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Review

Roles of m^{6}A RNA methylation in hematopoiesis and leukemogenesis

Xinjian Mao[1] and Linheng Li[1,2,*]

[1]Stowers Institute for Medical Research, Kansas City MO, USA; [2]Department of Pathology and Laboratory Medicine, and Division of Medical Oncology, Internal Medicine, University of Kansas Medical Center, Kansas City KS, USA.

ABSTRACT

Hematopoietic stem cells (HSCs), characterized as having self-renewal and multi-lineage differentiation potentials, maintain the hematopoietic system through an entire lifespan. Comprehensive understanding of how HSCs are regulated will facilitate the development of new strategies to manage hematopoietic diseases. Past decades of effort unraveled a pivotal role of epigenetic modification *via* DNA or histone in the regulation of HSC behaviors; however, effects of RNA modification on HSC behaviors have been largely neglected. N6-methyladenosine (m^{6}A), as the most prevalent RNA modification in eukaryote, shows versatile functions in various physiological processes. Recent reports demonstrate a critical role of m^{6}A mRNA methylation in the determination of HSC fate, including HSC specification during embryogenesis and HSC proliferation and differentiation in adults. Furthermore, dysregulation of m^{6}A RNA methylation has been reported to be associated with leukemogenesis. Elucidation of the underlying mechanisms by which m^{6}A RNA methylation regulates HSC fate and promotes leukemogenesis may provide an insight for the translation of basic discoveries into clinical practice in treating hematopoietic disorders. Here we review the recent advances in understanding the regulation of m^{6}A RNA methylation in hematopoiesis and leukemogenesis.

*Corresponding author: lil@stowers.org

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Introduction

The hematopoietic system plays a pivotal role in developing the immune system and producing red blood cells for oxygen delivery. The hematopoietic system in an adult human is estimated to produce 500 billion hematopoietic cells every day [1]. How can the hematopoietic system meet this high demand of regeneration throughout an entire lifetime? The answer is due to a specific property of hematopoietic stem cells (HSCs). Although the actual number of HSCs is low (1-5 cells per 10^5 marrow cells) [1], HSCs are characterized with self-renewal capability and multi-lineage differentiation potential to produce all mature blood cell types [2]. There are long-term (LT) and short-term (ST) HSCs; the former enriches quiescent HSCs and the latter enriches actively cycling HSCs. At steady state, the quiescent feature presumably protects HSCs from extrinsic insults [3, 4], whereas the proliferative ST-HSCs are responsible for replenishing mature hematopoietic cells. Under stress, however, ST-HSCs decline and LT- HSCs re-enter into cell cycle, replacing lost ST-HSCs and giving rise to multipotent progenitor cells for hematopoietic regeneration [3, 4]. Thus, the balance between quiescent and cycling HSCs regulates HSC maintenance and its progeny production, thus maintaining the blood system throughout an entire lifetime. Although

HSCs and the associated microenvironment (so called niche) have been extensively investigated over past decades [3, 4], the regulatory mechanism of HSC fate, particularly self-renewal, is still far from fully understood, which impedes translational medicine development such as the *in vitro* expansion of human hematopoietic stem and progenitor cells (HSPCs) for clinical applications [5].

Gene mutation and chromosomal aberration dysregulate proliferation and differentiation of HSPCs, which creates risk for malignant development that can lead eventually to leukemogenesis [6]. For decades, epigenetic modifications of DNA or histone (e.g., histone methylation or acetylation and DNA methyltransferase) have been known to regulate HSC fate [7]. Mutations of DNA epigenetic modifiers are frequently found in patients with leukemia and other blood disorders. For instance, DNMT3A, a critical DNA methyltransferase, is frequently mutated in patients with acute myeloid leukemia (AML), leading to hypermethylation of oncogenes and poor prognosis [8, 9]. However, it is not until recently that emerging evidence indicates an essential role of epigenetic modification of RNA (epitranscriptomics) in normal HSCs as well as in AML [10, 11]. In this review, we introduce basic concepts regarding N6-methyladenosine (m^6A) of mRNA [12]. Then we review recent advances on the role of m^6A RNA modification in hematopoietic development, maintenance, and malignancy. Although the m^6A epigenetic modification regulates various RNAs (e.g., long non-coding RNA, circular RNA, tRNAs) [12], in this review we mainly summarize the recent progress regarding m^6A modification of message RNA (mRNA), and discuss its potential applications in translational medicine including HSC expansion and leukemia treatment.

m^6A RNA modification

m^6A is a reversible and dynamic nucleotide modification abundantly detected in ~20% of cellular mRNAs and approximately 0.4% of adenosines within mRNA in mammalian cells [13]. m^6A regulatory components include m^6A 'writers', 'erasers', and 'readers' based on their roles in adding, recognizing, or erasing the methylation, though some of them may have versatile functions [14]. Methyltransferase-like protein 3 (METTL3) containing a catalytic unit is the main 'writer' of m^6A modification [15]. Methyltransferase-like protein 14 (METTL14) facilitates RNA binding and m^6A deposition *via* forming the METTL3/METTL14 complex [16, 17]. The METTL3/METTL14 complex specifically recognizes the 'RRACH' motif (R=A/G, H=A/C/U), at which m^6A modification is deposited [18-21]. Of note, the affinity of METTL3/METTL14 complex to the motif is weak, which can be enhanced by the m^6A-METTL-associated complex (named MACOM). The MACOM is composed of multiple proteins, including Wilms tumor 1-associated protein (WTAP) [16], Vir-like m 6 A methyltransferase-associated (VIRMA, also known as KIAA1429), Cbl proto-oncogene like 1 (CBLL1, also known as Hakai), RNA-binding motif 15 (RBM15), and zinc finger CCCH-type containing 13 (ZC3H13) [22]. In addition, m^6A modification is frequently detected nearby the stop codon, 3′-untranslated regions, and long internal exons [23, 24], raising the question of how the specificity of m^6A methylation is achieved among the ubiquitous distribution of METTL3/METTL14 recognition motif sequence across all mRNAs. MACOM was reported to contribute to this process, although the underlying mechanism remains to be further elucidated [22]. More recently, another m^6A methyltransferase-like protein-16 (METTL16) has been discovered that differentially deposits m^6A on U6 small nuclear RNAs (snRNAs), and in the 3' UTR of human methionine adenosyltransferase 2A mRNA (MAT2A) by binding preferentially to a UAC(m^6A)GAGAA sequence in the bulge of a stem-loop structure [25-27].

The deposited m^6A in mRNA could also be 'erased' by demethylases, including fat mass and obesity-associated proteins (FTO) [28-30] and AlkB homologue 5 (ALKBH5) [30-32]. FTO has been demonstrated to demethylate not only m^6A but also N^6, 2-O-dimethyladenosine (m^6A_m) at the 5' cap in mRNA [33] and N1-methyladenosine (m^1A) in tRNA [34]. FTO could be in either nucleus or cytoplasm depending on cell types, status of RNA demethylation, and RNA splicing stages. ALKBH5 is colocalized with nuclear speckles to mediate mRNA export and demethylation. In addition, FTO and ALKBH5 show tissue specific distribution and are associated with

different pathophysiological phenotypes [28-32], implying their different mechanisms pertaining to RNA demethylation.

Translation of m^{6}A mRNA to proteins involves regulation by m^{6}A 'reader' proteins containing YT521-B homology (YTH) domain responsible for the specific binding to m^{6}A, including YTH domain family 1-3 (YTHDF1-3) and YTD domain containing 1-2 (YTHDC1-2) [23, 35-40]. Although all YTHDF1-3 are in cytoplasm, they perform different functions. YTHDF1 and YTHDF3 function mainly to promote translation of m^{6}A methylated mRNAs *via* recruiting translation initiation factors [36, 37], while YTHDF2 promotes the decay of target mRNAs *via* specifically recognizing Gm^{6}AC consequence motif in the presence of the CCR4-NOT deadenylase complex [36]. In contrast, YTHDC1-2 are commonly found in nucleus and play multiple roles, including mRNA splicing, exporting, stability, and translation [38, 39].

The m^{6}A RNA methylation is associated with various biological behaviors ranging from plants to human, suggesting its conserved role in multicellular eukaryotes [11]. Dysregulation of m^{6}A is linked to multiple human diseases, for example, invasiveness of cancer cells [41-43], obesity, and depressive disorders [32, 44-46]. Embryonic stem cells (ESCs) are tightly regulated by a plethora of pluripotent genes (e.g., *Smad3, Nanog, Sox2, Myc*) and developmental factors (e.g., Foxa2, Sox17), both of which have m^{6}A modification on their corresponding transcripts [47, 48]. Knockout of METTL3 substantially increased self-renewal and blocked differentiation of mouse ESCs by boosting the expression of pluripotent-promoting genes [47, 48]. Interestingly, METTL3 knockdown in mouse epiblast stem cells, a primed pluripotency state of ESCs, enhanced their lineage priming, through upregulating lineage commitment factors [48]. These indicate that the function of m^{6}A RNA methylation is in a context-dependent manner in association with maintenance and differentiation of stem cells.

The m^{6}A regulation and hematopoietic maintenance

HSCs have well-defined developmental trajectories, and their abnormal differentiation is a common feature of myeloid hematological malignancies. Given the pivotal role of m^{6}A-deposited mRNA in self-renewal and differentiation of stem cells, the hematopoietic system provides an ideal model to further explore the roles of m^{6}A RNA methylation. Interestingly, METTL3 knockdown substantially inhibited proliferation and increased myeloid differentiation of human cord blood enriched CD34$^+$ HSPCs, without inducing apoptosis [49]. In contrast, overexpression of METTL3 significantly increased the proliferation and inhibited myeloid differentiation of CD34$^+$ HSPCs [49]. Heather Lee *et al*. investigated the hematopoietic relevant phenotype in METTL3 conditional knockout mice and found that deficiency of METTL3 prominently accumulated HSCs in the bone marrow, without impacting myeloid cell number or function [50]. Although the cell number of HSCs was increased, the repopulation potential of METTL3-deficient HSCs was substantially compromised due to blockade of HSC differentiation [50]. Using m^{6}A-tagged mRNA immunoprecipitation sequencing analyses, researchers discovered MYC as a direct target of m^{6}A. Decline of MYC blocked differentiation of METTL3-deficient HSCs without inducing apoptosis [50]. This finding is consistent with another report showing that METTL3 is a critical mediator of HSCs self-renewal *via* maintaining HSCs quiescence [51]. Furthermore, MYC decline in METTL3 knockout HSCs was recently reported to be associated with enhanced symmetric division [52]. The controversy of effects of METTL3 on human cord blood-derived HSPCs or on genetic mouse model-derived HSCs could be attributed to 1) different phenotypes of HSCs and progenitor cells in response to METTL3-deficiency, or 2) different approaches (shRNA knockdown of METTL3 in human cord blood-derived CD34$^+$ HSPCs versus Mx1-cre; METTL3$^{fl/fl}$ knockout in bone marrow HSCs from mice). In fact, the controversy mainly focuses on number change of HSPCs in response to METTL3 deficiency, while METTL3-deficient HSCs, either from human cord blood or conditional knockout mice, exhibit more like multipotent progenitor-like cells with compromised repopulation potential.

METTL14 was found to be expressed at a much higher mRNA level in mouse bone marrow HSCs than in their progeny, implying its potential role in

HSCs [51, 53]. METTL14 knockout mice showed similar number but compromised repopulation potential of HSCs compared to wild type HSCs, although such decline of transplantation potential was not as substantial as deficiency of METTL3 in HSCs [51]. Knockdown of METTL14 substantially promoted the myeloid differentiation of human $CD34^+$ HSPCs [53]. In contrast to the indispensable role of METTL3 with catalytic activity of methyl-transferring, METTL14 mainly facilitates METTL3 binding to RNAs [51, 53].

In respect to the m^6A readers, it was reported that YTHDF2 conditional knockout in mouse bone marrow resulted in increased number and repopulation potential of HSCs without skewing lineage differentiation or leading to hematopoietic malignancies [54, 55]. YTHDF2 deficiency delayed the decay of m^6A-deposited mRNAs encoding transcription factors critical for stem cell self-renewal (e.g., TAL1, GATA2, RUNX1, MYC, HOXB4, and STAT5a) [54]. This interesting finding prompted the authors to further test the role of YTHDF2 in cord blood-derived HSPCs, given that expanding HSPCs for clinical application is a long-sought but unachieved goal in the hematopoietic field. 7-day *ex vivo* culture of human cord blood-derived $CD34^+$ HSPCs transfected with lentivirus containing shRNA of YTHDF2 achieved 10-fold increase of HSPC phenotypical number, compared to that transfected with lentivirus containing scramble RNA [54]. Furthermore, these YTHDF2 knockdown HSPCs exhibited substantially enhanced repopulation potential as evidenced by *in vitro* colony-forming unit (CFU) assay and *in vivo* transplantation experiment [54]. Of note, the role of other m^6A readers in HSCs has not been reported.

The m^6A regulation and hematopoietic development

Compared to extensive investigation on the regulation of adult HSCs, the process of hematopoietic development remains largely unknown, partially due to technical challenge. With the advance of cutting-of-edge techniques (e.g., real time *in vivo* imaging, single cell RNA-sequencing), exploring embryonic hematopoietic development becomes achievable and a hotspot in the hematopoietic field [56]. There are three waves of hematopoiesis during embryogenesis. The first wave of hematopoiesis occurs at E7 to produce primitive erythroid progenitors, macrophages, and megakaryocytes [57]. The second wave is from E8.5 that produces additional lymphoid cells in yolk sac. However, it is not until the third wave of embryonic hematopoiesis from E9.5 that hemogenic endothelial cells undergo dramatic change in the aorta-gonad-mesonephros (AGM) region to hematopoietic cells (so called endothelial-to-hematopoietic (EHT) transition), among which only very few of cells are pre-HSPCs [57-59]. Intriguingly, there is emerging evidence in revealing a critical role of m^6A mRNA in hematopoiesis in both zebrafish and mice. Liu and Yu Labs found that deletion of METTL3 by morpholino in zebrafish substantially impaired the presence of HSPCs *via* blocking EHT. The EHT process is negatively regulated by the YTHDF2-mediated mRNA decay of the arterial endothelial genes *Notch1a* and *Rhoca* [60]. They also discovered increasing expression of m^6A components (e.g., METTL3, METTL14, WTAP, YTHDF2) from endothelial cells to hemogenic endothelial cells, then to HSPCs [60]. Knockdown of METTL3 declined the CFU cells from AGM of mouse E10.5 embryo, potentially due to the impaired EHT *via* sustained expression of arterial genes, including *Notch1, Ephrinb2, Hes1,* and *Hey1* [60]. In a following study, Liu group further applied METTL3 conditional knockout mice to demonstrate the critical role of the m^6A modification in definitive hematopoiesis *via* YTHDF2-mediated Notch signaling pathway [61]. These two studies shed light on the role of m^6A modification in embryonic hematopoiesis.

The m^6A regulation and hematopoietic malignancy

Acute myeloid leukemia (AML) is characterized as highly proliferative immature leukemic cells with blocked differentiation, the so-called blasts. There are different strategies regarding leukemia treatment including widely used inhibition of proliferation and induction of differentiation. For the latter, using *all-trans* retinoid acid and phorbol-12-myristate-13-acetate is an excellent example to treat patients with acute polymyelotic leukemia (APL) [62-64]. Since m^6A methylation

plays a critical role in cell differentiation and development, it raised a possibility that m^{6}A might be associated with AML leukemogenesis [49]. Indeed, significantly higher METTL3 mRNA expression was found in some AML samples [49]. In addition, multiple AML cell lines showed prominently increased protein expression of METTL3 than over cord blood-derived CD34$^+$ HSPCs [49]. METTL3 knockdown decreased the fraction of m^{6}A deposition in mRNAs of MOLM-13 AML cells, which correlates with reduced proliferation and increased differentiation of MOLM-13 cells [49]. Interestingly, inhibiting METTL3 led to apoptosis of MOLM-13 AML cells, but had less apoptotic effect on cord blood-derived CD34$^+$ HSPCs. This described difference implies that HSPCs may be more tolerant than AML cells in response to METTL3 inhibition, thus opening a therapeutic window of targeting AML by inhibiting METTL3. Mechanistically, METTL3 maintains AML cells with stem cell property *via* sustaining expression of self-renewal related mRNAs (e.g., c-MYC, BCL2, and PTEN) [49]. METTL3 depletion increased the phosphorylation of AKT that subsequently promoted differentiation of AML cells [49]. Interestingly, expression of BCL2 and c-MYC mRNAs was recovered a few days after METTL3 inhibition, though proliferation arrested and apoptosis persisted, suggesting additional mechanisms involved underlying the observed cellular phenotypes [49]. The *ex vivo* genome-wide CRISPR dropout screen also identified METTL3, METTL14 and METTL16 as essential regulators of the survival of mouse AML cells carrying KMT2A-MLLT3 (referred as MLL-AF9) fusion gene and Flt3 internal tandem duplication [65].

During transcription of given genes, CAATT-box binding protein zeta (CEBPZ, also called CHOP) recruits METTL3 to the transcriptional start sites of active genes on chromatin to facilitate m^{6}A deposition on the associated mRNAs (e.g., SP1, c-MYC) [49]. Although METTL3 is supposed to deposit m^{6}A in the nucleus, it was also found to be localized to the cytoplasm of AML cells to promote the translation of specific mRNA independent of its 'writer' role [66, 67]. On the other hand, METTL14 was upregulated in multiple AML cell lines, but was downregulated after the treatment of differentiation-inducing agents [53]. Depletion of METTL14 substantially decreased the *in vitro* CFU capacity and *in vivo* repopulation potential of AML cells with MLL-AF9 fusion gene, *via* promoting apoptosis and differentiation [53]. Using anti-m^{6}A to conduct RNA-seq, researchers identified MYB and MYC as direct targets of METTL14 [53]. In addition, chromatin immunoprecipitation sequencing (ChIP-seq) revealed that hematopoietic transcription factors GABPA and ELF1 are positive, and SPI1 is negative upstream regulators of METTL14 in AML cells [53]. The MACOM components, including WTAP and RBM15, were also upregulated in AML and associated with AML progression. Downregulation of WTAP inhibited proliferation and induced apoptosis of AML cells as well as delayed leukemia progression [68]. While RBM15 is known to mediate the alternative splicing of transcripts of several critical hematopoietic differentiation genes (TAL1, RUNX1, GATA1 and c-MPL) [69], deletion of RBM15 blocked B cell differentiation and myeloid and megakaryocytic expansion. Whether the described functions of RBM15 are mediated by m^{6}A modification needs further investigation [70].

Though m^{6}A 'writers' are critical, the level of m^{6}A is also balanced by the activity of 'erasers'. For example, upregulated m^{6}A demethylase, FTO, increased cell transformation and leukemogenesis in AML cells with various mutations (e.g., MLL-AF9 fusion, t(15;17)/PML-RARA, FLT3-ITD, and NPM1 mutation) [71]. In addition, upregulation of FTO also led to the resistance of AML cells to the differentiation-induction therapy, *via* promoting the expression of leukemic oncogenes, such as ASB2 and RARA [71]. The demethylation role of FTO revealed by these above observations seems in contrast to that of m^{6}A 'writers', METTL3/METTL14, as methylase. There is evidence showing that FTO mainly demethylates the m^{6}A at the 5' cap of mRNA but has negligible effect on m^{6}A in other sites [33]. Most likely, METTL3/METTL14 and FTO may be associated with leukemic progression through different mechanisms. R-2-hydroxyglutarate (R-2HG), an oncometabolite that has been demonstrated as an inhibitor of FTO [72], in combination with standard chemotherapy, showed promising

anti-cancer effects against AMLs. This was due to the inhibition of R-2HG to demethylation of m^6A-marked mRNAs of critical oncogenes (e.g., MYC, CEBPA), which were then rapidly degraded by a m^6A reader YTHDF2 [72]. This observation also suggests that YTHDF2 could be a promising target for treating AML. Several other small molecule inhibitors of FTO have been developed; the majority of them, however, showed limited cellular activity [73-76]. FB23-2 is an exception, which was optimized from Meclofenamic acid, and has greater activity and selectivity to FTO [77]. FB23-2 substantially prohibited leukemia progression and prolonged the survival of mice transplanted with human AML cell lines and patient-derived AML cells, through suppressing proliferation and increasing myeloid differentiation [77].

The functional role of m^6A readers in AML progress has been less reported and awaits further exploration. YTHDF2, a m^6A reader, was found to be upregulated at both mRNA and protein levels in several AML cell lines owing to various gene mutations or chromosome aberrations 55]. YTHDF2-deficient c-kit$^+$ cells from Ythdf2$^{fl/fl}$; Vav-iCre mice dampened the leukemic induction by overexpression of Meis1/Hoxa9, suggesting a role of YTHDF2 in leukemogenesis [55]. On the other hand, YTHDF2-depleted leukemic stem cells showed prominently declined engraftment in mice, resulting in significantly extended survival of mice [55]. Knockdown of YTHDF2 also reduced CFU capacity of human AML cell line THP-1. Mice transplanted with YHTDF2-depleted THP-1 cells showed prolonged survival compared to that of wild type THP-1 [55]. RNA-seq and m^6A-seq analyses revealed that YTHDF2 deficiency upregulated genes associated with generic metabolic processes and downregulated genes implicated with immune response processes [55]. Particularly, TNF receptor 2 was discovered as a direct target of YTHDF2 by which m^6A-deposited mRNAs of TNF receptor 2 was decayed [55]. As a result, the accumulated TNF receptor 2 rendered AML cells more sensitive to TNF-induced apoptosis.

Conclusions and perspectives

Accumulating evidence suggests versatile roles of m^6A RNA methylation in various biological activities in a context-dependent manner [78]. This is evidenced by studies regarding the roles of m^6A methylation in normal hematopoietic system and AML. Recently, remarkable progress has been made in the understanding of m^6A RNA methylation. However, it remains largely unclear for the regulatory roles of m^6A methylation in different biological settings. It is still elusive in terms of the mechanism of the site selectivity for m^6A (RRACH motif) that are broadly distributed in RNAs. How the m^6A complex dynamically fine-tunes thousands of m^6A-deposited RNAs during complicated biological processes, such as stem cell differentiation and development [79], needs further investigation. Environmental stimulations (e.g., heat shock, hypoxia) might regulate the activity of m^6A components [30, 80]. Future work is required to elucidate the mechanism by which m^6A components sense and respond to the environmental stimulations alone or in coordination with other signaling pathways. These include unfolded protein response (UPR) signaling pathway that orchestrates a balance with protein synthesis under homeostasis or under stress. CEBPZ, a critical mediator of the UPR pathway that induces apoptosis under robust or chronic stress of misfolding protein synthesis, was reported to recruit METTL3 to chromatin for m^6A methylation [65]. It would be intriguing to explore the role of m^6A methylation under stress condition, in a CEBPZ-dependent or independent manner.

Many components of m^6A have been shown to play a critical role in the regulation of self-renewal and differentiation of HSCs, although the underlying mechanism is far from being fully understood. Intriguingly, genetical inactivation of YTHDF2 was proved in principle as a practicable way to expand HSCs without lineage biases [54]. Developing a small molecule that inhibits YTHDF2 activity holds the promise as a new strategy to expand human cord blood-derived HSCs for clinical application. On the other hand, although the number of HSCs in METTL3-deficient mice was substantially increased, they were actually more like multipotent progenitors with compromised repopulation potential. In contrast, over-expression of METTL3 also led to *ex vivo* expansion of human umbilical cord blood-derived HSCs. Therefore, looking for a way to

interfere with METTL3 may provide an alternative strategy to expand HSCs.

The evidence showing a role of m^{6}A methylation in the regulation of embryonic hematopoietic development is just emerging. There are many remaining questions to be addressed in the future. For example, how do m^{6}A components dynamically and spatially orchestrate the fine-tuned mRNA translation during hematopoiesis? What is the mechanism by which the specificity of m^{6}A modification on transcripts is determined? Revealing the regulation of m^{6}A on hematopoietic development may shed light on *in vitro* blood regeneration [81-83].

Upregulation of m^{6}A components has been found in multiple AML cells, and they also play a pivotal role in leukemogenesis and are associated with poor prognosis. Genetic interference (e.g., knockdown) as proof-of-principle revealed the feasibility of targeting AML cells by inhibiting m^{6}A components, thus motivating the development of small molecule inhibitors of the corresponding m^{6}A components. A small molecule inhibitor of FTO already shows a promising effect on preclinical AML models [71]. On the other hand, it is reasonable to combine the m^{6}A pathway inhibitors with differentiation-induction therapy or chemotherapy to target both leukemic stem cells and blast cells simultaneously. Innovative treatment strategy for AML has been limited over past decades, despite increased understanding of AML. Decades of research on DNA epigenetic modification on normal hematopoiesis and leukemia pave the way to develop epigenetic drugs targeting epigenetic modifiers. Some of these drugs are currently in clinical trial for the treatment of AML [7-9]. We expect that, with the further in-depth understanding of m^{6}A RNA methylation on hematopoietic development and leukemia, targeting epitranscriptomic RNA methylation may provide an innovative way to treat AML in the future.

CONFLICT OF INTEREST STATEMENT

L. L. serves as a SAB member for Elixell in Boston.

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