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Review Article

Systematic review of epigenetic targets in acute myeloid leukemia

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Abstract: Acute myeloid leukemia (AML), although genetically and morphologically distinct from other B and T cell ALL subtypes, has one of the most rapidly progressing course and worse outcomes. The current diagnostic classification of AML offers best curative intent, the outcomes are not usually those that are expected at the start of therapy. This is partly attributed to the complex mechanism of leukemogenesis and resistance to chemotherapy. The underlying genetic mechanism of resistance is as complex as is the disease etiopathogenesis. Recent advances in therapy of drug resistant AML highlight the role of epigenetic targets. New FDA approved targeted therapy has also provided some evidence at improving outcomes in clinical trials. This review provides a detailed review of FDA approved targets and ongoing clinical trials for targeting CRISPER, CAR-T and other intestinal modalities for approach to epigenetictargets. However, this group of epigenetic targeted therapy needs more validation to prove its clinical efficacy. A systematic review of all published research on these targets, investigational agents and FDA approved targeted therapy summarizes this evidence. It also takes us through a brief review of mechanism of action and targets for therapy.

Keywords: Acute myeloid leukemia, targeted therapy, epigenetic targets, methylation, histone deacetylation, ubiquitination

Introduction

Acute myeloid leukemia (AML), a genetically dissimilar hematopoietic malignant condition is characterized by the accumulation of blast cells in bone marrow and blood with its genome having dysregulated epigenetic regulators and recurrent mutations [24].

DNA and histone proteins undergo various dynamic and reversible post-translational modifications known as epigenetic mutations. They play crucial role in various processes like opening, closing, remodeling of chromatin for euchromatin or heterochromatin state formation which is responsible for the gene regulation [25]. These modifications are divided into groups based on their action like phosphorylation, SUMOylation, methylation, acetylation, ubiquitination, and ADP-ribosylation. Epigenetic enzymes present add, read and remove substrate to specific target. Dysregulations in these modifications directly or indirectly lead to malignancy [27].

Cytotoxic agents were used as a traditional AML treatment. New molecular techniques like next generation sequencing (NGS) help in identification of genetic alterations and therefore has led to development of new drugs targeting these specific gene mutations. In the past few years the AML treatment has evolved rapidly, flow cytometery can be used for detection of minimal residual disease (MRD) along with NGS.

Leukemogenesis is complex mechanism and recent studies have paved path of many novel FDA approved agents and targeted therapies

like midostaurin and gilteritinib targeting activated FLT3 [46], venetoclax in along with azacitidine or dectabine targeting BCL2 and ivosidenib and enasidenib targeting mutated IDH1/2 [32].

Targeting therapies can be divided into following three groups: Group 1: agents that act on oncogenic effectors for AML associated mutations. Group 2: agents that cause disruption in normal metabolic activity without directly damaging DNA and repair. Group 3: agents that meant for some targeted cytotoxic agents.

In this article we did a systematic review of clinical trials and published literature on experimental human studies on epigenetic targets currently under evaluation for AML therapy (Lysine demethylase LSD1, the protein methyltransferase EZH2, DOT1L and PRMT5, and the BET bromodomain protein) and FDA approved IDH1/2 targets.

Methodology

Detailed search was carried out from Pubmed (1990-2020), EMBASE (1990-2020), Google Scholar (1990-2020), Cochrane Library, and Archives of Indian oncology journals (1990-2020), Latest data from PBCR (India), and GLOBOCAN databases, with the search terms (epigenetic target) and (acute myeloid leukemia) and (IDH1/IDH2/BET/DOT1L/PRMT5/EZH2/LSD1).

The search strategy that was followed was 'Epigenetic regulation in AML by IDH1/2, LSD1, PRMT5, EZH2, DOT1l and BET Bromodomain Protein (BRD protein)'.

Types of studies included were human experimental case studies, cohort studies, case control studies, study cases, systematic and literature review. Pre-clinical studies with trials in animals and trials not reporting patient-centred outcome, editorials, conference proceedings and books were excluded from the study.

Inclusion criteria

Adult and Patients diagnosed with AML (De novo, refractory, relapsed), case control and cohort studies which showed significant effect on patient survival and outcome variables as described by study, on either of epigenetic targets.

Exclusion criteria

Secondary and treatment induced AML were excluded as the etiopathogenesis is divergent from De Novo AML. Mixed cohorts, consisting of both solid cancer and haematologic malignancy, pre-clinical studies, conference proceedings, articles that were not valid statistically on effect of epigebnetic regulators were excluded on study.

Results and discussion

As we examined our search results the older studies were targeted towards preclinical/preliminary studies that could identify game changing or sensitive single targets in epigenetics or small molecules in the machinery, governed by epigenetics that could alter disease process or change the patient outcome drastically. As the understanding of epigenetics improved the studies started streamlining towards alternate strategies or approaches for adjuvant therapy that could leverage existing therapy in relapsed and resistant disease. This review includes FDA approved agents, their recent mechanism of actions and current trials approved and ongoing towards the target.

The results were independently reviewed by 2 reviewers, results of clinical trials and the mechanism of epigenetic regulation was summated and described as below.

Epigenetic modifications

Methylation

Transfer of methyl group to the target that like DNA or histone is known as methylation. Depending on state and position of protein, methyltransferase enzyme leads to changes in chromatin rearrangement causing either inhibition or activation of a gene [1]. DNA methyltransferase (DNMT3A), IDH1, IDH2, TET2 and Histone Methyltransferase (HMT) are genes involved in DNA and Histone methylation respectively [39]. Dysregulated HMT expression and activity is common leukemia characteristic. Also, HMTs are responsible for methylation of Arginine and lysine groups of histone residues. Arginine undergoes mono- and dimethylation and Lysine exists in all three methylated states because of arginine and lysine methyltransferases respectively [34] (**Figure 1**). Lysine methyltransferase (KMT) are divided into

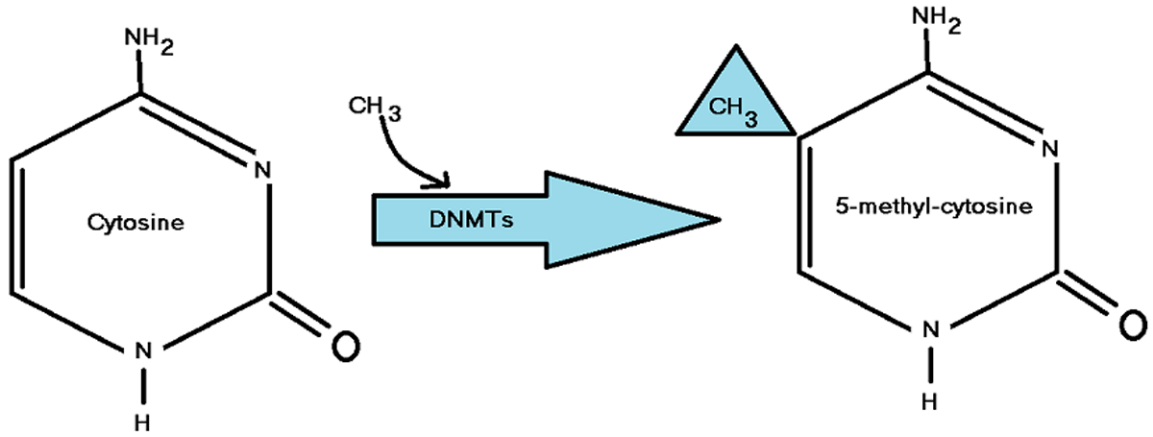

Figure 1. DNA Methylation.

two main groups based on the catalytic sites [2]. First group include EZH2 which contain evolutionary conserved catalytic Sure (car)3-9 Enhancer-of-Zeste and Trithorax (SET) domain [3-9]. The second group is doesn't contains SET domain. However, these enzymes have catalytic sites like DNMTs and PRMT1 which are responsible for methylation. Here S-adenosyl-L-methionine (SAM) is used as a cofactor.

IDH1/2

Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) causes catalysis of isocitrate to alpha ketoglutarate (αKG) while reducing NADP to NADPH. IDH enzyme family contains three proteins IDH1, IDH2 and IDH3 [10]. Out of these, IDH1 is found in cytoplasm and peroxisomes whereas IDH2 and IDH3 in mitochondria. Various cellular processes such as mitochondrial oxidative phosphorylation, glutamine metabolism, regulation of cellular redox status and glucose sensing are governed by these proteins [11]. IDH3 catalyses conversion of isocitrate to αKG in TCA cycle which is NAD+-dependent. IDH1 and IDH2 are similar enzymes they both form homodimers and catalyze the reversible oxidative decarboxylation of isocitrate to αKG which is NADP+-dependent. NADPH is a reducing agent which plays crucial role in detoxification process by reduction of glutathione, thioredoxin and activation of catalase. Hence, protecting against reactive oxygen species (ROS) and oxidative DNA damage [10, 12].

The point mutations in IDH1/2 genes can lead to neomorphic enzymatic activity, which is responsible for conversion of NADPH and αKG to NADP+ and D-2-hydroxyglutarate (D-2HG). IDH1 (R132) or IDH2 (R140 and R172) mutations are frequently observed in AML patients, R140 mutation being the most common of them all [11].

Increased histone and DNA methylation is often associated with increased D-2HG, It is seen to inhibit demethylase like JMJD2A, thereby modifying cancer cell differentiation and promoting proliferation via TET2 inhibition. High levels of 2HG accumulation is seen to results in differentiation blockage contributing to AML pathogenesis [52].

mutIDH inhibitors

The discovery of mutIDH1/2 gave rise to numerous novel therapeutic approaches, which helped to either restore or block IDH1/2 mutation or inhibit downstream effects of D2-HG (**Figure 2**). Some hypomethylating agents (HMAs) like Azacitidine and decitabine, are DNA methyltransferase (DNMT) inhibitors that have significant role in both AML and MDS. Treatment with HMAs has been seen to reduce cell proliferation and cause tumor repression in patients having IDH1 mutation these approaches are been evaluated in the phase trials (NCT02223052 and NCT02332889) [21].

AG-881, an IDH inhibitor developed by agios pharmaceuticals shows an inhibition of both IDH1/2 mutations. It binds to IDH in similar way at a different residue of different allosteric inhibitory regions. Since, it can easily pass-through blood brain barrier it makes it more promising candidate as an IDH inhibitor [53]. It acts faster when combined with IDH1 R132H

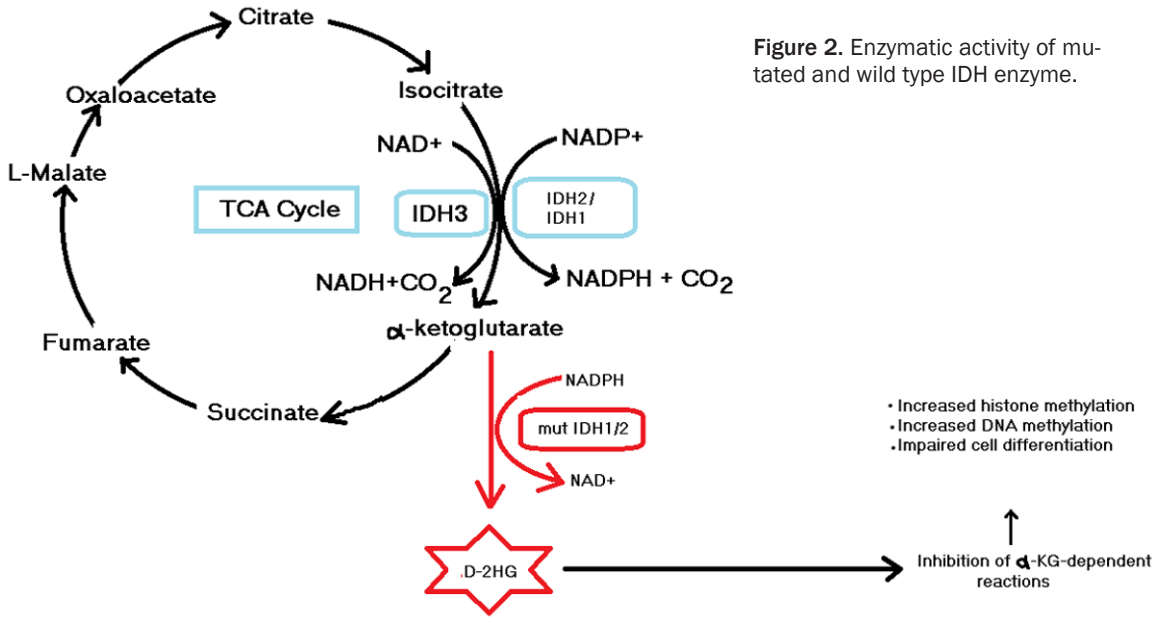

Figure 2. Enzymatic activity of mutated and wild type IDH enzyme.

and IDH2 R172K than IDH2 R140. A phase 1 clinical trial is presently going on for myeloid malignancies with mutated IDH1/2 (NCT02-492737) for evaluation of safety and efficacy [50].

AG-120 is a common oral and selective inhibitor for IDH1 mutations. It is seen to reduce intracellular 2-HG levels, inhibit growth factor independent proliferation and restore erythropoietin induced differentiation in IDH1 cells [16]. It is an FDA approved drug for adult R/R AML treatment with IDH1 mutations with the recommended dose usually being 500 mg orally once daily until the occurrence of unacceptable toxicity or HSCT but can be adjusted according to the patient's condition [13]. 1/2 phase clinical trial of ivosidenib monotherapy in IDH1 mutated R/R AML patient was responsible for its FDA approval (NCT02074839) and concluded that ivosidenib monotherapy was safe, effective and it could reduce or eliminate variant allele frequency (VAF) for effective remission in some patients [26].

IDH305 is another selective allosteric inhibitor of IDH1 mutation. Preclinical studies and phase 1 clinical trial of IDH305 for AML with IDH R132 is ongoing (NCT02381886). Initial results showed good clinical effect that it could reduce the level of 2-HG and induced differentiation of AML cells [12]. The adverse effect of this inhibitor includes raised level of bilirubin and lipase [14].

BAY1436032 is an allosteric inhibitor for all IDH1 mutation protein which is administered orally. It is can pass through blood-brain barrier [20] and it's monotherapy can reverse the abnormal histone methylation, decreased 2-HG level, self-renewal inhibition and LSCs proliferation (Chaturvedi A et al., 2017). When taken with Azacitidine, it also induces AML cell differentiation by EGR-GFI1-NFκB pathway regulation (Chaturvedi A. et al., 2017). Although, phase 1/2 clinical trials of BAY1436032 for the treatment of advanced AML has been completed, but the results of them hasn't been reported yet (NCT03127735) [26].

IFT-2102 is another mutIDH1 inhibitor with phase1/2 clinical trial presently going on for monotherapy and in combination with Azacitidine or cytarabine (NCT02719574). Results from phase 1 trial indicated safety and efficacy with adverse effects such as thrombocytopenia, febrile neutropenia, anemia, pneumonia, etc. with no deaths. Based on data obtained from phase 1 trials, phase 2 trials have started/ongoing with 150 mg administration dose, twice a day [45]. An additional trial is being undertaken with DNA methyltransferase (DNMT) inhibitor for the treatment of R/R AML with IDH R132 mutation [26].

Enasidenib (AG-221) is a selective oral allosteric inhibitor of IDH2 mutation. Although, it shows inhibitory effects for both mutIDH1 and 2 but it has more significant effects on IDH2

R172K than IDH1 R140Q mutation [19]. FDA has approved Enasidenib for treatment of IDH2 mutation [15] with 100 mg being the recommended dosage per day for at least 6 months or until intolerable adverse effects or disease progression [51]. It is able to reverse abnormal epigenetic changes, reduce more than 90% of 2-HG serum levels and can cause mutant AML cell differentiation [3]. Enasidenib monotherapy in Clinical trial (NCT-01915498) showed partial efficacy in adult R/R AML.

Olaparib is an immunosuppressive selective inhibitor of the poly (ADP-ribose) polymerase enzyme family (e.g., PARP-1, PARP-2, and PARP-3). When it binds to receptors, it creates breaks in DNA by inhibiting single [47] stranded excision repair leading to cell death in cells that is not able to repair double-stranded breaks accurately (e.g., tumours with BRCA1/2 mutation) [30].

(AGI-6780) is a selective allosteric inhibitor of IDH2 R140Q mutation, it targets abnormal methylation in histones and DNA and reduces 2-HG levels that are caused by the mutIDH2 R140Q [23] in primary AML cells, and further leads to the AML cell differentiation [44].

PRMT5

Arginine methylation leads to increased hydrophobicity and reduced hydrogen bond donor, it significantly alters protein-protein, protein-DNA and protein-RNA interactions. Hence, It regulates chromatin organization, transcriptional functions, RNA processing and DNA damage repair. In total, nine protein arginine methyltransferases have been identified and have been classified as type 1 and type 2 enzymes based on the asymmetric and symmetric dimethylarginine formation. PRMT5 is a type 2 enzyme, having multiple substrates like histones (H3, H4 and H2A) and also other non-chromatin proteins such as p53, p65 and HOXA9. Post-translational histone modifications by PRMT5 effects gene expression and induce abnormal cell growth and proliferation. PRMT5 Overexpression causes hematologic and solid malignancies. However, role of PRMT5 in AML has not been fully investigated but its dual function as suppressor and activator of genes like miR-29b and FLT-3 respectively is seen to contribute towards leukemia growth [41].

PRMT5 inhibitor

PRMT5 has a reversible, potent, specific inhibitor called GSK3326595. It is peptide competitive and SAM uncompetitive inhibitor, that inhibits induces cell death and inhibits proliferation. It also shows anti-tumor activity in hematologic tumor cell lines and acts by inhibiting cellular mRNA splicing and upregulating tumor suppressor genes which might lead to synthetic lethal phenotype in splicing mutant disease [22].

EZH2

Some proteins like EED, SUZ12 and RBBP4 along with EZH2 form a multi-subunit polycomb repressive complex (PRC)-2.SET domain methylation in EZH2 (H3K27me3) leads to transcriptional repression of target gene. A second polycomb complex PRC 1 is recruited by histone which maintains gene repression by ubiquitination of H2AK119. EZH2 hence, is responsible for regulation of cell fate decisions and coordination of gene expression to control the balance between self-renewal and differentiation (**Figure 3**). The gain and loss of function mutation of EZH2 is generally observed in lymphoid and myloid malignancies respectively and are often associated with poor prognosis. EZH2 inhibits differentiation in MLL-AF9 AML, thus mutation or deletion in EZH2 can leads toloss of LSCs and increased differentiation. Mutation in EZH2, ASXL1, splicing genes, U2AF and SRSF2 which cause dysfunctional processing of pre-mRNA can lead to Loss of function of EZH2 in AML.

In some aml patients, EZH2 phosphorylation by CDK1 can also decrease EZH2 protein expression. Disease relapse, multiple drug resistance and hox gene depression are frequently observed in this inactivation. EZH2 is more frequent mutation in RUNX1-mutated and secondary AML patients, it is also associated with increased HOXA9 expression as it occurs as a result of inactivation by EZH2 mutation or by deletion of entire EZH2 locus.

EZH2 inhibitors

DZNep (3-deazaadenosine) is a non-specific EZH2 inhibitor, it leads to the reduction of EZH2 protein expression level and H3K27me3 marks, thus exhibits anti-tumor effect in malignancies. Preclinical studies conducted shows efficacy but toxicity as well [29].

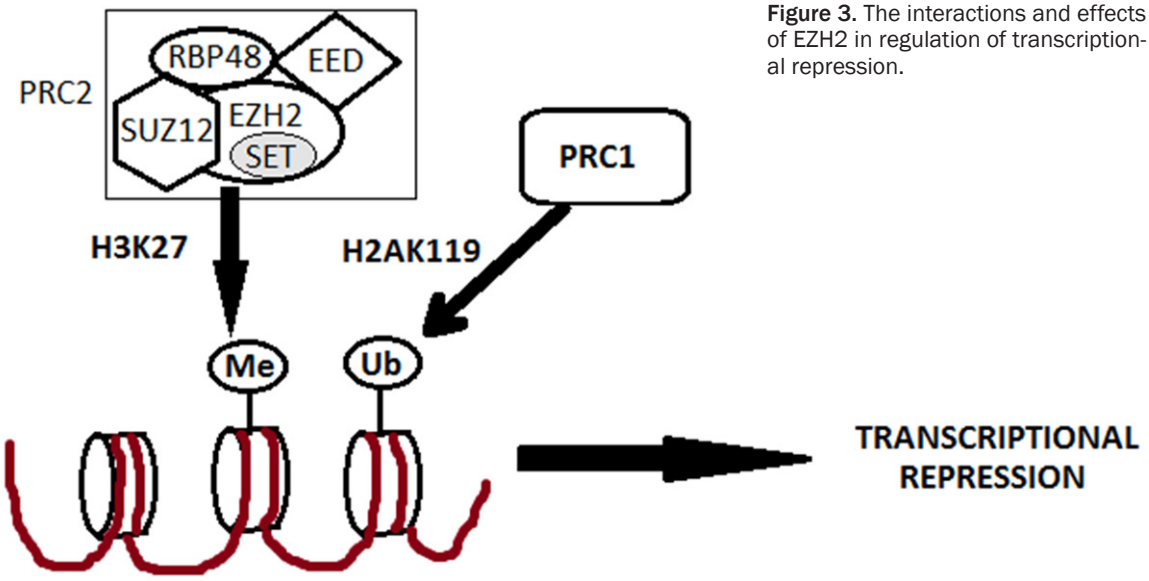

Figure 3. The interactions and effects of EZH2 in regulation of transcriptional repression.

GSK126 and EPZ005687 are small, potent, highly selective SAM competitive EZH2 inhibitor molecules. They effectively inhibit proliferation of mutEZH2 by binding to wild type or mutEZH2 which leads to decreased H3K27me3 expression levels and upregulated expression of silenced gene transcription.

Tazemetostat (EPZ6438), an oral EZH2 inhibitor reduces H3K27me3 levels and therefore reduces tumor growth in mutEZH2. Non-Hodgkins lymphoma is currently under evaluation in phase 1/2 clinical trials.

Recent studies suggests inhibiting the interaction between EZH2 and PCR2 complexes can serve as a specific and efficient approach to decrease toxicity. The stabilized aloha-helix in EZH2 (SAH-EZH2) selectively Inhibits H3K27me3 by disrupting EZH2-EED complex, thereby reducing EZH2 protein levels, leading to differentiation and growth arrest of MLL-AF9 leukemia cells (Kim et al.). Astemizole is another small molecule inhibitor that inhibits EZH2/EED interaction, It destabilizes PRC2 complex which leads to decreased proliferation of lymphoma cells [29].

Venetoclax is another oral inhibitor still in ongoing phase, 1/2 clinical trials (NCT0433-0820). It binds to BCL2, which releases pro-apoptotic proteins such as BIM and BAX, facilitating apoptosis of AML cells. Similarly, DS-3201b is another inhibitor which targets parts of EZH2 protein that shows oncogenicity. It is in its phase 1 clinical trial (NCT03110354). Bortezomib is another inhibitor that induces apoptosis in AML cells.

DOT1L

DOT1L is a gene involved in numerous crucial processes like transcriptional regulation, cell cycle progression and DNA damage repair. Hence, it is implicated in several cancers. Upregulation of DOT1L is observed in AML with mixed-lineage leukemia (MLL), Translocations in 5% of AML cases results in MLL-AF9 and MLL-ENL fusion gene. Out of these, MLL-AF9 occurs more frequently with higher incidence i.e. 70% in pediatric patient [49]. Crystallographic studies revealed SAM binding site (position 186) close to lysine substrate site and catalytic domain in C-terminal. The oncoprotein possesses the ability to directly bind MLL target gene via N-terminal sequences and to recruit additional proteins to bind MLL target gene via C-terminal sequences. DOT1L is the only protein known to mono-, di- and trimethylate H3K79 and this modification is responsible for its active transcription. H3 contains H3K79me in its globular domain which makes its methylation more influenced by other modifications like ubiquitination of H2BK120 and acetylation of H4K14 by histone deacetylase (HDAC) Rpd3 and by silent information regulator (SIR) complex [17] (**Figure 4**).

Hyper recruitment of DOT1L results in abnormally high H3K79 expression levels and meth-

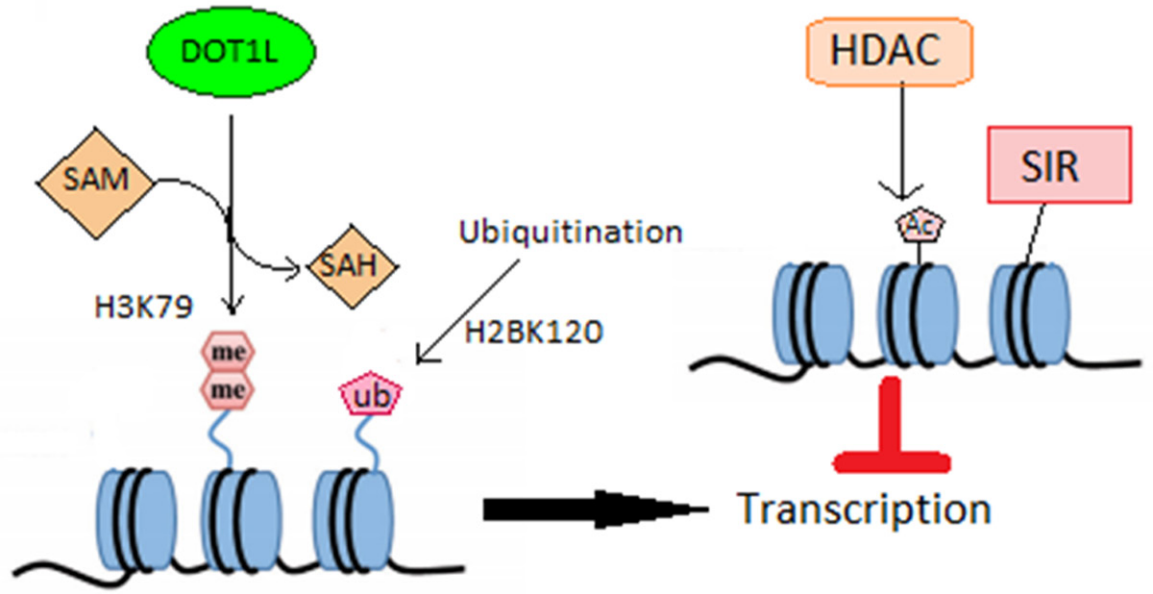

Figure 4. Mechanism of action of DOT1L and infuences of other epigenetic modifications.

ylation in promotor Gene and subsequently hyper expression of proteins like HOXA cluster and cofactor MEIS1 homeobox gene. HOXA causes segmentation and hematopoiesis and MEIS1 is involved in leukemia growth and is associated with oncogenic factors. Thus the dysregulation or hyperexpression of HOXA and MEIS1 lead to hematopoietic transformation and promote their oncogenic potential.

DOT1L inhibitors

Pinometostat (EPZ-5676) is a selective DOT1L inhibitor. Preclinical studies shows, single agent antiproliferative drug activity for MLL-r leukemia (Daigle et al., 2013). It also acts in a synergistic manner when administered along standard of care (SOC) AML chemotherapeutic agents like Ara-C and daunorubicin and DNMT inhibitors like azacitidine and dectabine. This synergy can be seen only when coadministered to cells [17]. Some preclinical studies also suggest that NOM1 mutated leukemogenesis depends upon HOX and MEIS1 expression levels, DOT1L inhibits/blocks NPM1 mutated leukemia and causes differentiation [18].

Demethylase

LSD1: The largest family jumonji C (JMJCs) can acts as tri-, di- and mono-methylated lysine depending on presence of alpha ketoglutarate and Fe2+. Lysine-specific demethylase (LSD) is a smaller family which contains only two members, LSD 1 and 2. LSD 1 is a flavin adenine dinucleotide (FAD) dependent amine oxidase (AO) demethylase (**Figure 5**). It is responsible for demethylation into mono-, di-methyl groups on H3K4 and H3K9 and other non-histone protein targets like DNMT1, p53 etc. [17].

It has three structural domains that regulate the enzyme etiquette and binding activities with several proteins. N terminal of enzyme contains a SWIRM domain and the C terminal contains FAD-dependent AO domain which surrounds the tower domain. AO domain has a catalytic region which consist of two lobes, the first one has the FAD-binding site and substrate recognition site while the other one is at close proximity to SWIRM domain that form a hydrophobic groove, it allows accommodation of larger portion of H3 tail. The tower domain is a site for RCOR1 binding (a member of CoREST transcriptional repressor complex) [18].

LSD 1 acts as a transcriptional Co-repressor by demethylating H3K4 which is a mark of active transcription. It binds with different proteins like CoREST transcriptional repressor complex, Mi-2/nucleosome remodeling and deacetylase (NuRD) complex to promote demethylation. LSD1/NuRD complex targets are involved in cell signaling pathways, regulating cell proliferation and survival. It is also the regulator of haematopoiesis and leukemogenesis. Thus it

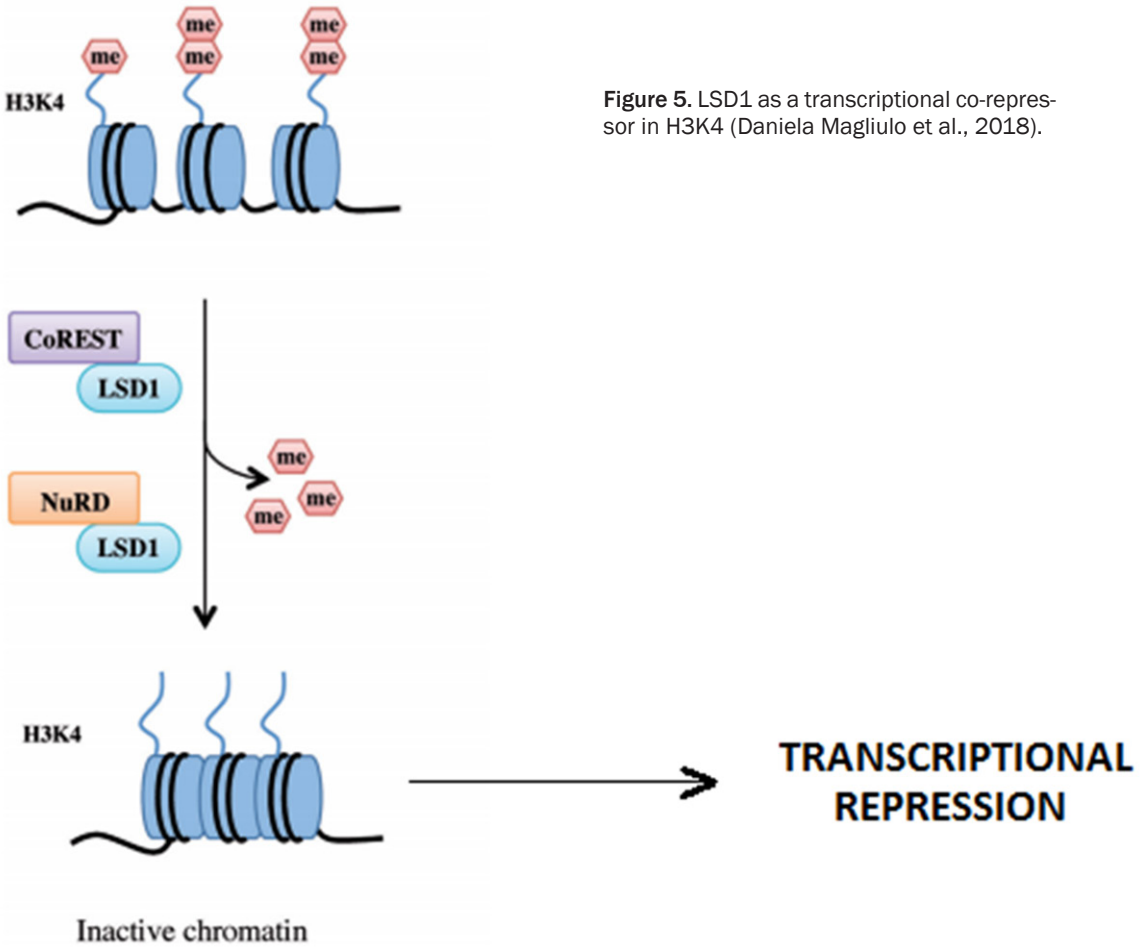

Figure 5. LSD1 as a transcriptional co-repressor in H3K4 (Daniela Magliulo et al., 2018).

plays a crucial role in differentiation and maturation of hematopoietic stem cells. By blocking differentiation and apoptosis, MLL-rearranged AML LSD1 maintains leukemic stem cell potential [31]. LSD1 although not found mutated in AML but is highly expressed in leukemic blast in 60% of AML patients [17].

LSD1 inhibitors: Lysine-specific demethylase 1 (LSD1) is a histone demethylase expressed in leukemic cells and is responsible for regulation of differentiation blockage in AML [23, 51]. Drugs like tranylcypromine (TCP) alone or in combination ATRA [43] can be used to target the enzyme and is currently in phase 1 and phase 1/2 for adults with R/R AML or MDS (NCT02273102, NCT02261779, NCT02-717884). Also, Selective TCP derivative LSD1 inhibitors, (GSK2879552 and ORY-1001), have passed many early-phase trials for patients with R/R acute leukemia (NCT02177812, EudraCT number 2013-002447-29). An open-label phase 1 trial of IMG-7289 with or without ATRA is also initiated (NCT02842827). Although, most of LSD1 inhibitors were designed by modifying structure of tranylcypromine (TCP) [48].

Tranylcypromine (TCP), an irreversible and weak LSD1 inhibitor targets monoamino oxidase (MAO). Many studies are registered in clinical trial for evaluation of therapeutic efficiency in AML and MDS are ongoing for LSD 1 inhibitor. Presently, A phase 1/2 study is going on for evaluation of pharmacodynamics, safety, feasibility, and effectivity of ATRA/TCP along with probability of relapsing AML for patients that aren't eligible for intensive treatment (NCT02261779). Also, another phase 1 study is ongoing for adult AML patients and high grade MDS to find tolerability and safety of TCP/ATRA drug combination (NCT02273-102) [19].

Till date, ORY-1001 (iadademstat) is the most potent and selective LSD1 inhibitor. In AML cells having MLL translocations, it is capable of inducing cell differentiation and reduction in

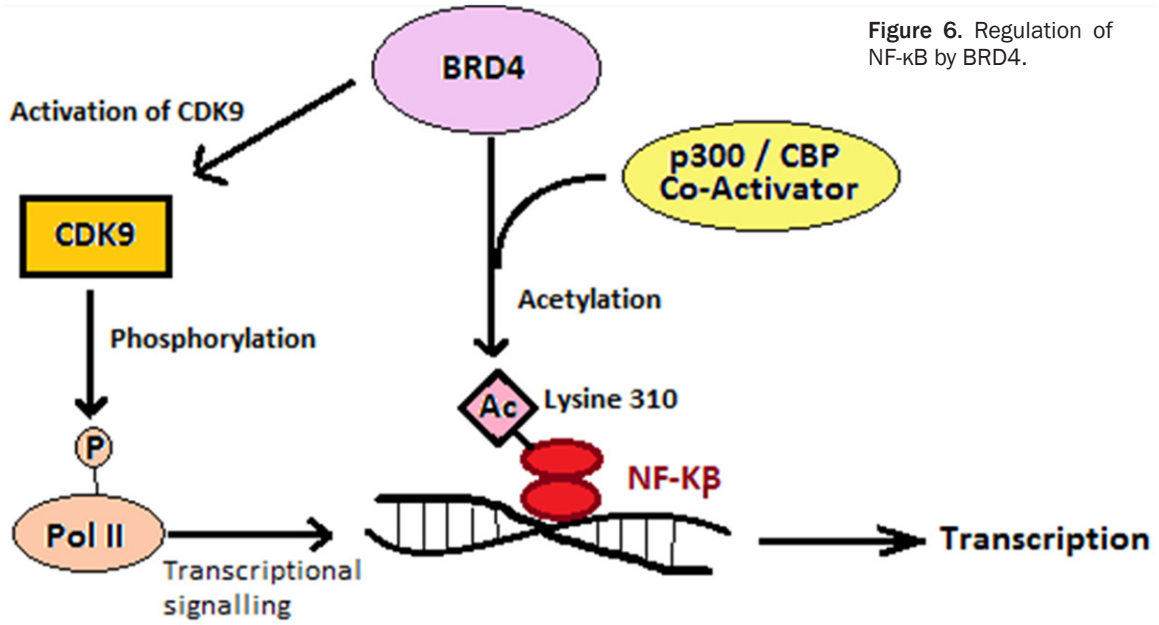

Figure 6. Regulation of NF-κB by BRD4.

cell growth and clonogenicity. It is seen to have synergistic interactions with usual standard of care drugs like ATRA, Ara-C, selective epigenetic and target inhibitors.

Two clinical phase 1 trials were terminated which were designated for investigation of GSK2879552 for its safety, pharmacodynamics and clinical activity with relapsed AML (NCT02034123 and NCT02177812) but although it showed remarkable proliferative activity, it also had risk benefit in relapsed refractory AML, hence the study was discontinued.

IMG7289 (Bomedemstat) is another potent, selective, orally available small molecule inhibitor, which is under investigation as single agent or in combination therapy with ATRA in AML and MDS patients (NCT02842827). The compound has shown efficacy by myeloid differentiation induction in MLL-r AMLs and gain in transcription factor accessibility.

INCB059872 (Incyte) and T3775440 (Takeda) are two untested irreversible LSD1 inhibitors. INCB059872 a potent and selective inhibitor can be used to induce differentiation and delay cellular proliferation in AML cell lines. T3775440 is seen to show selectivity against mega karyocytic and erythroid cell lines cell lines and for granulocytic cells, It is seen to promote growth inhibition and transdifferentiation.

Although, all of these irreversible LSD1 inhibitors have long lasting effect on target but if the target is non-specific, they can also show long lasting off target effects [19]. Hence, the reversible non-covalent inhibitors have a safer metabolic profile with high potential for antileukemic properties and low nonmolar potency like SP2509 (Salarius Pharmaceuticals). Without induction of unrelated toxicity, it promotes cell differentiation and apoptosis and inhibits cellular proliferation. SP2509 is lethal against AML with MLL translocations and NMP1 mutation [36].

Epigenetic reader

BRD4/BET protein: BRD4 is a member of bromodomain and extra terminal motif (BET) protein family [40], it acts as histone acetyltransferase and leads to nucleosome destabilization and clearance by acetylating H3K122 [28]. Increase of gene transcription and chromatin remodeling can be observed when BRD4 is upregulated (**Figure 6**). Other than these it is also seen to be involved in many crucial processes like DNA damage checkpoint signaling, DNA repair and regulating expression of several regulators of cell cycle [37]. MYC when accumulates into promoter regions is usually associated with transcriptional activation and also regulates about 60 to 70% of all cancers overexpression of BET protein. Nucleophosmin (NPM1) is another pro-

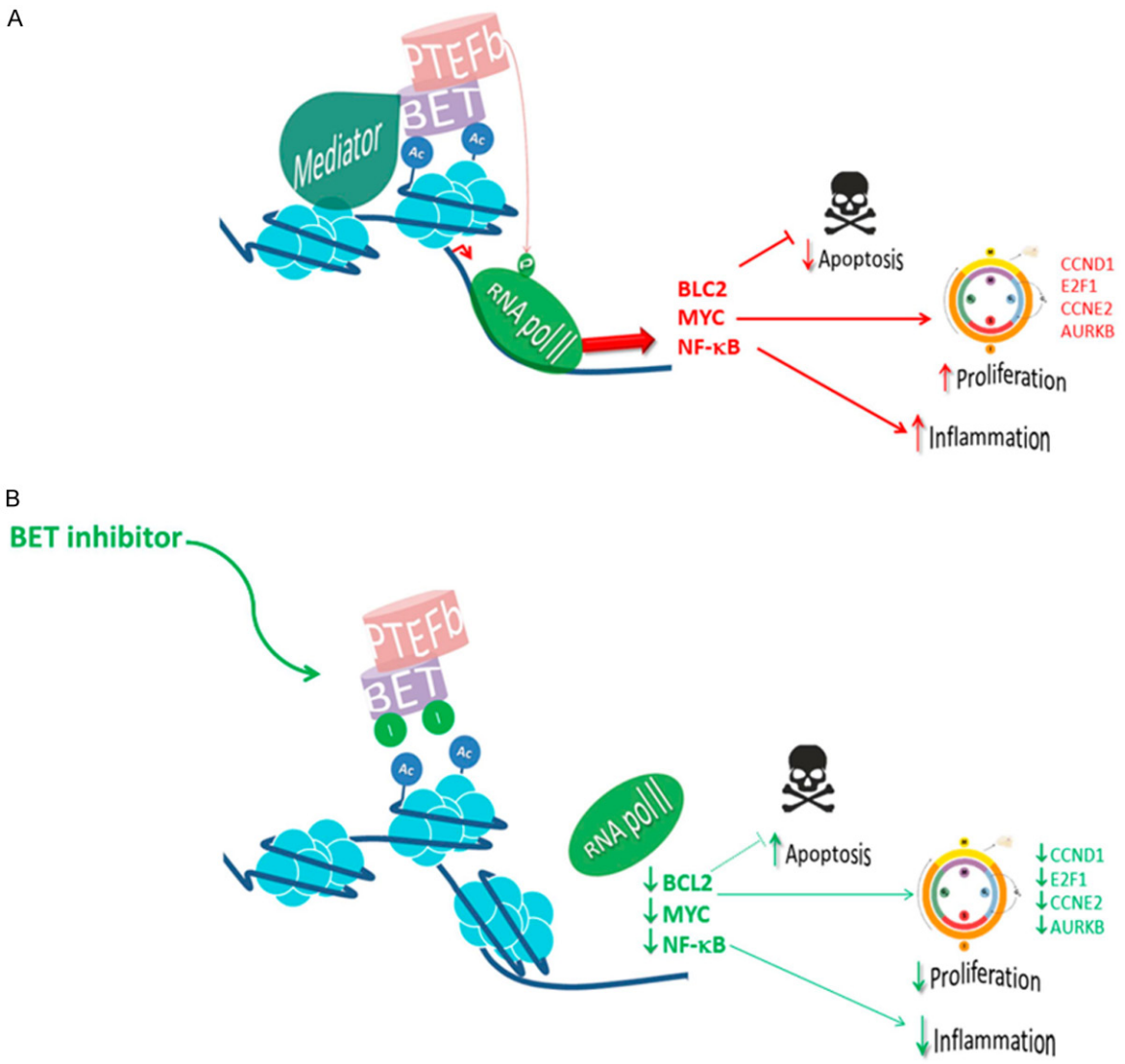

Figure 7. Mechanism of action of BET inhibitor. A. BET protein regulates transcription of gene related to various functions as shown. The BET family member, BRD4 plays important role in oncogenic expression of MYC in malignancies. B. BET inhibitors release BRDs from chromatin, reduce RNA-Pol II blocking transcription of downstream gene. (Diana Reyes-Garau et al., 2019).

tein that regulates BRD proteins in about 35% of AML cases. Transcriptional repression of BRD4 is associated with loss of function mutation in NPM1 which leads to increased expression of BCL2 and MYC. Children are the most common AML patients (22% cases) in which Chromosomal translocation involving lysine methyltransferase 2A (KMT2A) is observed. Different transcriptional elongation regulators like super elongation complex (SEC) fuses with KMT2A after translocation which results in regulated transcription further leading to leukemogenesis. BRD3, BRD4 and SEC components play crucial roles in KMT2A localization and transcription [35].

BET inhibitors: Many experimental studies have been conducted for BET inhibitors having BRD affinity for therapeutic approaches for various malignancies (**Figure 7**). BRD2 and BRD4 acetyl-lysine binding motifs has a competitive interactor, Thieno-triazolo-1,4-diazepine (JQ1), in almost all cell lines is able to inhibit 50% of the cell proliferation. By promotion of BRD2/4 displacement from MYC promoter region, it downregulates MYC expression in leukemic cell lines, because of its high

potential many structurally similar JQ1 BET inhibitors were developed [37].

Remarkable anti-tumor activity was shown by CPI203 and (Benzoisoxazoloazepine) that correlated to BET-driven reduction in MYC expression. In phase 1/2 of clinical study, PI0610 was recruited (NT02158858). Good capacity to bind reversibly with BET proteins and BRDs was also shown by two inhibitors GS-5829 and GS-626510 that prevents their recruitment for acetylated histones.

Binding of BRDs with acetylated histone H4 can also be inhibited by a compound called Thienotriazolodiazepine compound MK-8628/OTX015. In AML and ALL cell lines It promotes cell cycle arrest, upregulation of Pol-II negative regulation, HEXIM1 and downregulation of MYC, BRD2, 4 levels. Diarrhea and hyperbilirubinemia are some of the adverse side effects observed in OTX015 treatment [33].

By impairing their clonogenic growth, inducing apoptosis and downregulation of MYC expression compound like dihydroquinazolinone PFI-1 (PF-6405761) exhibits activity against MLL rearranged leukemic cell lines. Similar to PFI-1, in order to prevent interaction with acetylated histone tails benzodiazepine I-BET762 binds to BRD2/4 acetyl-lysine binding domain [33].

In AML and various tumor derived cell lines ABBV-075 a BRD2/4-selective BET inhibitor is able to trigger apoptosis and anti-proliferative activity. After evaluation of INCB054329 a novel BET inhibitor in both preclinical and phase 1 clinical trials/(NCT02431260), it was demonstrated that hematological malignancies like AML, MMs etc. Is the source of anti-proliferative effect in over 32 cell lines. Displacement of BRD4 from MYC drives these anti-proliferative and pro-apoptotic effects that at leads to increased myeloma cell sensitivity to JAK inhibitors and reduction of corresponding mRNA levels [42]. Hence, responsible for therapeutic benefits.

Conclusion

Epigenetic regulators play a very significant role in maintenance and initiation of various hematopoietic malignancies. Countless opportunities for development of targeted therapies lie in observations of these regulations. Influencing gene expression and ability to correct dysregulated transcriptional errors of AML are some common benefits to all epigenetic modifying drugs. Optimum strategy for leukemia can be determined with small molecule inhibitors targeting epigenetic regulators, conventional chemotherapeutic drugs and for clinical trial evaluation for single agent activity or in combination with other novel therapies or with standard of care chemotherapies for mutIDH1/2 therapy, some drugs like ivosidenib and enasidenib are already approved by FDA. While others such as Pinometostat are being investigated for different phase for pediatric and adult patients with AML harboring MLL-r for other mutated epigenetic regulators. Unique role of “oncometabolite” D-2HG in cancer was Highlighted after discovery of IDH1/2 mutation. On other hand, dysregulation and blockade of other regulators such as PRMT5, LSD1 are some areas where knowledge is still limited.

In conclusion, the findings of this review in present times reflect ongoing and additional pathways that are being explored for improving patient outcomes in relapsed and resistant diseases. It is summation of ongoing clinical trials and experimental research with interdisciplinary novel approaches towards realization of targets by-CRISPER/CAS, Artificial intelligence, Bio-informatic tools, big data analytics and other parallel technologies. A comprehensive approach rather than one targeted towards single agent is likely to succeed.

Results of ongoing and upcoming clinical studies and trials, future classification of these regulators based on molecular function in leukemia can provide a clear understanding of epigenetic regulatory mechanism of AML and hence improve treatment of patients. The interdependence of immunological responses and cancer epigenetic is also under evaluation. Epigenetic therapies in tumor cells induce some cellular responses that can contribute in immune efficiency by interacting with immune system [38]. Thus, for development of additional therapeutic options for AML combination of epigenetic and immunotherapies hold huge potential.

Disclosure of conflict of interest

None.

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