, Soejima A, Nakano K, et al. Polycomb-dependent epigenetic landscape in adult T-cell lymphoma. Blood. 2016;**127**(14):1790-1802. DOI: 10.1182/blood-2015-08-662593

[20] Shah UA, Chung EY, Giricz O, Pradhan K, Kataoka K, Gordon-Mitchell S, et al. North American ATLL has a distinct mutational and transcriptional profile and responds to epigenetic therapies. Blood. 2018;**132**(14):1507-1518. DOI: 10.1182/blood-2018-01-824607

[21] Tse E, Kwong YL. The diagnosis and management of NK/T-cell lymphomas. Journal of Hematology Oncology. 2017;**10**(1):85. DOI: 10.1186/s13045-017-0452-9

[22] Zhang Y, Li C, Xue W, Zhang M, Li Z. Frequent mutations in natural killer/T-cell lymphoma. Cell Physiology and Biochemistry. 2018;**49**(1):1-16. DOI: 10.1159/000492835

[23] Roy DM, Walsh LA, Chan TA. Driver mutations of cancer epigenomes. Protein and Cell. 2014;**5**(4):265-296. DOI: 10.1007/s13238-014-0031-6

[24] Maruyama D, Tobinai K, Makita S, Ishida T, Kusumoto S, Ishitsuka K, et al. First-in-human study of the EZH1/2 dual inhibitor DS-3201b in patients with relapsed or refractory non-Hodgkin lymphomas–Preliminary results. Blood. 2017;**130**:4070

[25] Yamagishi M, Uchimaru K. Targeting EZH2 in cancer therapy. Current Opinion in Oncology. 2017;**29**:375-381. DOI: 10.1097/CCO.0000000000000390

[26] Daisuke S, Hiroo H, Hiroaki T, Imaizumi Y, Naoki U, Yoshitomo M, et al. EZH2 inhibitor DZNep induces apoptosis in adult T-cell leukemia/lymphoma cells by BCL2 suppression via regulation of Mir-181a. Blood. 2013;**122**:4265

[27] Yan J, Ng SB, Liang-Seah J, Lin B, Koh TL, Tan J, et al. EZH2 overexpression in natural killer/T-cell lymphoma confers growth advantage independently of histone

methyltransferase activity. Blood. 2013;**121**(22):4512-4520. DOI: 10.1182/blood-2012-08-450494

[28] Kumar A, Kumar V, Lightner JW, Little AL, Jutabha P, Endou H, et al. EZH2 inhibitor GSK126 for cancer treatment: Metabolism, drug transporter and rat pharmacokinetic studies. Medical Research Archives. 2015;**SI**(3). Available from: https://www.journals.ke-i.org/index.php/mra/article/view/273

[29] Lue JK, Prahbu SA, Liu Y, Verma A, Elemento O, Amengual JE. Dual inhibition of EZH2 and HDAC is synergistic in EZH2 dysregulated lymphomas. Hematological Oncology. 2017;**35**(S2):254-255

[30] Ji MM, Huang YH, Huang JY, Wang ZF, Fu D, Liu H, et al. Histone modified gene mutations in peripheral T-cell lymphoma not otherwise specified. Haematologica. 2018;**103**:679-687. DOI: 10.3324/haematol.2017/182444

[31] Kang-Decker N, Tong C, Boussouar F, Baker DJ, Xu W, Leontovich AA, et al. Loss of CBP causes T cell lymphomagenesis in synergy with p27Kip1 insufficiency. Cancer Cell. 2004;**5**(2):177-189

[32] Lasko LM, Jakob CG, Edaji RP, Qiu W, Montgomery D, Digiammarino EL, et al. Discovery of a potent catalytic p300-CBP inhibitor that targets lineage-specific tumors. Nature. 2017;**550**(7674):128-132. DOI: 10.1038/nature24028

[33] Liu Y, Fiorito J, Gonzalez Y, Zuccarello E, Calcagno E, Mazzeo A, et al. Development of First-in-Class Histone Acetyltransferase Activators for Precision Targeting of Epigenetic Derangements in Lymphoma. In: American Society of Hematology Annual Meeting: Oral Presentation; 2018. Available from: https://ash.confex.com/ash/2018/webprogram/Paper110447.html

[34] Stein EM, Garcia-Manero G, Rizzieri DA, Tibes R, Berdeja JG, Savona MR, et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. Blood. 2018;**131**(240):2661-2669. DOI: 10.1182/blood-2017-12-818948

[35] Kumar V, Kumar A, Lightner JW, Rice PJ, Nebout M, Peyron JF, et al. DOT1L inhibitor EPZ-5676: Synthesis, pharmacokinetic and tissue distribution studies in Sprague-Dawley rats. Medical Research Archives. 2015;**3**

[36] Delarue R, Dupuis J, Sujobert P, Barbieux S, Marcais A, Tournilhac O, et al. Treatment with hypomethylating agent 5-Azacytidine induces sustained response in angioimmunoblastic T-cell lymphomas. Blood. 2016;**128**:4164

[37] Falchi L, Lue JK, Amengual JE, Sawas A, Deng C, Marchi E, et al. A phase ½ study of oral 5-Azacitadine and Romidepsin in patients with lymphoid malignancies reveals promising activity in heavily pretreated peripheral T-cell lymphoma. Blood. 2017;**130**:1515

[38] Lemmonier F, Couronne L, Parrens M, Jais JP, Travert M, Lamant L, et al. Recurrent TET2 mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. Blood. 2012;**120**(7):1455-1459. DOI: 10.1182/blood-2012-02-408542

[39] Willemsen M, Schouten HC. Inappropriate costimulation and aberrant DNA methylation as therapeutic targets in angioimmunoblastic T-cell lymphoma. Biomarker Research. 2017;**5**:6. DOI: 10.1186/s40364-017-0085-8

[40] Rao A. Novel Therapeutic Strategies for Peripheral T-Cell Lymphoma by Targeting TET2. 2014. Available from: https://www.lls.org/content/

novel-therapeutic-strategies-for-peripheral-t-cell-lymphoma-by-targeting-tet2-and-rhoa [Accessed: October 25, 2018]

[41] Rodriguez-Cortes J, Palomero T. The curious origins of angioimmunoblastic T-cell lymphoma. Current Opinion in Hematology. 2016;**23**(4):434-443. DOI: 10.1097/MOH.0000000000000261

[42] Jagadeesh D, Smith MR. Novel targeted therapies in peripheral T cell lymphoma. Disoveries in Medicine. 2013;**15**(85):367-378

[43] Kamijo H, Sugaya M, Takahashi N, Oka T, Miyagaki T, Asano Y, et al. BET bromodomain inhibitor JQ1 decreases CD30 and CCR4 expression and proliferation of cutaneous T-cell lymphoma cell lines. Archives of Dermatology Research. 2017;**309**(6): 491-497. DOI: 10.1007/s00403-017-1749-9

[44] Boi M, Bonetti P, Ponzoni M, Tibiletti MG, Stahis A, Cvitkovic E, et al. The Brd-inhibitor OTX015 shows pre-clinical activity in anaplastic large T-cell lymphoma (ALCL). Blood. 2012;**120**:4872

[45] Kim SR, Lewis JM, Cyrenne BM, Monico PF, Mirza FN, Carlson KR, et al. BET inhibition in advanced cutaneous T cell lymphoma is synergistically potentiated by BCL2 inhibition or HDAC inhibition. Oncotarget. 2018;**9**(49):29193-29207. DOI: 10.18632/oncotarget.25670

[46] Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA menthylation: A review of molecular mechanisms and the evidence for folate's role. Advances in Nutrition. 2012;**3**(1):21-38. DOI: 10.3945/an.111.000992

[47] Wood GS, Wu J. Methotrexate and pralatrexate. Dermatologic Clinics. 2015;**33**(4):747-755. DOI: 10.1016/j.det.2015.05.009

[48] Wu J, Wood GS. Reduction of Fas/CD95 promoter methylation, upregulation of Fas protein, and enhancement of sensitivity to apoptosis in cutaneous T-cell lymphoma. Archives of Dermatological Research. 2011;**147**(4):443-449. DOI: 10.1001/archdematol.2010.376

[49] Izbicka E, Diaz A, Streeper R, Wick M, Campos D, Steffen R, et al. Distinct mechanistic activity profile of pralatrexate in comparison to other antifolates in in vitro and in vivo models of human cancers. Cancer Chemotherapy and Pharmacology. 2009;**64**(5):993-999. DOI: 10.1007/s00280-009-0954-4

[50] Amengual JE, Lichtenstein R, Lue J, Sawas A, Deng C, Lichtenstein E, et al. A phase 1 study of romidepsin and pralatrexate reveals marked activity in relapsed and refractory T-cell lymphoma. Blood. 2018;**131**(4):397-407. DOI: 10.1182/blood-2017-09-806737