 doi:10.20944/preprints202206.0046.v1

Review

Epigenetic Modifications and Targeted Therapy in Pediatric Acute Myeloid Leukemia

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Abstract: Acute myeloid leukemia (AML) is a hematological malignancy that is the culmination of genetic and epigenetic alterations in the hematopoietic progenitor cells, leading to uncontrolled proliferation at the expense of normal hematopoiesis and bone marrow exhaustion. Although the outcomes for pediatric AML have improved in recent decades, at least one third of children still have relapses. Recent studies have notably highlighted the important role of dysregulated epi-genetic mechanisms in myeloid leukemogenesis. Epigenetic modifications are frequently reversible compared to genetic alterations, thus providing opportunities for targeted epigenetic therapy. In this review, we summarize the landscape of epigenetic alterations and the progress to date in epigenetic targeted therapy, and focus on the future role of epigenetic abnormalities in predicting relapse and the precision therapy in pediatric AML.

Keywords: Acute myeloid leukemia; Pediatric; Epigenetics; DNA methylation; Histone modification; Non-coding RNAs; Therapy

1. Introduction

Acute myeloid leukemia (AML) is a hematological malignancy that is the culmination of genetic and epigenetic abnormalities in the hematopoietic stem/progenitor cells, causing dysregulation of critical signal transduction pathways and the expansion of undifferentiated myeloid cells [1]. Pediatric AML accounts for about 25% of children leukemias. One third of children have relapses and relapsed/refractory AML accounts for half of childhood leukemia-related deaths, although considerable improvements in overall survival (OS) have been achieved over the decades, mainly due to intensified treatment strategy, enhancements in supportive care and progress in risk-adapted patient stratification [2]. Consequently, there is an urgent need for developing more specific and less toxic drugs to improve the prognosis of children with AML.

In recent decades, rapid advances in sequencing technologies have led to tremendous progress in defining the molecular pathogenesis of AML, revealing enormous genetic and epigenetic alterations and paving the way for precision medicine approaches. The term "epigenetics" refers to the changes in gene expression that are inheritable through cell division rather than caused by changes in the DNA sequence itself [3]. Epigenetic modifications include DNA methylation, histone modification, and RNA-associated silencing, which contribute to initiate and sustain epigenetic silencing [4]. Recent studies have shed light on the important role of dysregulated epigenetic mechanisms in the pathogenesis of AML, and some recurrent somatic alterations have been proved to play an important role in epigenetic regulation [5]. Epigenetic modifications are frequently reversible, thus offering potential avenues for epigenetic targeted therapy using specific inhibitors [6]. Epigenetic therapy has become a promising strategy with many novel inhibitors being applied in adult AML [7]. Therefore, it is timely to consider the important role of epigenetic

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 June 2022 doi:10.20944/preprints202206.0046.v1

alterations and the potential targeted therapy in pediatric AML, so as to promote the development of precision therapy and improve the outcomes of children with AML.

2. Epigenetic Regulation and Dysregulation in Adult and Pediatric AML

The transition from hematopoietic stem cells (HSCs) to lineage differentiation and maturation follows a distinct hierarchy, which is tightly controlled at the level of transcriptional regulation. And the transition is regulated by multiple epigenetic mechanisms, including DNA methylation, histone modifications and non-coding RNA interactions. Epigenetic dysfunction is common to most cancers, and mechanisms of epigenetic dysregulation have been widely studied in AML, though AML has few mutations compared to other cancer types. The current advances of epigenetic modifications in AML are briefly summarized below (Table 1).

Table 1. Recurrently mutated or translocated genes in epigenetic modification in adult and pediatric AML.

Gene	Epigenetic funcion	Type of mutation	Frequency of AML	Prognostic role
DNMT3A	De novo DNA methylation	Missense, nonsense, and frameshift, 60% heterozygote mutation at R882 residue	20-22% of adult AML; 1-2% of pediatric AML	Adverse prognosis, especially in intermediate-risk AML and with FLT3-ITD
TET2	Conversion of 5-methylcytosine to 5-hydroxymethylcytosine	Missense, nonsense, and frameshift mutations	8-23% of adult AML; 1.7% of pediatric AML	Uncertain
IDH1/IDH2	Conversion isocitrate to α-ketoglutarate (α-KG), a cofactor for TET2	Heterozygous mutations, primarily missense mutations affecting arginine residues	5-33% of adult AML; 1-4% of pediatric AML	Uncertain
CREBBP	Histone lysine acetyltransferase	Rearrangements (fusion genes)	Rare	Uncertain
KAT6A	Histone lysine acetyltransferase	Rearrangements (fusion genes)	Rare	Uncertain
EP300	Histone lysine acetyltransferase	Rearrangements (fusion genes)	Rare	Uncertain
HDAC2/HDAC3	Histone deacetylase	Missense mutations	Rare	Uncertain
KDM5A	Histone lysine demethylase	Rearrangement involving NUP98	10% of pediatric acute megakaryoblastic leukemia	Uncertain
KDM6A	Histone lysine demethylase	Missense mutations	Rare	Uncertain
KMT2A	H3K4 methyltransferase	Gene fusion with > 70 fusion partners, partial tandem duplications	Fusion: 1-10%; Tandem duplications: 4-7%	Adverse prognosis
EZH2	H3K27 methyltransferase, enzymatic component of PRC[1]2	Missense, nonsense, and frameshift loss-of-function mutations	1-5% of adult AML; Rare in pediatric AML	Uncertain
NSD1	H3K36 methyltransferase	Rearrangement involving NUP98	2-5%	Uncertain
ASXL1	Recruitment of PRC2 to target loci	Missense, nonsense, and frameshift loss-of-function mutations	3-17% of adult AML; 1-9% of pediatric AML	Adverse prognosis, especially in intermediate- and low-risk AML

 doi:10.20944/preprints202206.0046.v1

ASXL2	Homolog of ASXL1; function unknown	Mutations	23% of AML with RUNX1-RUNX1T1	Uncertain
SUZ12	Member of PRC2	Missense mutations, insertions and deletions	unknown	Uncertain
JARID2	Recruitment of PRC2 to target loci	Deletion in transformation of MDS[2]/MPN[3] to AML	unknown	Uncertain

[1]PRC: polycomb repressor complex; [2]MDS, myelodysplastic syndrome; [3]MPN, myeloproliferative neoplasm.

DNA methylation

DNA methylation is by far the most well-characterized epigenetic modification, and methylation of the C5 position of cytosine residues in DNA to form 5-methylcytosine (5-mc) have been recognized as an epigenetic silencing mechanism [8]. Abnormal patterns of DNA methylation in malignancies were originally investigated specifically in the context of so-called "CpG islands" in gene promoters. Most CpGs are methylated (70-80%), apart from the CG-dense regions termed CpG islands (CGIs) [9]. The methylation of CpG sites within the human genome is maintained by several DNA methyltransferases (DNMTs) and demethylases. De novo methylation is mediated by DNMT3A and DNMT3B, while demethylation is carried out by the ten-eleven translocation (TET) family of demethylases (TET1, TET2 and TET3) [10].

Hematopoietic stem cell differentiation requires widespread epigenome remodeling, and DNA methylation patterns are strongly related with specific cell types throughout hematopoiesis [11]. Abnormal methylation patterns are the hallmark of AML, and several studies have implicated both hypermethylation and hypomethylation in malignant transformation [12-14]. DNMT3A is one of the most frequently mutated genes in adult AML [15], which have been found in pediatric AML at lower frequencies (20%-22% vs. 1%-2%, respectively) [16]. DNMT3A mutations appear to be early events in leukemogenesis and are predominately heterozygous R882H in AML [17]. Some studies demonstrated that the mutation confers increased self-renewal, impaired differentiation and a repopulation advantage over wild-type hematopoietic stem cells (HSCs) [18, 19]. Meanwhile, DNMT3A mutations play an important role in resistance to chemotherapy, giving rise to a population of cells primed for relapse [20]. Although the mechanism by which mutant DNMT3A contributes to leukemic transformation have not been completely understood, targeting DNMT3A mutations could be a highly attractive treatment approach, particularly as this abnormality confers a poor outcome [21].

TET2 mutations are anther pathway to abnormal DNA methylation. The oxidation of 5-methylcytosine (5-mc) yields 5-hydroxymethylcytosine (5-hmc), which is catalyzed by TET2, resulting in DNA demethylation and reversal of methylation-driven gene silencing. Mutations in TET2 are observed in 8%-23% of patients with AML, but these mutations are rare in pediatric AML(Table1) [16]. TET2 mutations are associated with reduced levels of 5-hmc, which confer a poor prognosis in intermediate-risk AML [22, 23]. Li et al. found that TET2 knockout HSCs undergo expansion in vivo and outcompete wild-type HSCs in serial transplantation assays [24]. Besides, Rasmussen et al. demonstrated that loss of TET2 caused DNA hypermethylation at active enhancers, which was associated with downregulation of tumor suppressor genes (e.g., MTSS1, LAS2, LXN and CTDSP1) and upregulation of oncogenes, such as NOTCH3 and IGFLR [25]. Therefore, these studies suggest that the loss of TET2 leads to aberrant DNA methylation, increased HSC self-renewal and impaired differentiation, finally contributing to leukemogenesis. Subsequently, Shih et al. revealed that FLT3-ITD mutations and TET2 defect synergistically remodeled DNA methylation and gene expression to an extent not observed with either mutant allele alone, including at the Gata2 locus. Then they found that re-expression of Gata2 induced differentiation in AML stem cells and attenuated leukemogenesis. Consequently, they concluded that TET2 and FLT3-ITD mutations cooperatively induce AML

 doi:10.20944/preprints202206.0046.v1

with defined leukemia stem cell population characterized by site-specific changes in DNA methylation and gene expression [26].

Isocitrate dehydrogenase 1/2 (IDH1/2) catalyze the conversion of isocitrate to α-ketoglutarate (α-KG), which a co-factor required by TET2 for conversion of 5-mc to 5-hmc and subsequent DNA demethylation [27]. Mutations in IDH1/2 are frequently observed in adult AML (5-33%), less observed in pediatric AML (1-4%) [16, 28, 29]. Mutations in IDH1/2 result in loss and gain of function. And cells harboring these mutations can't catalyze α-KG and, instead, synthesize the tumor metabolite 2-hydroxyglutarate (2-HG) in a neomorphic form that leads to aberrant DNA methylation [30]. Mutational epigenetic profiling of a large AML patient cohort study revealed that IDH1/2-mutant AMLs display global DNA hypermethylation and a specific hypermethylation signature, particularly at promoter regions [31]. Figueroa et al. found that IDH1/2 mutations were mutually exclusive with mutations in the α-KG-dependent enzyme TET2, and TET2 loss-of-function mutations were associated with similar epigenetic defect as IDH1/2 mutants. Furthermore, either expression of IDH1/2 mutations or TET2 depletions impaired hematopoietic differentiation and increased stem/progenitor cell marker expression, leading to a shared leukemogenic effect [31]. Rampal et al. observed that WT1 mutant AML patients have reduced 5-hmc levels similar to TET2 or IDH1/2 mutant AML and the overexpression of WT1 increased global levels of 5-hmc, whereas 5-hmc levels were reduced when WT1 was silenced [32]. They also demonstrated that WT1 physically interacts with TET2 and TET3, and loss-of-function WT1 caused a similar hematopoietic differentiation phenotype as observed with TET2 defects [32]. Although the mechanism of 5-hmc decrease upon WT1 silencing or mutation is not completely understood, the TET2, IDH1/2, and WT1 mutations define a novel AML subtype defined by dysregulated DNA hydroxymethylation.

Histone acetylation

Histone acetylation involves the transfer of acetyl groups to lysine residues in histone proteins, which is regulated by histone lysine acetyltransferases (KATs) and histone deacetylases (HDACs) and is associated with gene transcription, chromatin structure, and DNA repair [33]. Acetylation of lysine residues results in open chromatin confirmations and gene activation, whereas deacetylation leads to condensed and closed chromatin, causing gene inactivation [34]. KAT rearrangements, rather than mutations, have been identified in AML at exceptionally low frequencies [14]. Although mutations in HDACs are also extremely rare in AML, HDACs can be aberrantly recruited by myeloid oncoproteins and leukemia-associated fusions, such as EVI1, PML-RARA and AML/ETO, so as to shut down differentiation gene expression programs and maintain the AML phenotype [35].

Histone lysine methylation

Methylation of histone lysine residues can give rise to mono-, di-, or trimethylation, which is processed by lysine methyltransferases (KMTs)[36]. Histone lysine methylation changes the affinity of reader proteins to the methylated histones [37]. The effect of methylation on transcription is determined by the residue targeted and the degree of methylation. And methylation state (e.g. mono- vs trimethylation) may have different functional consequences in the same lysine residue [38]. For instance, marks associated with activation include methylation of H3K4, H3K79, and H3H36. And the methylation of H3K9, H3K27, and H4K20 is associated with silenced gene transcription [38]. In AML, KMTs include mixed-lineage leukemia (MLL) proteins and components of the polycomb repressor complexes (PRCs), which are frequently involved in translocations or are mutated.

MLL, or called KMT2A, is a member of the family of SET domain-containing KMTs, which is characterized by chromosomal translocations affecting the MLL gene and its binding partners, most commonly ALL1-fused gene from chromosome 9 (AF9) [39]. MLL can make transcription activation by targeting H3K4. In AML, MLL fusion proteins recruit a histone methyltransferase, DOT1, so as to cause aberrant methylation of H3K79 at MLL

 doi:10.20944/preprints202206.0046.v1

gene targets and enhanced expression of leukemia-associated genes [39]. MLL translocations occur more frequently in pediatric than adult AML (30-50% vs. >10%, respectively), and are the most common aberration in infant AML [40, 41]. Besides, partial tandem duplications of MLL are found in 5%-7% of de novo AML [6]. MLL translocations result in fusion proteins that lack the wild-type SET domain and are frequently replaced by members of genes encoding super elongated complex nuclear proteins, such as AF9, AF10, and ENL [42]. It is a promising area to develop the inhibitors of this complex and its enzymatic co-factors for pediatric and infant AML.

Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) is part of the PRC2 polycomb repressor complex, which is an H3K27 methyltransferase to catalyze di- and trimethylation of H3K27, resulting in transcriptional repression [43]. EZH2 mutations result in loss of function in myeloid malignancies [44]. Neff et al. found that EZH2 was required for tumor progression rather than leukemogenesis in MLL-AF9 leukemia [45]. Other member of the PRC2 complex associated with the development of AML include additional sex combs like transcriptional regulator 1/2 (ASXL1/2), Jumonji AT-rich interactive domain 2 (JARID2), and SUZ12 polycomb repressive complex 2 subunit (SUZ12) [46-49]. Mutations of ASXL1/2 occur in adult and pediatric AML at different frequencies (3-15% vs. 1-9%, respectively) [50, 51]. The PRC2-associtaed protein ASXL1 can recruit PRC2 to its target loci, and mutations of ASXL1 lead to loss of PRC2-mediated H3K27 methylation. ASXL1 mutations have been associated with adverse prognosis that is correlated to the presence of RUNX1 mutations [52]. Besides, ASXL2 mutations are frequently observed in patients with the RUNX1-RUNX1T1 fusion gene and are mutually exclusive with ASXL1 mutations [47]. JARID2 similarly recruits PRC2 to specific target loci. Puda et al. reported that deletions of PRC2 complex members, especially JARID2, play an essential role in the leukemic transformation of chronic myeloid disorders [48]. Then Beekman et al. revealed that SUZ12 is mutated in progression from severe congenital neutropenia to AML [49]. Another polycomb repressor group gene, BMI1, is part of the PRC1 polycomb repressor complex in AML. Studies have reported that increased BMI1 expression was associated with poor prognosis in AML and MDS [53, 54].

Demethylation of histone proteins is regulated by lysine demethylases (KDMs), that include the amine oxidases and the α-ketoglutarate-dependent Jumonji domain (JmjC) containing proteins. And the amine oxidases include lysine-specific demethylase 1 (LSD1/KDM1A). LSD1 has specificity for H3K4 and H3K9 as a transcriptional activator or a transcriptional repressor [55]. The JmjC lysine demethylase, KDM5A (JARID1), is involved in a fusion protein with NUP98 in about 10%of pediatric acute megakaryoblastic leukemia [56, 57]. And another JmjC family member, KDM6A, can be altered by inactivating mutations in AML [58].

Apart from the histone modifications described above, other modifications include methylation of arginine residues in histones and tyrosine phosphorylation at histone tails. The former is mediated by the family of protein arginine methyltransferases (PRMTs), and may be associated with transcriptional activation as well as with silencing [59]. PRMT5 that is recognized as a transcriptional repressor, is required for sustaining normal hematopoiesis [60]. The latter has been reported, but the function mechanism of histone phosphorylation is poorly understood [61].

Epigenetic readers

The bromodomain and extra-terminal (BET) protein family member mediates crosstalk between chromatin organization and gene transcription, including BRD2, BRD3, and BRD4[62]. As epigenetic readers, these proteins bind to acetylated lysine residues on histone tails and initiate chromatin-mediated signal transduction to carry out normal or cancer-dependent functions [63, 64]. Some studies have reported that BRD4 could facilitate the aberrant expression of key oncogenes, such as c-Myc and Bcl-2 at a high level in AML [65, 66]. Although BRD4 and other BET proteins ubiquitously express at gene promoters and enhancers, inhibition of BET proteins give rise to disproportionately large changes in

 doi:10.20944/preprints202206.0046.v1

expression of particular genes [67]. And Zuber et al. indicated that inhibition of BET proteins can block aberrant transcription elongation of these leukemia-relevant oncogenes, thus preventing upregulation of leukemic stem cell (LSC) self-renewal programs and inducing differentiation [68]. Furthermore, Dawson et al. demonstrated that BET inhibition could exhibit profound anti-leukemic effects against human and murine MLL-fusion leukemic cell lines and mouse models of murine MLL-AF9 and human MLL-AF4 leukemia [69]. It is a promising therapeutic strategy to target the addiction of tumor cells to high oncogene expression by using BET inhibition, malignant cells could potentially be eliminated in a therapeutic window that retains normal hematopoietic cells.

Non-coding RNAs (ncRNAs)

With the development of RNA-seq technology, more and more ncRNA have been discovered that are closely associated with AML leukemogenesis. There is accumulating evidence that ncRNAs actively participate in the pathogenesis of hematological malignancies, especially AML [70]. And the discovery of ncRNAs opens up new prospects for the diagnosis, treatment, and prognosis of AML. ncRNAs are functional small RNA molecules that are not translate into a protein, including microRNAs (miRNAs, typically 19-24bp long), long non-coding RNAs (lncRNAs, ＞200bp) and circular RNAs (circRNAs) [71]. ncRNA can be classified as housekeeping RNA and regulatory RNA based on their functions. The latter includes miRNAs, circRNAs, and lncRNAs, which are extensively involved in gene transcription and translation.

MiRNAs are small RNA molecules of approximately 22 nucleotides that binds to the 3′-untranslated regions (3′-UTR) of the target and posttranscriptionally suppress the expression of the target gene [72]. Numerous studies have implicated miRNAs in regulating hematopoiesis. Oshima et al. demonstrated that EZH2 cooperated with miRNA let-7 to suppress HSC function and is repressed by PRC2-mediated H3K27me3[73]. Bolouri et al. performed miRNA-seq of 152 samples from pediatric AML patients, and found an association between miRNA expression and genomic alterations, including high expression of miRNA-10a with NPM1-mutations and high miRNA-21 expression in Core Binding Factor (CBF)-AMLs [74]. Some studies have shown that miRNA-155 was associated with poor prognosis of adult and pediatric AML [75]. Zhu et al. analyzed the miRNA data and corresponding clinical data of 229 patients, and identified that the high expression of has-miR-509 and has-miR-542 were independent poor prognostic factors, whereas has-miR-146a and has-miR-3667 had a trend to be favorable factors [76]. Mittal et al. reported that ectopic viral integration site 1 (EVI1) upregulation was associated with methylation of the miR-9 promoter and related with downregulation of miR-9 in human AML cell lines and bone marrow cells from pediatric patients with AML. Then they demonstrated that repression of miR-9 delayed disease progression in EVI1high leukemia -xenograft mice. They concluded that EVI1-induced hypermethylation and downregulation of the miR-9 play an important role in leukemogenesis in EVI1high pediatric AML [77].

CircRNAs are ubiquitous, stable, and conserved ncRNAs, which are single-stranded RNA molecules and have roles in transcriptional regulation and as miRNA sponges [78]. Nicolet et al. analyzed circRNA expression in human hematopoietic progenitors and in differentiated lymphoid and myeloid cells, and they showed that the expression of circRNA is cell-type specific and increase during hematopoietic differentiation [79]. Liu et al. investigated the expression pattern of circRNAs in pediatric AML using a circRNA microarray and identified that circRNF220 was specifically abundant in and accumulated in peripheral blood and bone marrow of pediatric patients with AML [80]. Subsequently, they demonstrated that circRNF220 could be highly efficient and specific for the accurate diagnosis of pediatric AML. Meanwhile, they showed that the expression of circRNF220 independently predicted prognosis and high expression of circRNF220 was an unfavorable prognostic marker for relapse of pediatric AML. Recently, Wang et al. showed that circ_0040823 inhibited proliferation and induced apoptosis of AML cells by sponging miR-516b to diminish the regulatory effect of miR-516b on its downstream target [81].

 doi:10.20944/preprints202206.0046.v1

LncRNAs are more than 200 nucleotides in length and lack a meaningful open reading frame [82]. Relatively fewer studies have investigated lncRNAs in pediatric AML. lncRNA urothelial carcinoma-associated 1(UCA1) has been reported to sustain the proliferation of AML cells [83, 84]. Recently, Liang et al. found an escalation of UCA1 expression and suppression of miR-204 expression in pediatric AML. Besides, the downregulation of UCA1 suppressed cell proliferation and promoted apoptosis through inactivating SIRT1 signals by upregulating miR-204 in pediatric AML [85]. Ma et al. identified that lncRNA LINC00909 promoted cell proliferation and metastasis via miR-625-mediated modulation of Wnt/β-catenin signaling in pediatric AML [86].

Currently, the role of miRNAs in AML is the most studied. However, the mechanism of miRNAs in AML is still complex and unclear since the target genes of miRNAs range from dozens to hundreds and involve different signaling pathways. Recently, lncRNAs and circRNAs have been introduced into the miRNA network, which can act as ceRNAs and miRNA sponges for miRNAs to regulate the expression of miRNAs in AML [70]. It is important to find the crossover miRNAs of the three ncRNAs to help illustrate the connection between these three ncRNAs.

3. Epigenetic targeted therapy in pediatric AML

The pivotal role of epigenetic regulators in AML has stimulated efforts to study epigenetically targeted drugs. Besides, aberrant epigenetic regulation poses the chance to apply epigenetically targeted therapies thanks to the inherent reversibility of epigenetic marks. Numerous clinical trials are ongoing to study the epigenetic targeted therapies in adults. Although mutations in epigenetic regulators occur less frequently in pediatric AML, more and more clinical trials are focusing on the role of epigenetic regulators in pediatric AML, and a range of small molecules that inhibit epigenetic enzymatic activity are now at varying stages of clinical development (Table 2).

Table 2. Clinical trials of epigenetic targeted therapies in pediatric AML.

Target	Drug	Phase	Study Start	Clinical trial	Status
DNA methyltransferases	Azacitidine	1	2013	NCT01861002	Completed
	Azacitidine	2	2017	NCT03164057	Recruiting
	Azacitidine	2	2018	NCT03383575	Active, not recruiting
	Azacitidine	2	2015	NCT02450877	Completed
	Azacitidine	2	2013	NCT01700673	Completed
	Azacitidine	2	2014	NCT02275663	Unknow
	Decitabine	2	2017	NCT03164057	Recruiting
	Decitabine	1	2017	NCT03132454	Recruiting
	Decitabine	2	2006	NCT00416598	Completed
	Decitabine	1/2	2018	NCT03453255	Unknow
	Decitabine	2	2018	NCT03417427	Recruiting
	Decitabine	2	2006	NCT00414310	Completed
	Decitabine	1	2017	NCT03263936	Active, not recruiting
	Decitabine	1/2	2013	NCT01853228	Terminated
	Decitabine	2	2011	NCT01177540	Completed
IDH1	Ivosidenib		2017	NCT03245424	Approved for marketing
IDH2	Enasidenib	2	2018	NCT03383575	Active, not recruiting
Histone deacetylases	Vorinostat	1	2005	NCT00217412	Completed
	Vorinostat	1	2017	NCT03263936	Active, not recruiting

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	Vorinostat	1/2	2012	NCT01422499	Completed
	Panobinostat	1	2016	NCT02676323	Terminated
	Panobinostat	1	2011	NCT01321346	Completed
	Valproic acid	2	2012	NCT02124174	Recruiting
DOT1L	Pinometostat	1	2014	NCT02141828	Completed
	Pinometostat	1/2	2019	NCT03724084	Active, not recruiting

DNMT inhibitors

DNA methyltransferase inhibitors (DNMTi) or called hypomethylating agents (HMAs) includes 5-azacytidine (azacitidine) and 5-aza-2′-deoxycytidine (decitabine), which alter DNA methylation pattern, resulting in increased expression of tumor suppressors and apoptosis [87]. HMAs are the best-established epigenetic therapies in adult AML, which have shown efficacy and safety in older patients with AML. HMAs form irreversible covalent bonds with DNMTs upon incorporation into DNA, which leads to proteasomal degradation of DNMTs, resulting in hypomethylation and transcriptional repression, and direct cytotoxic effects through DNA damage (Figure 1) [88]. HMAs have been demonstrated to prolong overall survival (OS) compared to standard treatment in adult patients [89], and Stahl et al. identified the important role of HMAs in relapsed/refractory AML in a large international patient cohort study of 655 cases [90]. Despite the low frequency of mutations in DNMTs in pediatric AML, some studies have shown that DNMTi might be efficient in pediatric AML. Gore et al. demonstrated that decitabine prior to standard combination chemotherapy is feasible and well tolerated in children with newly diagnosed AML [91]. Subsequently, Sun et al. identified that azacitidine can be used safely in sequence with intensive chemotherapy in pediatric relapsed/refractory AML [92]. Some studies have indicated that HMAs only have limited efficacy and rarely lead to sustained remission when used as a single agent in adult AML [93], thus further preclinical and clinical studies should focus on the combination therapy with other chemotherapy agents, targeted drugs, and immunotherapy.

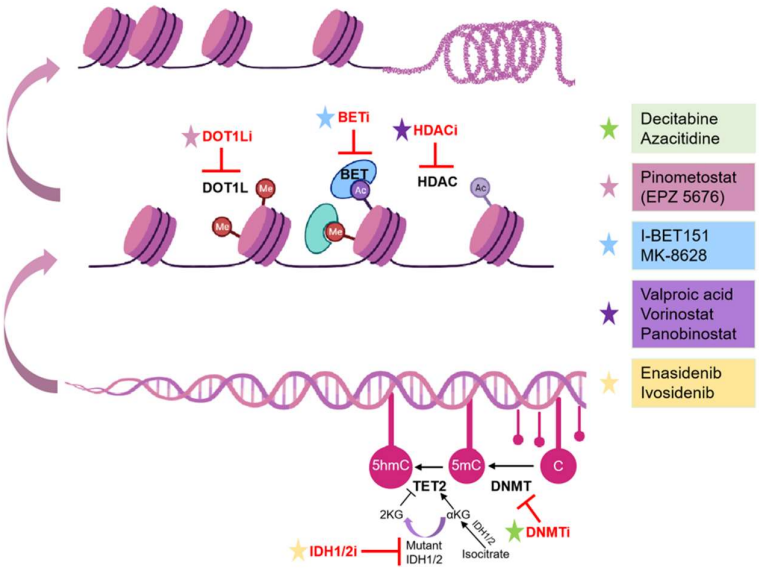

Figure 1. Epigenetic inhibitors and mechanisms in pediatric AML. Epigenetic inhibitors are highlighted in red. C=cytosine; 5mc=5-methylcytosine; 5hmc=5-hydroxymethylcytosine; Ac=acetylation.

IDH inhibitors

 doi:10.20944/preprints202206.0046.v1

IDH inhibitors have been reported to alleviate histone demethylase inhibiton and promote the terminal differentiation of abnormal myeloid cell [94]. Stein et al. firstly reported the IDH inhibitor, enasidenib, could be used in relapsed/refractory IDH2-mutant AML in a phase 1/2 clinical trial (NCT01915498) [95]. Then DiNardo et al. reported that ivosidenib, a small-molecule inhibitor of IDH1, promoted durable remission and molecular remission in patients with CR in a phase 1 clinical trial of patients with advanced IDH1-mutant relapse/refractory AML [96]. However, few clinical trials accept children with AML at present. The only clinical trial currently that accept pediatric AML patients is investigating the synergistic effect of azacytidine and enasidenib in IDH2-mutant myelodysplastic syndrome (MDS)(NCT03383575), with no active trial in pediatric AML yet.

HDAC inhibitors

HDAC inhibitors (HDACi) can activate epigenetically silenced tumor suppressor genes and promote tumor cell killing, which have been evaluated in clinical trials for adult AML patients with limited efficacy, either alone or in combination with chemotherapy [97]. Leukemia associated fusion proteins have been reported to block gene expression by recruitment of HDACs, which could be alleviated by inhibiting HDACs, causing differentiation of leukemic blasts. And these fusion proteins are more frequent in pediatric AML, raising the possibility that children with AML may benefit more from HDAC inhibitors than adult patients. Karol et al. reported that panobinostat could be safely administrated with chemotherapy and increased blast histone acetylation in a phase 1 clinical trial [98]. Recently, Pommert et al. demonstrated that the combination therapy of decitabine and vorinostat with fludarabine, cytarabine, and G-CSF (FLAG) was well-tolerated and effective in pediatric patients with relapsed/refractory AML in phase 1 clinical trial (NCT02412475) [99]. The results of these trials will be the first step in evaluating the utility of HDAC inhibitors in pediatric patients.

DOT1L inhibitors

Owing to more frequent MLL translocations of pediatric AML than adult AML (30-50% vs. $>$10%, respectively), targeting vulnerabilities of MLL-associated leukemias is a highly promising therapeutic strategy for children with AML. Inhibition of DOT1L blocks MLL target gene expression by regulating the aberrant methylation of H3K79. Pinometostat (EPZ-5676), a DOT1L inhibitor, has been initiated in pediatric patients with relapsed/refractory AML harboring MLL rearrangements in a phase 1 clinical trial [100]. However, the study reported only transient reductions in leukemic blasts in 7 of 18 patients (NCT02141828). Despite the lack of clinical benefit of EPZ-5676 as a single agent, preclinical and clinical studies are warranted to test the utility of DOT1L inhibitors and conventional cytotoxic regimens in relapsed/refractory AML

BET inhibitors

BET inhibitors have an important role in inhibiting histone acetylation at oncogene-associated enhancers and super-enhancers. Dawson et al. showed that a small-molecule inhibitor of the BET family, GSK1210151A (I-BET151), has profound efficacy against murine and human MLL-fusion leukemic cell lines by inducing early cell cycle arrest and apoptosis [69]. Then they indicated that IBET151 significantly promoted survival in two distinct mouse models of murine MLL-AF9 and human MLL-AF4 leukemia [69]. These results provide a promising epigenetic therapy target for pediatric AML. Other BET inhibitors, including MK-8628 (OTX015) and CPI-0610, also are currently being assessed in phase 1 and 2 trials in adult patients with AML (NCT02698189, NCT02158858) [101].

4. Future Prospective

Although the prognosis for pediatric AML have improved in recent decades, outcomes of children with AML remain suboptimal due to high rates of relapse and few options for treatment when initial regimens fail. There remains an urgent need for better and more precise therapeutic strategies for patients with AML, especially for pediatric AML.

Rapid advances in next-generation sequencing technologies have led to tremendous progress in understanding the molecular pathogenesis of AML, revealing enormous genetic and epigenetics and paving the way for precision medicine approaches. Based on the clinical efficacy of epigenetic therapies in adult AML, the low frequency of epigenetic abnormalities in pediatric AML is not a reason to limit the preclinical and clinical development of epigenetic targeted therapies. Some inhibitors of epigenetic targeted therapies have benefited many children with the appropriate patient stratification. Meanwhile, it is important to consider that more personalized medicine will require more precise and appropriate patient stratification with different genetic and epigenetic alterations. One drug is unlikely to be curative in AML, either epigenetic targeted drugs or other inhibitors, which provides reservoirs for drug resistance and subsequent relapse due to the clonal heterogeneity. Further preclinical and clinical studies should focus on the combination therapeutic strategies of epigenetic targeted drugs with other inhibitors and immunotherapy to yield greater cell kill and improve the outcomes for pediatric AML.

Author Contributions: H.X. and Y.W. collected the data and wrote the manuscript. H.C. and R.J. reviewed and finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (No. 31701207).

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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