

Review

Immunotherapeutic Potential of m6A-Modifiers in Controlling Acute Myeloid Leukemia

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Abstract: Epigenetic alterations have contributed greatly to human carcinogenesis. Conventional epigenetic studies have been predominantly focused on DNA methylation, histone modifications and chromatin remodelling. However, recently, RNA modification (m6A-methylation) also termed 'epitranscriptomics' has emerged as a new layer of epigenetic regulation due to its diverse role in various biological processes. In this review, we have summarized the therapeutic potential of m6A-modifiers in controlling haematological disorders especially acute myeloid leukemia (AML). It is a type of blood cancer affecting specific subsets of blood-forming hematopoietic stem/progenitor cells (HSPCs) which proliferate rapidly and acquire self-renewable capacities with impaired terminal cell-differentiation and apoptosis leading to abnormal accumulation of white blood cells, and thus an alternative therapeutic approach is required urgently. Here, we have described how RNA m6A-modification machineries EEE (Editor/writer: Mettl3, Mettl14; Eraser/remover: FTO, ALKBH5 and Effector/reader: YTHDF-1/2) could be reformed into potential druggable candidate or as RNA modifying drug (RMD) to treat leukemia. Moreover, we have shed-light on the role of microRNA and suppressor of cytokine signalling (SOCS/CISH) in increasing anti-tumor immunity towards leukemia. We anticipate, our investigation will provide a fundamental knowledge in nurturing the potential of RNA modifiers in discovering novel therapeutics or immunotherapeutic procedures.

Keywords: Epitranscriptomics, acute myeloid leukemia, microRNA, CISH, Immunotherapeutics.

1. Introduction

There are several conventional drugs, including epigenetic-based drugs targeting DNA and histone modifications, have been already approved by the US food and drug administration (FDA) for the treatment of AML. However, targeting RNA-modification machineries as RNA modifying drugs (RMDs) refuelled the scientific interest to discover novel therapeutic drugs. Although RNA modification was discovered as early in 1970s [1,2] but it refuelled the passion of scientific research after the discovery of first m6A-demethylase in 2008 and 2011 [3,4]. To date more than 150-170 chemical modifications have been identified [5,6] in methylating approximately 7,000 human transcripts; among them, 50% is conserved in mouse [7]. The abundance of total RNA in a single cell of 10-30 pg consisting of 85% r-RNA, 15% t-RNA and 1-5% m-RNA, where the majority of m6A-modification takes place in the coding region (CDS) of mRNA enriching near 3'UTR and 5'UTR stop codon requiring conserved DRACH (D = G/A/U; R = G/A; H =

A/C/U) sequences [8-10]. The three major RNA epigenetic modifiers reforming the whole biological functions are writers, erasers and readers. The detailed mechanism of m6A-modification and gene regulation is well described in several excellent journals. However, this review mainly focuses on reforming RNA epigenetic machineries into druggable form to treat haematological disorders especially AML.

2. AML Therapeutics

AML is a group of neoplastic diseases involving bone marrow with or without the involvement of peripheral blood. It is the most common type of haematological disorder prevalent in children & adults and globally affecting nearly 3-million people every year [11]. For some time now, several conventional therapies have been employed to treat AML including chemotherapy and rarely surgery and radiation [11]. Cytotoxic chemotherapy or remission-induction with chemotherapeutic agents (anthracycline and cytarabine) followed by consolidation-therapy involving an allogeneic stem cell transfer (SCT), bone-marrow transplant (BMT) or hematopoietic stem cell transplantation (HSCT) have been used as a standard therapy regimen for prolonged remission of AML [12]. However, due to the delayed diagnosis of AML and the therapy-related morbidity and mortality, these conventional means of treating AML have fallen short when it comes to “do no harm” approach. Recently, with the advancement of NGS and gene-mutational analysis, several new strategies have been under development to minimize the global effects of these conventional therapies [13,14]. One of these new strategies includes the ‘targeted-drug therapy’ which is cell, gene or marker specific and allows the option of treatment in patients where invasive chemotherapy is not feasible. This targeted therapy includes biological (monoclonal antibodies), epigenetic or combination therapies [15] (Figure 1, Table 1). Several antigen-specific monoclonal antibodies have now been approved by the FDA for AML therapy. Among these, CD33-directed Gemtuzumab ozogamicin (GO) mAb was the first to be used for AML therapy [16]. In addition, several studies have now emerged marking DNA methylation as one of the hallmarks of AML carcinogenesis [17] involving DNA methyltransferases (DNMTs) and Ten-eleven-translocation (TET) dioxygenases [18]. Various DNMT inhibitors have been investigated for AML therapy, however only two of these (azacitidine and decitabine) are FDA approved [19]. On the other hand, any mutation in the TET family members results in altered DNA methylation [20]. For example, isocitrate dehydrogenases (IDH1 and IDH2) can inhibit TET2 and can cause hypermethylation resulting in AML progression [21]. Therefore, IDH inhibition can resolve this TET2 reduction-induced hypermethylation [22]. The FDA has approved two oral IDH inhibitors: ivosidenib (AG-120) and enasidenib (AG-221) for AML treatment [23,24]. In addition to DNA modifiers, histone modifiers (HATs and HDACs) have also been implicated in AML treatment [25] by regulating tumor suppressor genes (TSGs) [26,27]. At present, four HDAC inhibitors have been approved by the US FDA including three as panHDACi: belinostat, vorinostat, panobinostat, and romidepsin as a selective HDACi [28]. In this review, we have summarized the therapeutic potential of m6A-modifiers in controlling AML. Furthermore, we have also summarized the current biopharmaceutical companies developing AML therapeutics as RNA modification drugs (RMDs) [6] Figure 1.

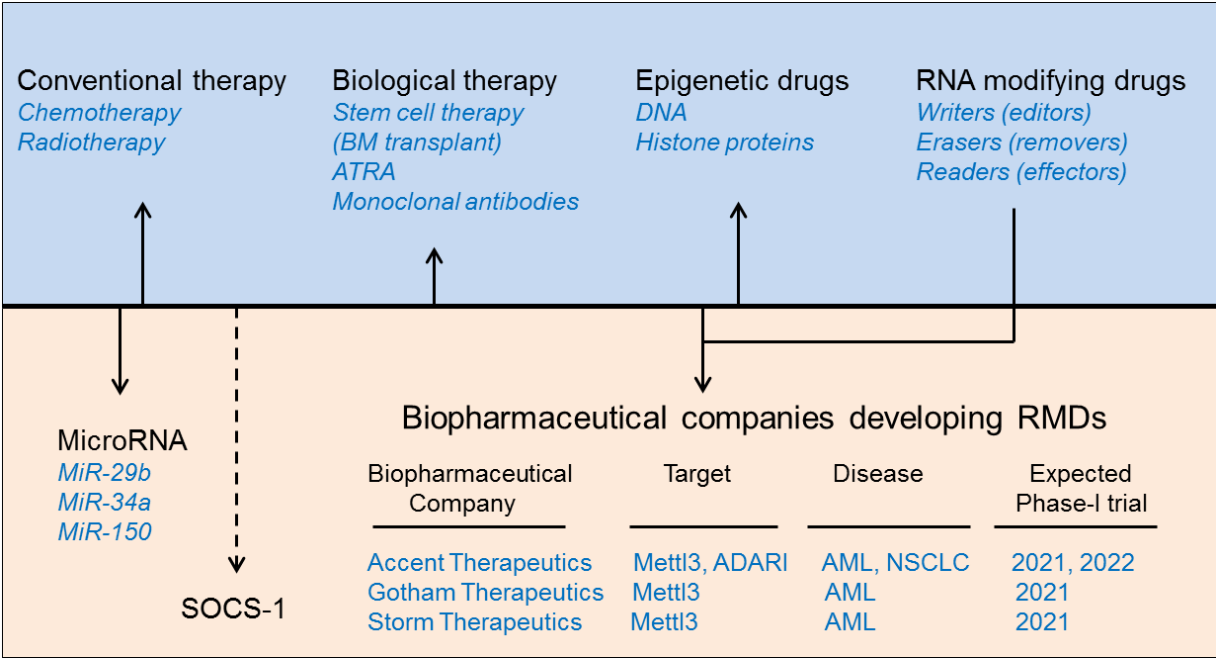


Fig 1. Development of AML therapeutics. The major AML therapeutics are depicted in the diagram above which includes; Conventional therapy (chemotherapy, radiotherapy and surgery), Biological therapy (stem cell therapy or bone marrow transplant), Epigenetic drugs (DNA and histone modifier-based drugs), RNA-modifying drugs (writers/editors, erasers/removers and readers/ effectors), MicroRNAs (miR-29b, miR-34a and miR-150) and Suppressor of cytokine signalling (SOCS1/CISH). Additionally, we have mentioned some biopharmaceutical companies developing RMDs and estimated phase-I clinical trials.

3. Epitranscriptomics in AML therapy

The development of advanced molecular techniques potentiates the opportunities to discover immune cell-based therapeutics over conventional pharmacological-based treatments. This section describes the strategies to treat haematological disorders, especially AML by targeting intracellular RNA-modification machineries, like EEE (Editor/writer: Mettl3, Mettl14; Eraser/remover: FTO, ALKBH5 and Effector/reader: YTHDF-1, YTHDF-2), microRNAs and Suppressor of Cytokine Signalling family of proteins (SOCS/CISH) and as a personalized medicines (Figure 1).

3.1. Editors (Writers):

3.1.1. Mettl3 in AML: The author has demonstrated the role of m6A writer enzyme ‘Mettl3’ in AML progression [29] supported by *Barbieri et. al.*,[30] and enlightened the molecular mechanism to control leukaemia. He found that ‘Mettl3’ is normally expressed in CD34+ hematopoietic stem/progenitor cells (HSPCs) but aberrantly expressed in leukemia cells as compared to the other tumor-types. However, Mettl3-silencing (shRNA) in HSPCs promoted HSPCs differentiation and apoptosis but inhibited self-renewal capacity & proliferation. Conversely, Mettl3 overexpression inhibited cell-differentiation and apoptosis but allowed cell-proliferation and self-renewal capacities. These results clearly suggest that Mettl3 facilitates AML cell growth but resist cell-differentiation. Furthermore, it was also reported that the adoptive transfer of Mettl3-deficient (shMettl3) human AML cell line (MOLM-13) into immunodeficient (recipient) mice induces cell-differentiation and apoptosis but remarkably delayed leukemia progression. This result further supports the key role of Mettl3 in AML progression. Mechanistically, he justified (via m6A-mapping and ribosome profiling) that Mettl3 increases the expression of c-Myc, Bcl2 (apoptosis regulator) and pTEN (a proto-oncogene or AKT regulator) genes by m6A-modification in human leukemic MOLM-1 cell-line. Moreover, Mettl3 regulates AKT-RICTOR signalling (PI3K-AKT) pathway and contributes to AML pro-

gression by increasing cell-proliferation and decreasing cell-differentiation capacities. However, the loss of Mettl3 activates pTEN-mediated AKT phosphorylation and controls AML by reducing cell-proliferation and promoting cell-differentiation, confirmed after treating AML cells with PI3K-inhibitor in blocking cell-differentiation under Mettl3-deficient state [29]. Taken together, these investigations confirm the key role of Mettl3 in facilitating AML growth and survival and potentiate the therapeutic value of Mettl3 in controlling AML by means of either specific inhibition or selective intracellular silencing in AML. These findings were further supported by *Konstantinos et. al., 2019*[31]; by synthesizing two small molecule compounds of Mettl3-inhibitor in inhibiting AML cell expansion (at 30mg/kg oral/intraperitoneal dose in mouse model with no clinical toxicity and weight loss) Table 2. Moreover, targeting AKT-RICTOR signalling pathways by PI3K-inhibitors in combination with other forms of conventional (radio/chemo/stem cell transplant) treatment procedures could overcome AML progression. These hypotheses were well supported by *Batista et. al., 2017* [32] and *Omar et. al., 2018* [33].

3.1.2. METTL14 in AML: Weng et. al., 2018 [34]; demonstrated the role of m6A-writer enzyme 'Mettl14' in AML progression, and enlightened the intrinsic mechanism to control AML by targeting Mettl14. The authors have found that 'Mettl14' is aberrantly expressed in AML patients confirmed by Cancer Genome Atlas (TCGA)-datasheet, human leukemia cell lines (MM6 and NB4), immortalized BM-progenitor cells (MA9, AE9a, PML-RARa) and AML cells carrying t(11q23), t(15;17), or t(8;21) translocation)-mutations, and gradually started decreasing during myeloid differentiation, suggesting the prominent role of Mettl14-mediated m6A-modification in leukemia progression. Experimentally he showed that, the in-vivo depletion of Mettl14 in recipient conditional knockout mice (tamoxifen-induced silencing) Mettl14^{CKO} or Mettl14^{fl/fl}-CRE^{ERT} mice decreases cell proliferation and self-renewal capacity of CD45.2⁺ (of Mettl14^{CKO}) in peripheral blood as compared to the CD45.1⁺CD45.2⁺ (competitor) cells. However, in-vitro depletion of Mettl14 ('shM14' pLKO.1 lentiviral-mediated silencing) in human HSPCs/-CD34⁺ promoted cell-differentiation, which was confirmed by increase of differentiation marker (CD11b-monocyte and CSF1R-macrophage). This suggests that Mettl14 induces leukemia growth by increasing cell-proliferation and decreasing terminal cell-differentiation. Conversely, the addition of differentiation inducing agent; OP9 culture medium [35], PMA (0.5ng/ml/48h) [36] and all-trans retinoic acid (ATRA 500nM/72h) [37] significantly decreased the expression of Mettl14 and overall m6A-abundance in AML cells. Reciprocally, Mettl14 overexpression (Mettl14-R298P vector) inhibits myeloid differentiation as compared to the empty vector. These results clearly suggest that Mettl14 is playing a significant role in AML progression by decreasing cell-differentiation and increasing cell-proliferation, and thus designating 'Mettl14' as a potential therapeutic target for AML treatment. Mechanistically, they have justified that (via RNA-sequencing) Mettl14-methylated methylation of Myb and Myc increase mRNA stability [38] leading to highly upregulated level of these transcription factors (TcFs) in leukaemia's and lymphomas [39-41], confirmed by reduced half-life due to decreased polyribosomes 40S, 60S, 80S (ribosome profiling) and eukaryotic initiation factor-3A (EIF3A)-binding to the Myb and Myc transcript in Mettl14-silenced (shMettl14) MM6 and NB4 (human leukemic) cell lines as compared to the control (shCON) transfections. Furthermore, m6A mapping and immunoprecipitation (miCLIP) revealed increased m6A-binding to the Myb and Myc transcripts relative to the IgG iso-control, suggesting the significant role of Mettl14-mediated m6A-modification in increasing Myb and Myc level and further downstream signalling cascades rather than decaying respective mRNAs. However, they did not find significant involvement of YTHDF2 reader protein in regulating TcFs by mRNA-decay mechanism [38] in Mettl14-deficient AML cells. Collectively, these investigations suggests that Mettl14 promotes AML progression by increasing the activity of Myb (in increasing cell proliferation [42]) and Myc (in inhibiting cell differentiation [43,44]) gene functions. Moreover, they found that PU.1 (Spl1 encoded transcription factor, also known as master regulator of myeloid cells, or an oncogene

product) binds to Mettl14 at 5kb upstream of TSS and negative regulates its expression, confirmed by chromatin immunoprecipitation (ChIP-seq) followed by luciferase reporter assays. Furthermore, Spl1-knockdown increases both mRNA and protein level of Mettl14. Conversely, Spl1 overexpression decreases Mettl14 expression. These results further suggests that Mettl14 itself is targeted by ‘Spl1’ and an additional therapeutic target to control Mettl14 expression. In support of this *Konstantinos et. al., 2019*; recently developed a small molecule ‘inhibitor of Mettl3’ by structure-guided medicinal chemistry tool and confirmed m6A-inhibition and respective cellular effects comparable to the Spl1-inhibitor, This study further support the therapeutic potential of Mettl3-inhibitor in controlling AML progression [31]. Taken together, Mettl14 showed its immunotherapeutic potential by regulating Spl1-Mettl14-Myb/Myc signalling axis, and therefore intracellular silencing of Mettl14 in combination with standard ATRA (differentiation inducing agent) therapy would be a promising approach to control AML with the other forms of therapeutic approaches (radio/chemo/BM-transplantation) [34], (Figure 2, Table 2).

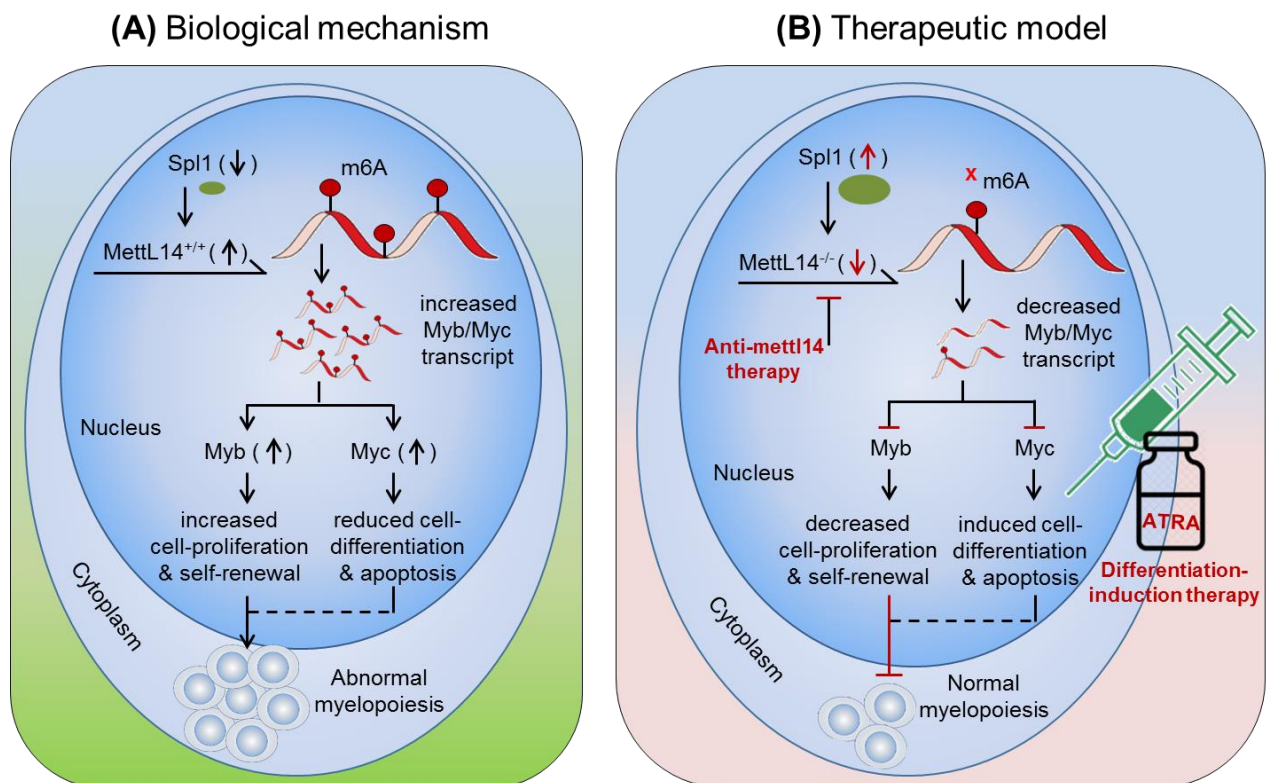


Fig 2. Therapeutic model targeting intracellular m6A-writer enzyme “Mettl14” in controlling AML. **(A)** Biological mechanism: Mettl14 methylates and enhances the expression of Myb and Myc transcription factor. The elevated Myb/Myc increases cell-proliferation and self-renewal capacity and reduces cell-differentiation and apoptotic processes, resulting in abnormal myelopoiesis. **(B)** Therapeutic model: Anti-Mettl14 therapy: intracellular silencing of Mettl14 decreases the expression of Myb/Myc TcFs resulting in decreased cell-proliferation and improved cell-differentiation and apoptotic processes. Differentiation-induction therapy: all-trans retinoic acid (ATRA) further enhances cell-differentiation and apoptotic processes and thus allows normal myelopoiesis. However, targeting other pathways like; Spl1, Myb/Myc, AKT pathways etc. has been also proposed previously [45,46] in controlling AML.

3.2. Erasers (Removers):

3.2.1. FTO in AML: Li, et. al., 2017 [47]; have demonstrated the oncogenic role of FTO, an m6A-demethylase, in facilitating leukemogenesis by promoting cell-proliferation (self-renewal) and reducing cell-differentiation and apoptotic processes, and enlightened the molecular mechanism to control APL by targeting FTO. They found

that, FTO is aberrantly expressed in a specific subset of hematopoietic stem/progenitor cells (HSPCs/CD34⁺CD38⁻) carrying t(11q23)/MLL-rearrangement and t(15;17)/PML-RARA mutations, called acute promyelocytic leukemia (APL), confirmed in one hundred 'affected' and nine 'control' human samples of (CD34⁺CD33⁺ and MNC) cells with Affymetrix GeneChip and qRT-PCR assays. In order to investigate the causative mechanism and the potential target of FTO in facilitating leukemogenesis, they overexpressed FTO in human leukemic 'MONOMAC-6' cell lines via retrovirus expression system (MSCV-PIG-FTO and MSCV-PIG-CON) and voted two stable clones under 0.5µg/ml puromycin selection marker. Before sequencing, they confirmed increase and decrease of FTO and m6A-abundance by western blot and dot-blot/methylene blue assays. Interestingly, the sequencing result revealed two potential target of FTO, namely ankyrin repeat and SOCS box protein 2 (ASB2), involved in controlling leukemia [48] by suppressor of cytokine signalling (SOCS/CISH)-mediated mechanism, and retinoic acid receptor alpha (RARA), a hallmark feature of APL [49], selected on the basis of MACS and exomePeak methods [50,51]. Moreover, conserved m6A-methylation motif 'GGAC' was also confirmed enriching >87% CDS as well as near stop codon vicinity. The authenticity of this potential target gene was validated by dual-luciferase reporter coupled with mutagenesis assays, where, [FTO cloning: pMIRNA1-FTO (wild-type), pMIRNA1-FTO-Mut (mutant) and pMIRNA1-Ctrl (empty vector); and target gene cloning: pMIR-REPORT-ASB2-3'UTR containing wild-type 'GGAC' and mutant 'GGTC' with four m6A-conserved motifs 464bp; RARA-3'UTR with five conserved motif 474bp and RARA-5'UTR with six conserved motif 395bp and transfected into HEK-293T cells/24h] the wild-type FTO significantly decreased the luciferase activity of ASB2 and RARA fragments containing wild-type m6A-methylated 'GGAC' motifs as compared to the other combinations of mutant and control transfections, indicating the requirement of m6A-methylation site for the regulation of FTO-mediated demethylase activity [52,53]. Furthermore, the FTO target was again validated by silencing FTO (shFTO) in MA9/FLT3-ITD leukemic cell lines in hyper-methylating (increased m6A-modification) ASB2 and RARA genes via m6A-seq and RNA-seq respectively. These results clearly suggest that (i) FTO regulate its target gene expression by its m6A-demethylase activity and (ii) the target gene must be m6A-modified. However, under hypo-methylated condition the exact mechanism of FTO-mediated negative regulation of its target gene expression was further justified by mRNA stability testing, where FTO overexpressing cell line (5µg/mL actinomycin D for 0, 3 and 6h) showed increased mRNA-decay (i.e. decreased half-life $t_{1/2} = \ln 2 / K_{\text{decay}}$) compared to the FTO silencing, suggesting the mechanism of target gene control by destabilizing the mRNA stabilities. These investigations clearly suggests that, under diseased state, the aberrantly expressed FTO negatively regulate its target gene expression; neither by directly decreasing m6A-level (hypo-methylation) of the target mRNA nor by m6A-effector protein YTHDF2-mRNA decay-dependent mechanisms, but by directly affecting the mRNA stability via its demethylase activity [38]. However, the possibility of m6A-reader protein (YTHDF1 and YTHDF2)-mediated regulation of ASB2 and RARA also cannot be denied completely due to the finding of increased target gene translation after YTHDF1 knockdown. Nevertheless, the FTO-mediated APL progression is not only by targeting mRNA destabilization, but also by affecting the efficacy of 'ATRA-mediated therapy' [54,55] justified by analysing NB4 cell (human acute promyelocytic leukemia cell line) differentiation marker. Where, the overexpression of FTO significantly inhibits ATRA-induced cell-differentiation of NB4 cells [56] characterized by decreased (differentiated: CD11b⁺ CD14⁺) and increased (un-differentiated: CD11b⁻ CD14⁻) markers compared to the FTO-silenced (shFTO) cells in reversing the both phenotypes. Conversely, the induction of 500mM ATRA significantly decreased FTO and thereby rescued/increased the expression of its target ASB2 and RARA genes [57,58]. Moreover, forced expression of ASB2 and RARA also increases cell-differentiation. Collectively, these results authenticate that FTO is playing a crucial role in promoting acute promyelocytic leukemia (i) by inhibiting the efficacy of ATRA-mediated induction of cell-differentiation and apoptosis, and (ii) by suppressing its

target gene expression. Taken together, these studies revealed the oncogenic property of FTO in promoting APL. Therefore, targeting FTO would be a recommendable approach to control APL with other forms of chemotherapeutic drug combinations [47,59] (Figure 3, Table 2, 3).

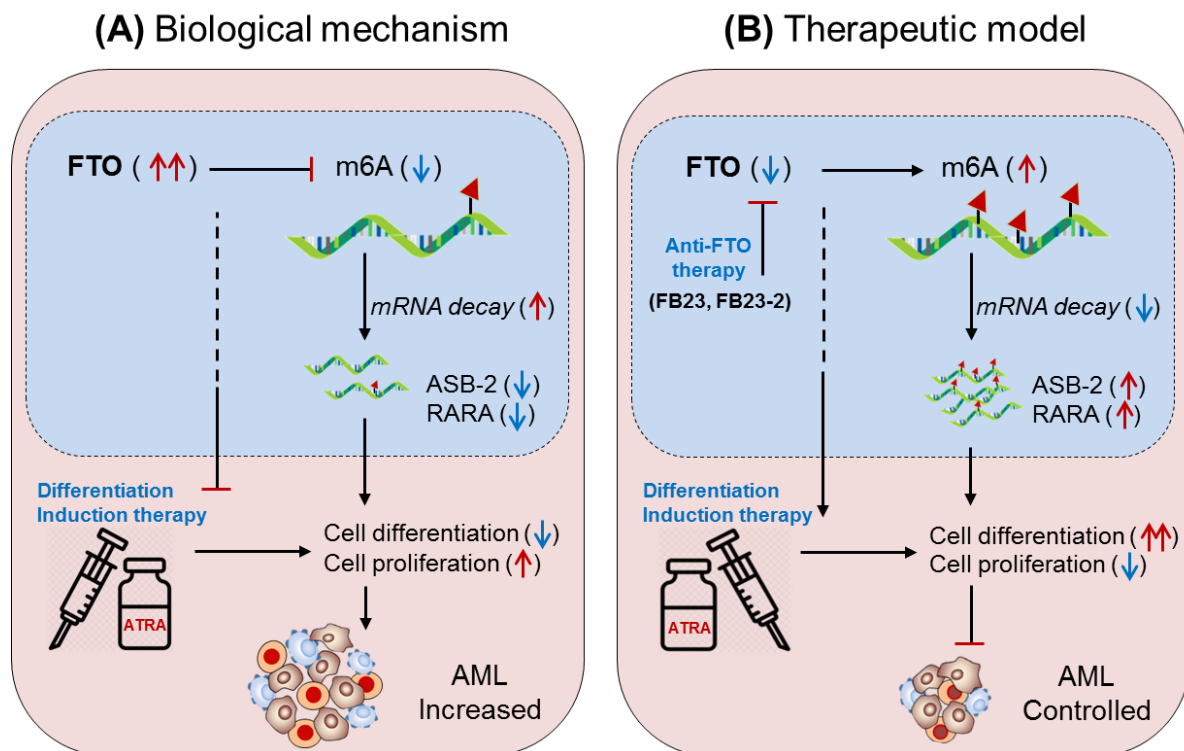


Fig 3. Therapeutic model targeting intracellular m6A-eraser protein FTO in controlling AML. **(A)** Biological mechanism: The m6A-demethylase FTO is abnormally expressed in APL and negatively regulates the expression of its target genes (ASB2 and RARA) causing decreased ATRA-induced cell differentiation and increased self-renewal capacity of APL cells, resulting in rapid APL progression. **(B)** Therapeutic model: Anti-FTO therapy: targeting FTO by means of either selective inhibitor (FB23 and FB23-2) [60] or intracellular silencing (shFTO) efficiently control APL by inducing m6A-mediated increase of ASB2 and RARA genes, as well as by enhancing the efficacy of ATRA-mediated differentiation and apoptosis [54].

3.2.2. ALKBH5 in AML: Shen et. al., 2020 [61]; have demonstrated the role of other m6A-eraser protein 'α-ketoglutarate-dependent dioxygenase AlkB homolog 5' (ALKBH5) in facilitating leukemia stem/initiating cells (LSCs/LICs) progression, a subsets of AML, characterized by high self-renewal capacity, and enlightened intrinsic mechanism to control LSCs by targeting ALKBH5. They found that, likewise the other m6A-eraser protein FTO [47], ALKBH5 also facilitates leukemogenesis, evidenced by significantly higher expression of ALKBH5 in various subtypes of AML cells carrying t(15;17), Inv(16), t(8;21) and t(11q23) mutations, independent of specific TP53-mutation (MONOMAC-6/MMC6, NOMO1 and NB4) and TP53-wild type (MA9.3-ITD and MOLM13) human cell lines, confirmed via GSE42519, GSE13159 Affymetrix, TCGA-sequencing and western blot analysis, as compared to the normal HSCs. However, in vitro silencing (lentiviral shRNA) of ALKBH5 in NOMO1 and MMC6 human cell lines significantly promotes apoptosis (AnnexinV^{hi}PI^{hi}) and restrict cell growth/proliferation with overall decreased % survival of TCGA-AML datasheet [62]. Furthermore, ex-vivo conditional knockout of ALKBH5 in MOLM13 (MOLM13-iKD) cells (doxycycline-inducible conditional knockdown model: where addition of doxycycline depletes ALKBH5, as similar to stable knockout) also promoted apoptosis and reduced cell growth/proliferation as compared to the KO (MOLM13-iCas9) cells. Conversely, overexpression of wild-type ALKBH5 (A5-WT) reversed this effect and promoted AML-cell progression as compared to the mutant (H204A) ALKBH5, suggesting the crucial role of ALKBH5 in facilitating leukemogenesis. In addition to this, significantly increased abundance of 'global m6A-level' was observed in the bone marrow of

ALKBH5-depleted (Exon-1 depletion by CRISPR-Cas9) mice compared to the wild-type mice. Moreover, marked increase in global m6A-level was also noticed in both in vitro/ex vivo ALKBH5-depleted cell lines confirmed by LC-MS/MS and dot-blot analysis as well. These results clearly suggest that (i) the m6A-demethylase ALKBH5 facilitate AML progression and required for its growth and survival. (ii) ALKBH5-depletion enhances global m6A-level. Next, to further validate the requirement of ALKBH5 in the promotion/transformation of AML cells, they used MLL-AF9 (MA9)-induced leukemogenesis model (where, MLL-rearranged fusion protein alone is sufficient to transform normal HSPCs into leukemic cells [63]) coupled with ALKBH5 knockout (ALKBH5^{KO}) model. Interestingly, ALKBH5-depletion significantly inhibited MA9-mediated cell immortalization. On the contrary, forced expression of ALKBH5 (A5-WT) significantly promoted MA9-mediated cell immortalization compared to the mutant ALKBH5 (A5-Mut), confirmed by in vitro colony formation/immortalization assays. Furthermore, adoptive transfer (or bone marrow transplantation-BMT) of ALKBH5-deficient immune population (donor: CD45.2⁺Lin⁻) co-cultured with MLL-AF9 cells into recipient (CD45.1) mice significantly delayed leukemia progression and prolonged survival with decreased splenomegaly, white blood cells count and immature blast cell (CD11b (Mac-1)⁺c-Kit⁺) populations. Moreover, ALKBH5-deletion inhibited the engraftment of MA9-transformed donor cells in the peripheral blood as compared to the ALKBH5-wild type donor under lethal irradiation conditions. These investigations clearly suggest that 'ALKBH5' plays a key role in promoting leukemogenesis of a specific subset of AML cells. The relevant experiments on leukemia xenograft mouse model further potentiate the involvement of ALKBH5 in self-renewal and maintenance of AML-cells. Next, In order to understand the molecular mechanism, ALKBH5-depleted MOLM13 and NOMO1 cells were sent for RNA sequencing (RNA-seq), which revealed TACC3 (transforming acidic coiled-coil-containing protein 3) as a positive target of ALKBH5. [Noticeably, RNA-seq of ALKBH5 [61] revealed: 623 up-regulated and 1237 down-regulated genes; whereas, RNA-seq of another eraser protein FTO by *Li, et. al., 2017* [47] revealed: 2279 up and 888 down-regulated genes. Interestingly, only 251 up-regulated and 119 down-regulated targets were common in both]. Surprisingly, from a total of 18 (12 positive and 6 negative targets of ALKBH5) only 'TACC3' was significant, which was not in-case of FTO, suggesting different target pattern of these two m6A-demethylases/eraser proteins in AML. Furthermore, m6A-sequencing (m6A-seq) revealed enrichment of m6A-abundance at TACC3 mRNA (from a total of 510 transcripts with maximum enrichment around protein coding region and 3'-UTR in ALKBH5-depleted AML cells) confirmed by RNA immunoprecipitation & sequencing (RIP-seq) followed by gene-specific m6A-qRT-PCR. Mechanistically they proved that, the ALKBH5 silencing (shALKBH5) decreases the expression of TACC gene, due to hyper-methylation of m6A, confirmed by decreased half-life of TACC3 (in MOLM13: 2.35h - 1.56h and NOMO1: 3.4h - 1.55h) compared to control (shNS) transfections. Conversely, forced expression of ALKBH5 (A5-WT) increases the half-life (3.63h - 7.47h) of TACC3 in NOMO1 cells compared to mutant (A5-Mut) transfections. Moreover, TACC3 has been seen targeting 'Myc' and 'P-21' genes as well [64,65], confirmed by decreased Myc and increased P-21 protein levels in ALKBH5-silenced NOMO1 and MMC6 cells (*Shen et. al., 2020* figure-6). These result clearly suggests that (i) TACC3 is the bona fide target of ALKBH5. (ii) ALKBH5/m6A/TACC3 axis regulates p53 apoptotic pathways in AML. Next, the functional analysis of TACC3 showed similar phenotypic effect like ALKBH5 justified by increased leukemia cell apoptosis (AnnexinV^{hi} PI^{hi}) and decreased cell-proliferation after silencing TACC3 (shTACC3) in NOMO1 and MMC6 cells confirmed by FACS and colony-formation and replanting capabilities. Collectively, these studies clearly suggests that the m6A-eraser protein ALKBH5 as well as also FTO promotes leukemogenesis, and therefore targeting ALKBH5 by means of either selective inhibitor or specific intracellular silencing could be a potential approach to control AML in humans (Figure 4, Table 2, 3) [61].

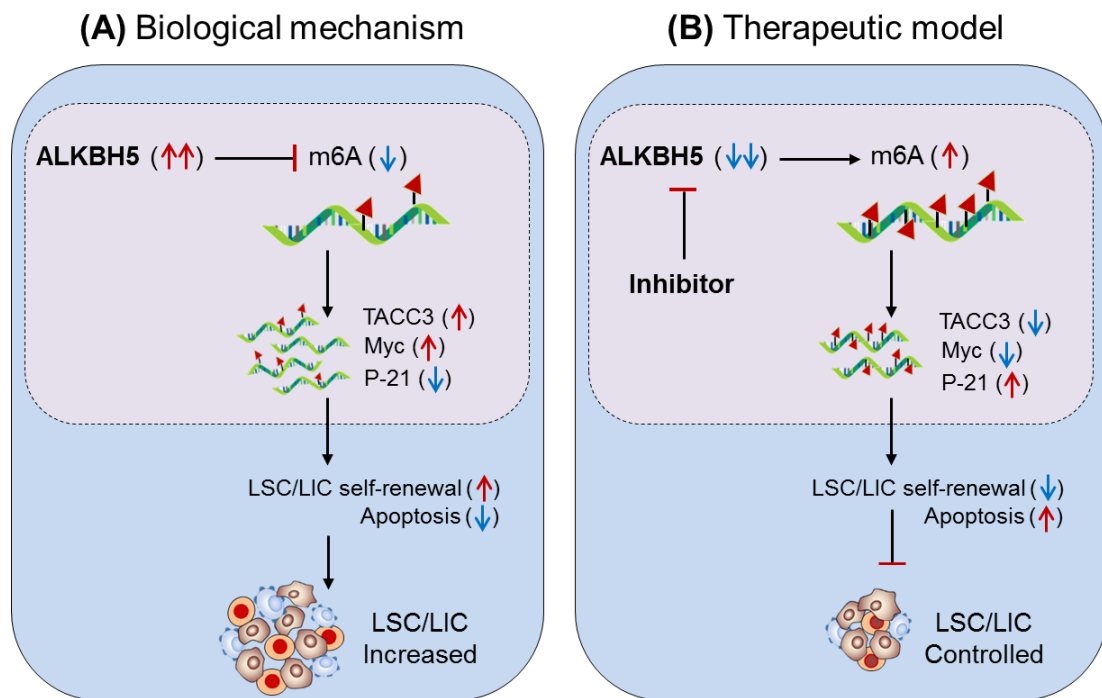


Fig. 4. Therapeutic model targeting intracellular m6A-eraser protein 'ALKBH5' in controlling AML. **(A)** Biological mechanism: The m6A-demethylase 'ALKBH5' is aberrantly expressed in AML, especially in leukemia stem/initiating cells (LSC/LIC) and facilitates its progression, indicating its requirement for development, self-renewal, maintenance and propagation. Biologically, the enhanced ALKBH5 in disease-state increases the expression of transforming acidic coiled-coil-containing protein 3 (TACC3) gene expression by m6A-mediated mechanism, resulting in increased self-renewal capacity and thereby increased progression of LSC/LICs. **(B)** Therapeutic model: Anti-ALKBH5 therapy: The selective inhibition of ALKBH5 decreases m6A methylation-mediated expression of TACC3 gene resulting in decreased self-renewal and increased apoptosis is well-efficient to control AML subsets.

3.3. Effectors (Readers):

3.3.1. YTHDF2 in AML: Paris et. al., 2019 [66]; demonstrated the role of m6A-reader protein 'YTHDF2' in promoting AML, and enlightened the molecular mechanism to control AML by targeting YTHDF2 in HSPCs. They found that, although, YTHDF2 is required for the development of leukemia stem cells (LSC) but also initiates AML development. However, selective depletion of YTHDF2 inhibits the self-renewal capacity of leukemic cells and promotes cell-differentiation and apoptosis, suggesting the therapeutic value of YTHDF2 in controlling AML progression. Experimentally, they found that, YTHDF2 targets tumor necrosis factor- α (TNF- α), required for cell-necrosis and apoptosis [67], and inhibits its expression by m6A-mediated mRNA decay mechanism [38]. However, selective removal of YTHDF2 increased the expression of TNF- α by lowering mRNA decay, confirmed by increased half-life of the target mRNA, and thereby increased terminal cell differentiation and apoptosis. This result clearly suggests that YTHDF2 plays a crucial role in promoting leukemogenesis [66] was also witnessed by Li et. al., 2019 [68]. Therefore targeting YTHDF2 would be a potential approach to control AML by means of either intracellular silencing or via specific inhibitor (Figure 5).

3.3.2. YTHDF2 in stem cell expansion: Li et. al., 2019 [68]; have demonstrated the therapeutic value of m6A-reader protein 'YTHDF2' in treating haematological disorders, especially AML including other types of cancer. The hematopoietic stem progenitor cell (HSPC/CD34⁺CD38⁻) population is believed to be major limiting issue during stem cell transplantation therapy, due to a lesser number of HSPCs populations from a single human umbilical cord blood (hUCB) donor. Therefore, ex-vivo expansion of HSPC population is a major and encouraging challenge for its widespread use. They found that under steady state, the YTHDF2 sequesters its target gene T-cell acute lymphocytic leukemia 1 (Tal1), which is required for the normal proliferation and self-renewal of HSPCs

[69,70] and inhibits its function by m6A-marked mRNA-decay mechanism [38]. Interestingly, the selective depletion of YTHDF2 (YTHDF2^{KO}) in HSPCs or hUCB-HSCs significantly boosted (10-fold increase) the number of HSPCs by rescuing the expression of Tal1 gene confirmed by increased mRNA stability. This result clearly suggests that YTHDF2 has therapeutic potential and can be clinically used to expand ex-vivo population of normal HSPCs by selective silencing and specific inhibitors of YTHDF2 [68]. However, the requirement of YTHDF2 in the maintenance of HSCs, by targeting pro-inflammatory cytokines, was also demonstrated by *Mapperley et. al.*, 2020 [71] (Figure 5).

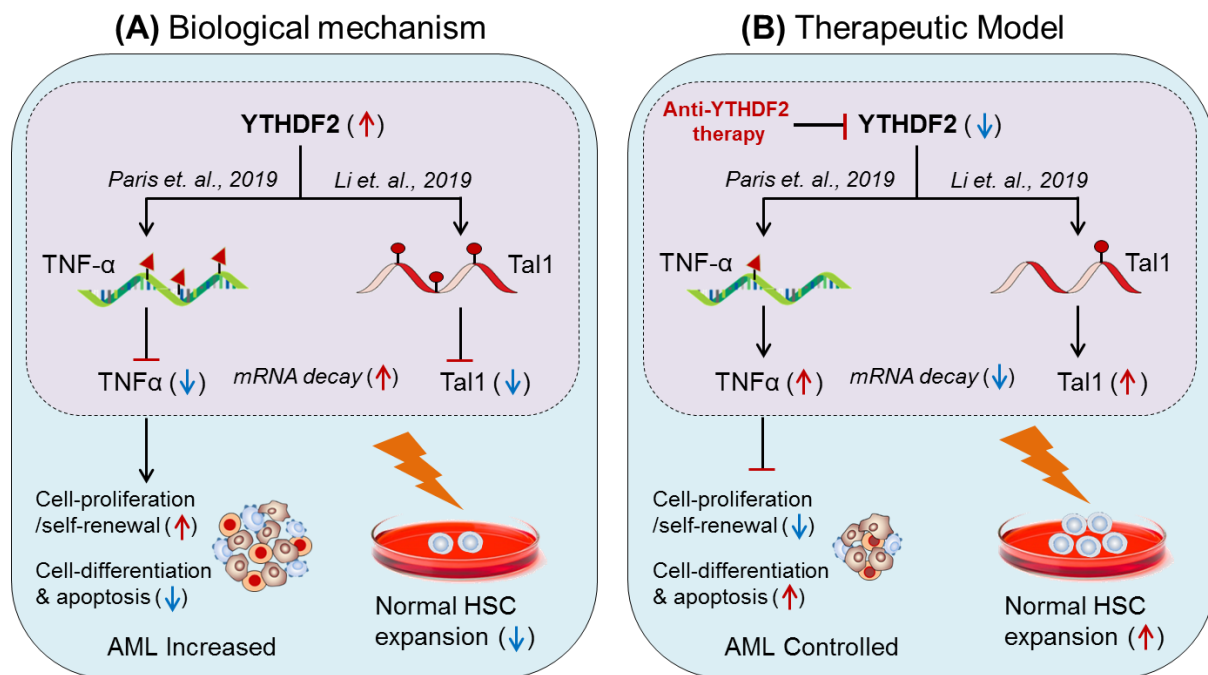


Fig. 5. Therapeutic model targeting intracellular m6A-reader protein 'YTHDF2' in controlling AML, and in expanding normal HSCs for BM-transplant. **(A)** Biological mechanism: YTHDF2 facilitates leukemia progression by suppressing TNF- α *Paris et. al.*, 2019 [66]. Whereas, hyper-methylation of Tal1 causes HSC-limitations during BM/stem cell transplant *Li et. al.*, 2019 [68]. **(B)** Therapeutic model: Anti-YTHDF2 therapy controls AML progression as well as allows normal HSCs expansion in vitro by targeting respective genes via mRNA-decay mechanism.

4. Therapeutic Strategies

4.1.1 Mettl3 Inhibitor: Recently, Eliza et. al., 2021[72] have discovered the selective inhibitor of Mettl3 and Mettl14 (STM2457, IC₅₀ = 16.9 nM) in controlling AML via high-throughput screening of 250,000 drug-like compounds. The binding affinity and specificity of STM2457 was confirmed by surface plasmon resonance (SPR) and X-ray crystallography as well as by intraperitoneal injection of STM2457 (50mg/kg) in selectively reducing m6A on poly-A⁺-enriched mRNA, with no effect on other mRNA modifications. Functionally, they showed significant effect of STM2457 in reducing clonogenic potential and inducing apoptosis in human and mouse AML model without affecting normal human cord blood (CD34⁺/HSPCs) and non-leukemia (HPC7) hematopoietic cells. Moreover, in vivo studies on 3-human AML patient derived xenograft (PDX) model and primary murine MLL-AF9/Flt3^{ltd/+} model showed convincing anti-leukemia effect compared to control STM2120 inhibitors [72]. Furthermore, the other researchers also suggested the significant effect of Mettl3/14-inhibitors as anti-leukemia effect (Table 3) [6,31,33].

4.1.2 FTO Inhibitor: Huang et. al., 2019 [60]; have examined the therapeutic efficacy of two synthetic small molecule inhibitor of FTO (FB23 and FB23-2) in controlling AML.

They found that the m6A-demethylase 'FTO' is highly upregulated in certain AML-subtypes and facilitates leukemogenesis by promoting cell-proliferation and inhibiting terminal cell differentiation. The FTO inhibitor especially 'FB23-2' dramatically suppresses cell-proliferation and promotes differentiation and apoptosis of both the cell lines as well as the primary cells in xeno-transplanted mice. Mechanistically, the synthesized inhibitors directly bind to the FTO and selectively inhibit its m6A-demethylase activity, mimicking FTO depletion similar to in vivo knockout. This result suggests that these FTO inhibitors (FB23 and FB23-2) could be a potential druggable candidate to treat leukemia [60], supported by Yang *et. al.*, 2019 [7] (Table 3).

4.1.3. FTO in anti-tumor immunity (AML): Su *et. al.*, 2020 [73] have demonstrated the clinical value of two synthetic small-molecule inhibitor of FTO, designed on the basis structural guided tool, (CS1 and CS2) in controlling AML, especially leukemia stem cell (LSC) progression in the aspect of anti-tumor immunity. They found that, the abundantly expressed FTO facilitates LSCs progression by impairing T-cell activity via increasing the expression of an immune inhibitory checkpoint molecule/gene 'LILRB4'. The application CS1 and CS2 significantly controls LSC progression in mouse model with low drug toxicity. Mechanistically, CS1 and CS2 were found to be selectively binds to the FTO and inhibit its demethylase activity, resulting in decreased expression of 'LILRB4' via inhibiting YTHDF2-mediated mRNA-decay mechanism. Moreover, increased T-cell cytotoxicity and reduced self-renewal abilities of AML cells were noted in the treated mice, suggesting that CS1 and CS2 is a potent inhibitors of FTO and can be developed as a potential therapeutics against LSC by increasing anti-leukemia T-cell activity [73]. This hypothesis was further supported by Olsen *et. al.*, 2020 [74], (Figure 6). Additionally, some other FTO-inhibitors are well described in these articles [75,76] and [77,78] (Table 2, 3).

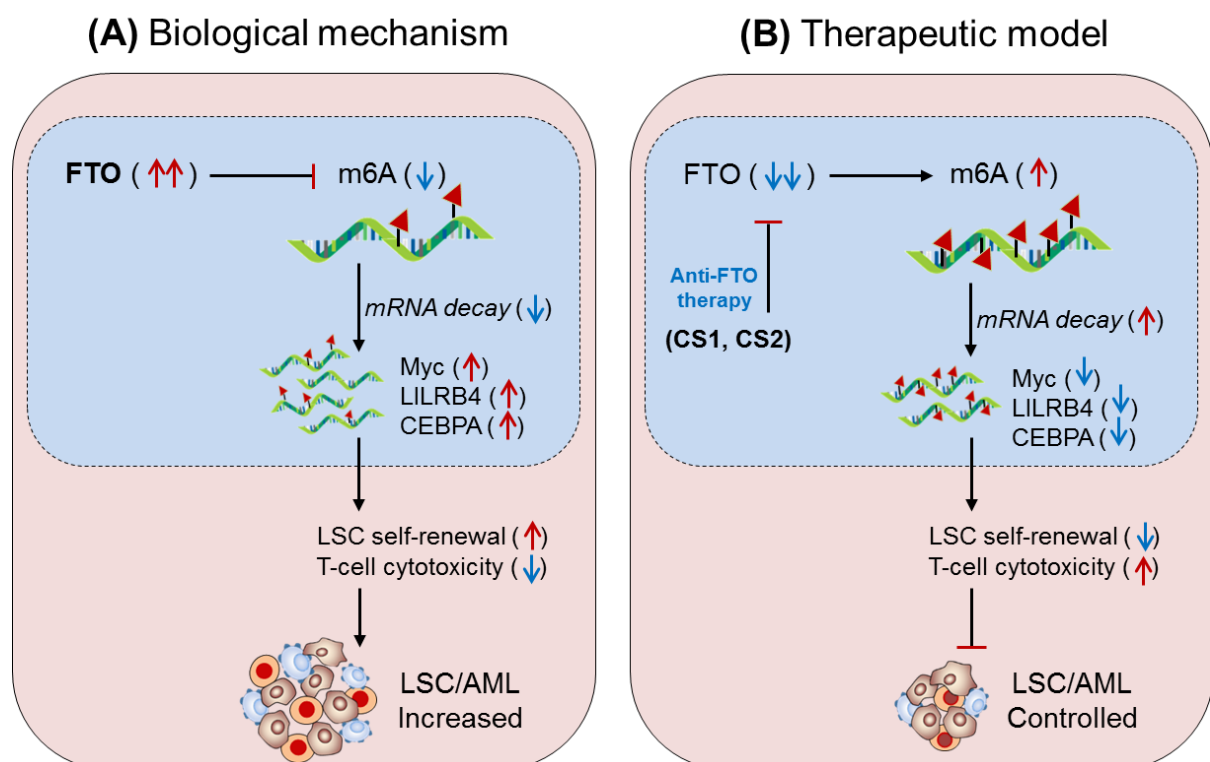


Fig. 6. Therapeutic model targeting m6A-eraser 'FTO' in controlling LSC progression by enhancing anti-tumor immunity. (A) Biological mechanism: FTO has been found to be aberrantly expressed in AML, especially LSC populations, and facilitates its progression. Mechanistically, the FTO enhance the expression of its target LILRB4 (immune checkpoint) and other Myc and CEBPA genes via its m6A-demethylase activity by inhibiting m6A-reader protein YTHDF2-mediated mRNA-decay mechanism, causing decreased T-cell activity and increased self-renewal capacity, resulting in enhanced LSC progression. (B) Therapeutic model: Anti-FTO therapy: The two synthetic small-molecule inhibitor of FTO (CS1 and CS2) selectively binds to the FTO domain and inhibits its demethylase activity, leading to decreased expression of its target (LILRB4, Myc and CEBPA) mRNA via reducing YTHDF2-mediated

mRNA-stability. The decreased expression of the targeted gene ultimately enhances T-cell cytotoxicity and immune evasion and thereby reduced self-renewal capacities, resulting in better control over LSC propagation.

5. MicroRNA in AML

5.1.1. MiR-150 in AML: In addition to m6A-modifiers, microRNAs also showed promising outcome in the treatment of acute myeloid leukemia (AML). *Fang and colleague*, 2016 [79] have demonstrated the importance of miR-150 in controlling leukemia progression. They showed that miR-150 is down-regulated in AML and CML patients, but normalized after complete remission/treatment. However, miR-150 restoration therapy (miR-150 mimic) significantly inhibited the AML by reducing cell-proliferation and promoting apoptosis of the leukemia stem cells, leading to reduced tumorigenicity in xenograft leukemia model. The underlying mechanism evidenced that miR-150 targeted genes are mainly associated with RNA metabolism (synthesis, export, splicing and stability), transcriptional regulation, wnt-signalling and mTOR-signalling pathways. Interestingly, knockdown of any of these miR-150 downregulated targets (TET3, EIF4B, FOXO4B and PRKCA) showed anti-leukemia activity similar to miR-150 restoration therapy [80]. Conclusively, these results authenticate the druggable value of miR-150 in treating AML and as a novel candidate for therapeutic drug development [79].

5.1.2. MiR-34a in AML: miR-34a was shown to be another microRNA playing a crucial role in controlling elderly AML, who were ineligible for conventional chemotherapy. The author [81] have found that, the combination of decitabine (dacogen/DAC; known as 5-aza-2'-deoxycytidine) and all-trans retinoic acid (ATRA; a well-known differentiation inducing agent) effectively control AML and prolong overall survival rate by 49.6% in a clinical trial of 36-elderly leukemia patients. The underlying mechanism demonstrates that the combination of DAC and ATRA inhibited the expression of DNA methyltransferase 1 (DNMT1) resulting in activation of miR-34a by hypo methylation. The activated miR-34a further inhibits the expression of Myc [43,44] resulting in cell cycle arrest and increased apoptosis in vitro. This result suggests that miR-34a could be a druggable candidate to control AML progression by modulating miR-34a/Myc axis [81,82].

5.1.3. MiR-29b in AML: *Liu et. al.*, 2019; demonstrated that miR-29b is also playing an important role in controlling (LSCs) leukemia stem cell progression, a subtype of acute myeloid leukemia, characterized by increased self-renewal capacity and decreased apoptosis, by targeting LSC-fucosylation. The author found that fucosyltransferase 4 (FUT4) is overexpressed in LSC population (CD34⁺CD38⁻) compared to the non-LSC (CD34⁺CD38⁺, CD34⁻CD38⁺, CD34⁻CD38⁻) populations isolated from MOLM13 and KG-1a cell lines determined by LTL lectin assays. However, selective depletion of FUT4 (shFUT4) significantly decreased cell-proliferation and induced apoptosis of the LSCs confirmed by reduced sphere formation ability (colony formation assay), reduced Ki67 cell-proliferation (immunofluorescence) and increased LSC apoptosis (TUNEL assays) indicated by increased cleavage of apoptotic (PARP and caspase3) markers. Moreover, in-vitro application of chemotherapeutic drug (ADR, Ara-C and Paclitaxel) in combination with shFUT4-LSC drastically reduced LSC cell-proliferation as compared to shFUT4 and drug effect alone. Furthermore, mouse xenograft studies showed decreased tumour growth when given in combination with shFUT4 and ADR relative to the individual treatments. This result clearly suggests that (i) FUT4-mediated fucosylation facilitate LSCs progression. (ii) FUT4 silencing overcome chemotherapeutic drug resistance in AML. Mechanistically, he proved that FUT4 is regulated by specificity protein 1 (Sp1) transcription factor confirmed by Sp1 binding to FUT4 promoter via chromatin immunoprecipitation (ChIP) and dual luciferase assays. Interestingly, miR-29b directly binds with Sp1 (bioinformatics analysis) and inhibits its expression along with Wnt/ β -catenin activation, confirmed by miR29b-mimic in 'inhibiting' and anti-miR29b in

'promoting' Sp1 expression and thus influences FUT4 expression accordingly, was further validated by partial restoration of FUT4 expression by co-transfecting anti-miR29b with siSP1. Moreover, Wnt/ β -catenin pathway inhibition by DKK inhibitor induces apoptosis and reduces cell-proliferation. Taken together, these investigations clearly suggest that miR-29b could be a potential candidate to control LSC progression by regulating miR-29b/Sp1/FUT4 axis [83].

6. Suppressor of cytokine signalling (SOCS/CISH) in AML

In addition to epigenetic modifiers (RMDs) and microRNAs; the role of cytokine-inducible SH2-domain containing protein (CISH or CIS) as anti-tumor and anti-leukemia activity, also, cannot be denied because of its versatile role in regulating cytokine signalling via sensitizing immune cells. For example, (i) CISH knockdown has been shown in increasing anti-tumor immunity by enhancing CD8⁺T-cell effector function [84]. (ii) CISH-knockdown also increases NK-cell fitness and cytotoxicity [85] and thus playing a crucial role in protecting tumor metastasis [86]. (iii) More relevantly, CISH has been shown in maintaining T-cell homeostasis via Mettl3-mediated methylation mechanism [87,88] and (iv) The m6A-reader protein 'YTHDF2' is involved in controlling NK cell-mediated anti-tumor immunity [89]. Furthermore, the specific function of CISH in association with acute myeloid leukemia was demonstrated by *Zhu et. al.*, [90], where the importance of CISH in NK cell-mediated anti-leukemia activity has been described. They have showed that selective depletion of CISH in NK cells (CISH^{-/-}) derived from human iPSC (induced pluripotent stem cells) significantly enhance the expansion as well as survival in the tumor microenvironment (TME), confirmed by improved metabolic fitness of NK-cells, characterized by increased glycolytic capacity and mitochondrial respiration (OxPhos activity) via mammalian target of rapamycin (mTOR) signalling response. Moreover, increased NK-cell cytotoxicity was reported against multiple tumor cell lines as well as in in-vivo leukemia xenograft model [90]. This result additionally supports the role of CISH as an alternative approach to control AML by encompassing immune cell-based therapeutics. Nevertheless, the role of professional antigen presenting cells like, dendritic cells (DCs) also cannot be circumvent for its proven role in improving anti-tumor immunity by targeting CISH [91,92]. One more relevant research outcome showed the involvement of suppressor of cytokine signalling 1 (SOCS1), one of the members of CISH/SOCS family, in mimicking AML like phenotype. Where, the overexpression of SOCS1 in zebrafish model showed increased myelopoiesis with distorted kidney and splenic morphology, suggesting the therapeutic inference of AML by targeting SOCS1 (Figure 1) [93].

7. Conclusions

In this review, we have described the potential of m6A-modifiers in developing cancer precision medicines and explained how 'epitranscriptomics' plays a central role in regulating the crucial genes associated with AML. Among these, the m6A-writer protein Mettl3 and Mettl14 has been found to be aberrantly expressed in specific sub-types of AML and promotes leukemogenesis by regulating Myb, Myc, Bcl2, pTEN and PI3K-AKT pathways [29,30,34]. Likewise, m6A-methylases (writer); m6A-demethylases FTO and ALKBH5 (eraser) have also been found to be aberrantly expressed in APL & LSCs/LICs and promote leukemogenesis by targeting ASB2, RARA, LILRB4, CEBPA, TACC3, Myc and P-21 gene [47]. Nevertheless, FTO single nucleotide polymorphisms (SNPs) has been also found to be associated with other cancers [94]. Likewise, writer and eraser; the reader protein 'YTHDF2' has been also found in promoting leukemogenesis by inhibiting its target Tla1 [68] and TNF- α [66] through universal YTHDF2-mediated mRNA decay mechanism [38]. These inventions suggest the different mechanism of action 'of these epigenetics modifiers' in controlling AML by regulating respective gene targets. However, still some questions remained unanswered, how all these epigenetic modifiers are up-regulated simultaneously and facilitates AML development. *For example, considering the balance mechanism of 'writer' and 'eraser' enzymes in regulating 'Myc' in the same cells by*

Mettl3-methylation as well as by FTO-mediated mechanism remains to be further justified. A justifiable answer seems to be possible by recruiting different gene set by inviting different RNA-machineries. Nevertheless, The microRNA; miR-150, miR-34a and miR-29b has also shown significant anti-leukemia effect by targeting wnt-signalling, mammalian target of rapamycin (mTOR)-signalling pathways [79] Myc [81] and Sp1/FUT4-fucosylations [83]. In addition to this, the immune-based therapeutics should also consider in AML treatment by utilizing dendritic cells, T-cells and NK cell-targeting CISH-mediated signalling [87,89,95]. Therefore, it is very critical to understand the stages of AML progression and so in implementing these exciting RMDs in eradicating AML with other forms of standard chemotherapeutic drugs.

8. Future Prospective

Targeting m6A-modification machineries has revolutionized the class of epigenetic research in discovering novel therapeutic drug targets. Co-targeting of intracellular genes along with m6A-modification machineries will improve the level of immunotherapy against those cancers which are resistant to the immune checkpoint-based therapeutics in patients with blood cancer. The future is not so far where targeted therapy in combination with other drugs which would become a universal panacea to control many diseases and other forms of cancer. Moreover, targeting suppressor of cytokine signalling family of proteins (SOCS/CISH) would further potentiate the immunotherapeutic potential of RNA modification inhibitors by targeting epigenetic regulator of dendritic cells. Pharmaceutical approaches pertaining to RNA epigenetic modification machinery are expected to shed light on the field of cancer immunotherapy for AML and other forms of blood cancer. Nevertheless, a human clinical trial is required under a medical setup to access the therapeutic value of these inhibitors by optimizing the initial doses or/if intracellular silencing (gene therapy). However, a proposed clinical trial is underway to conduct till 2021-2022 by several biopharmaceutical companies mentioned above.

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Table 1. Therapeutic drug for Acute Myeloid Leukemia (AML).

Conventional drugs

Drug	Brand/ other name	Drug type	Clinical trial	Ref.
Midostaurin	Rydapt (Novartis)	Multikinase FLT3 inhibitor	FDA approved	[96]
CPX-351	Vyxeos (Jazz Pharma)	cytarabine and daunorubicin combination (5:1 molar ratio)	FDA approved	[97,98]
Vosaroxin	Formerly SNS-595 (Sunesis Pharma)	Topoisomerase II inhibitor: anticancer quinolone derivative (AQD)	FDA approved	[99]
Gilteritinib	ASP2215, Xospata (Astellas Pharma)	Dual inhibitor of FLT3/AXL	FDA approved	[100,101]
Venetoclax	Venclexta, Venclyxto (AbbVie, Genentech)	Bcl2-inhibitor	Phase III	NCT02993523 NCT03069352 [102]
Epigenetic drugs				
Azacitidine	Vidaza	DNMT inhibitor (Hypomethylating agent)	FDA approved	[19]
Decitabine	Dacogen	DNMT inhibitor (Hypomethylating agent)	FDA approved	[19]
Ivosidenib	Tibsovo (AG-120)	IDH1 inhibitor	FDA approved	[23,24]
Enasidenib	Idhifa (AG-221)	IDH2 inhibitor	FDA approved	
Belinostat	Beleodaq (PXD101)	Pan-HDAC inhibitor	Phase II	[103]
Vorinostat	Zolin	Pan-HDAC inhibitor	Phase I/II	[104]
Panobinostat	-	Pan-HDAC inhibitor	Phase I/II	[105-107]
Romidepsin	Istodax	Selective HDAC inhibitor	Preclinical	[28]
Guadecitabine	SGI-110	Dinucleotide of decitabine and deoxyguanosine	Phase III	NCT02348489
Monoclonal Antibodies (mAbs)				
Gemtuzumab Ozogamicin	GO (Mylotarg, Wyeth Pharma)	CD33-targeted	Phase 2	NCT03374332 NCT00372593
Vadastuximab talirine	SGN-CD33A (Seattle Genetics)	CD33-targeted	Phase I	[108]
Daratumumab	Darzalex Faspro (Janssen Biotech)	CD38-targeted	Phase II	NCT03067571
RG7356		CD44-targeted	Phase I	[109]
Apamistamab	Iomab-B	CD45-targeted	Phase III, SIERRA	NCT02665065

Table 2. RNA epigenetic machineries as a therapeutic target in AML.

RNA modifiers	Disease condition	Target	Mechanism of action	Therapeutic strategies	Ref.
Writers Mettl3	up-regulated in AML	Myb/Myc, Bcl2, pTEN, Sp1 (PU.1), PI3K-AKT pathway	Inhibiting cell differentiation & apoptosis and promoting cell proliferation (self-renewal) capacity.	Selective Mettl-3/14 inhibitor or Targeted therapy	[29,32] [34,72]
Mettl-14					
Erasers FTO	up-regulated in APL and LSC/LICs	Myc, CEBPA	Regulated by FTO related with Leukemia	Targeted silencing or specific FTO/ALKBH5 inhibitor	[48]
		ASB2, RARA, Myc, LILRB4, CEBPA	By inhibiting ASB2 and RARA gene targets as well as ATRA-induced cell differentiation and apoptosis		[47]
		TACC3, Myc, P21	By impairing self-renewal capacities		[61]
Readers YTHDF2	up-regulated in AML	T-cell acute lymphocytic leukemia (Tal1)	YTHDF2 inhibit the expression of essential T-cell acute lymphocytic leukemia 1 (Tal1) gene.	Targeted silencing/therapy	[68]
YTHDF2	up-regulated	Tumor Necrosis Factor- α (TNF α)	YTHDF2 inhibit the expression of TNF α required for cell necrosis and apoptosis.		[66]

Table 3. Selective inhibitors of m6A-modifiers in AML treatment.

Name		Therapeutic application		Ref.
	Disease	Epigenetic Inhibitor	Function	
Writers Mettl3, Mettl14 Inhibitors	AML	STM2457 (STC-15)	By selective inhibition of Mettl-3/14 (<i>proposed human clinical trial in 2022 by Storm Therapeutics</i>)	[31,33,72]
	Metabolic disease	SAH (S-Adenosyl-homo cysteine)	Selective inhibition of Mettl-3/14 by targeting endogenous metabolite	[110]
	Cancer	UZH1/ UZH1a	Promotes apoptosis and enhance T-cell anti-tumor activity	[31,111]
Erasers FTO Inhibitors	Up-regulated in AML	FB23	Selective FTO inhibitor works by retaining m6A-demethylase activity in LSCs	[60,112]
		FB23-2		[7,60]
		R-2-hydroxyglutarate (R-2HG)	Selective FTO inhibitor targeting m6 A/MYC/ CEBPA signalling and increase anti-tumor immunity	[75]

		CS1 and CS2	Selective FTO inhibitor targeting LILRB4 immune checkpoint gene	[73,74]
	Glioblastoma Stem Cells	Non-steroidal anti-inflammatory drug, (MA2) an ethyl ester form of meclofenamic acid	Selective FTO inhibitor targeting ADAM19 gene by m6A hyper-methylation	[113,114]
	Triple-negative inflammatory breast cancer	MO-I-500	Selective inhibitor of FTO targeting IRX3 gene in SUM149-MA cells	[115]
	CNS (epilepsy)	BBB-penetrating small molecule inhibitor of FTO	First FTO inhibitor with anticonvulsant activity by targeting various microRNAs	[116]
Readers	AML	BET inhibitor (OTX015)	Bromodomain inhibition	[117-119]
YTHDF2 Inhibitors		MB-3 inhibitor of KAT2A	MB-3 Inhibit the expression of KAT2A gene	[120,121]

References

- Desrosiers R, Friderici K, Rottman F: **Identification of methylated nucleosides in messenger rna from novikoff hepatoma cells.** *Proc Natl Acad Sci U S A* (1974) **71**(10):3971-3975.
- Perry RP, and Kelley, D.E.: **Existence of methylated messenger rna in mouse l cells.** (January 1974) **Volume 1, Issue 1**(Pages 37-42.
- Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C: **N6-methyladenosine in nuclear rna is a major substrate of the obesity-associated fto.** *Nat Chem Biol* (2011) **7**(12):885-887.
- Jia G, Yang CG, Yang S, Jian X, Yi C, Zhou Z, He C: **Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and rna by mouse and human fto.** *FEBS Lett* (2008) **582**(23-24):3313-3319.
- Shi H, Wei J, He C: **Where, when, and how: Context-dependent functions of rna methylation writers, readers, and erasers.** *Mol Cell* (2019) **74**(4):640-650.
- Cully M: **Chemical inhibitors make their rna epigenetic mark.** *Nat Rev Drug Discov* (2019) **18**(12):892-894.
- Van Der Werf I, Jamieson C: **The yin and yang of rna methylation: An imbalance of erasers enhances sensitivity to fto demethylase small-molecule targeting in leukemia stem cells.** *Cancer Cell* (2019) **35**(4):540-541.
- Kane SE, Beemon K: **Precise localization of m6a in rous sarcoma virus rna reveals clustering of methylation sites: Implications for rna processing.** *Mol Cell Biol* (1985) **5**(9):2298-2306.

9. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR: **Comprehensive analysis of mrna methylation reveals enrichment in 3' utrs and near stop codons.** *Cell* (2012) **149**(7):1635-1646.
10. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R *et al*: **Topology of the human and mouse m6a rna methylomes revealed by m6a-seq.** *Nature* (2012) **485**(7397):201-206.
11. Pelcovits A, Niroula R: **Acute myeloid leukemia: A review.** *R I Med J* (2013) (2020) **103**(3):38-40.
12. Daver N, Wei AH, Pollyea DA, Fathi AT, Vyas P, DiNardo CD: **New directions for emerging therapies in acute myeloid leukemia: The next chapter.** *Blood Cancer J* (2020) **10**(10):107.
13. Leisch M, Jansko B, Zaborsky N, Greil R, Pleyer L: **Next generation sequencing in aml-on the way to becoming a new standard for treatment initiation and/or modulation?** *Cancers (Basel)* (2019) **11**(2).
14. DiNardo CD, Cortes JE: **Mutations in aml: Prognostic and therapeutic implications.** *Hematology Am Soc Hematol Educ Program* (2016) **2016**(1):348-355.
15. Walasek A: **The new perspectives of targeted therapy in acute myeloid leukemia.** *Adv Clin Exp Med* (2019) **28**(2):271-276.
16. Morsink LM, Walter RB: **Novel monoclonal antibody-based therapies for acute myeloid leukemia.** *Best Pract Res Clin Haematol* (2019) **32**(2):116-126.
17. Yu J, Xie T, Wang Z, Wang X, Zeng S, Kang Y, Hou T: **DNA methyltransferases: Emerging targets for the discovery of inhibitors as potent anticancer drugs.** *Drug Discov Today* (2019) **24**(12):2323-2331.
18. Yang X, Wong MPM, Ng RK: **Aberrant DNA methylation in acute myeloid leukemia and its clinical implications.** *Int J Mol Sci* (2019) **20**(18).
19. Thomas X: **DNA methyltransferase inhibitors in acute myeloid leukemia: Discovery, design and first therapeutic experiences.** *Expert Opin Drug Discov* (2012) **7**(11):1039-1051.
20. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A: **Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by mll partner tet1.** *Science* (2009) **324**(5929):930-935.
21. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS *et al*: **Leukemic idh1 and idh2 mutations result in a hypermethylation phenotype, disrupt tet2 function, and impair hematopoietic differentiation.** *Cancer Cell* (2010) **18**(6):553-567.

22. Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, Ng V, Xia B, Witkowski MT, Mitchell-Flack M, Grillo I *et al*: **Restoration of tet2 function blocks aberrant self-renewal and leukemia progression.** *Cell* (2017) **170**(6):1079-1095 e1020.
23. Golub D, Iyengar N, Dogra S, Wong T, Bready D, Tang K, Modrek AS, Placantonakis DG: **Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics.** *Front Oncol* (2019) **9**(417).
24. Zeng Z, Konopleva M: **Concurrent inhibition of idh and methyltransferase maximizes therapeutic efficacy in idh mutant acute myeloid leukemia.** *Haematologica* (2021) **106**(2):324-326.
25. Lee E, Koh Y, Hong J, Eom HS, Yoon SS: **Recent clinical update of acute myeloid leukemia: Focus on epigenetic therapies.** *J Korean Med Sci* (2021) **36**(13):e85.
26. Garcia-Manero G, Yang H, Bueso-Ramos C, Ferrajoli A, Cortes J, Wierda WG, Faderl S, Koller C, Morris G, Rosner G, Loboda A *et al*: **Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [sahal]) in patients with advanced leukemias and myelodysplastic syndromes.** *Blood* (2008) **111**(3):1060-1066.
27. Xu QY, Yu L: **Epigenetic therapies in acute myeloid leukemia: The role of hypomethylating agents, histone deacetylase inhibitors and the combination of hypomethylating agents with histone deacetylase inhibitors.** *Chin Med J (Engl)* (2020) **6**:699-715.
28. San Jose-Eneriz E, Gimenez-Camino N, Agirre X, Prosper F: **Hdac inhibitors in acute myeloid leukemia.** *Cancers (Basel)* (2019) **11**(11).
29. Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, Chou T, Chow A, Saletore Y, MacKay M, Schulman J *et al*: **The n(6)-methyladenosine (m(6)a)-forming enzyme mettl3 controls myeloid differentiation of normal hematopoietic and leukemia cells.** *Nat Med* (2017) **23**(11):1369-1376.
30. Barbieri I, Tzelepis K, Pandolfini L, Shi J, Millan-Zambrano G, Robson SC, Aspris D, Migliori V, Bannister AJ, Han N, De Braekeleer E *et al*: **Promoter-bound mettl3 maintains myeloid leukaemia by m(6)a-dependent translation control.** *Nature* (2017) **552**(7683):126-131.
31. Konstantinos T ED, Eliza Y, Justyna R, Demetrios A, Ana FD, Richard F, Alan H, Dan L, Yaara OR, Alexandra S, Cristina P, Mark A, Wesley B, Oliver R, George SV, Tony K: **Pharmacological inhibition of the rna m6a writer mettl3 as a novel therapeutic strategy for acute myeloid leukemia.** *Blood* (2019) **134**: 403(
32. Schmiechen AC, Batista PJ: **An epitranscriptomic vulnerability in myeloid malignancies.** *Nat Med* (2017) **23**(11):1252-1254.
33. Abdel-Wahab O: **Rna methyltransferases as a new therapeutic target in leukemia.** *The Hematologist* (2018) **15** (2)(

34. Weng H, Huang H, Wu H, Qin X, Zhao BS, Dong L, Shi H, Skibbe J, Shen C, Hu C, Sheng Y *et al*: **Mettl14 inhibits hematopoietic stem/progenitor differentiation and promotes leukemogenesis via mrna m(6)a modification.** *Cell Stem Cell* (2018) **22**(2):191-205 e199.
35. Choi KD, Vodyanik M, Slukvin, II: **Hematopoietic differentiation and production of mature myeloid cells from human pluripotent stem cells.** *Nat Protoc* (2011) **6**(3):296-313.
36. Zeng C, Wang W, Yu X, Yang L, Chen S, Li Y: **Pathways related to pma-differentiated thp1 human monocytic leukemia cells revealed by rna-seq.** *Sci China Life Sci* (2015) **58**(12):1282-1287.
37. Chim CS, Kwong YL, Liang R, Chu YC, Chan CH, Chan LC, Wong KF, Chan TK: **All-trans retinoic acid (atra) in the treatment of acute promyelocytic leukemia (apl).** *Hematol Oncol* (1996) **14**(3):147-154.
38. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia G, Ren B *et al*: **N6-methyladenosine-dependent regulation of messenger rna stability.** *Nature* (2014) **505**(7481):117-120.
39. Gopal V, Hulette B, Li YQ, Kuvelkar R, Raza A, Larson R, Goldberg J, Tricot G, Bennett J, Preisler H: **C-myc and c-myb expression in acute myelogenous leukemia.** *Leuk Res* (1992) **16**(10):1003-1011.
40. Mundo L, Ambrosio MR, Raimondi F, Del Porro L, Guazzo R, Mancini V, Granai M, Jim Rocca B, Lopez C, Bens S, Onyango N *et al*: **Molecular switch from myc to mycn expression in myc protein negative burkitt lymphoma cases.** *Blood Cancer J* (2019) **9**(12):91.
41. Sakai N, Kubota Y, Shuin T: **Statistically significant expression of b-myb in clinically advanced human renal-cell carcinomas.** *Int J Oncol* (1993) **2**(3):419-423.
42. Musa J, Aynaud MM, Mirabeau O, Delattre O, Grunewald TG: **Mybl2 (b-myb): A central regulator of cell proliferation, cell survival and differentiation involved in tumorigenesis.** *Cell Death Dis* (2017) **8**(6):e2895.
43. Melnik S, Werth N, Boeuf S, Hahn EM, Gotterbarm T, Anton M, Richter W: **Impact of c-myc expression on proliferation, differentiation, and risk of neoplastic transformation of human mesenchymal stromal cells.** *Stem Cell Res Ther* (2019) **10**(1):73.
44. Leon J, Ferrandiz N, Acosta JC, Delgado MD: **Inhibition of cell differentiation: A critical mechanism for myc-mediated carcinogenesis?** *Cell Cycle* (2009) **8**(8):1148-1157.
45. Carter JL, Hege K, Yang J, Kalpage HA, Su Y, Edwards H, Huttemann M, Taub JW, Ge Y: **Targeting multiple signaling pathways: The new approach to acute myeloid leukemia therapy.** *Signal Transduct Target Ther* (2020) **5**(1):288.
46. Delgado MD, Leon J: **Myc roles in hematopoiesis and leukemia.** *Genes Cancer* (2010) **1**(6):605-616.

47. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, Huang H, Nachtergaele S, Dong L, Hu C, Qin X *et al*: **Fto plays an oncogenic role in acute myeloid leukemia as a n(6)-methyladenosine rna demethylase.** *Cancer Cell* (2017) **31**(1):127-141.
48. Guibal FC, Moog-Lutz C, Smolewski P, Di Gioia Y, Darzynkiewicz Z, Lutz PG, Cayre YE: **Asb-2 inhibits growth and promotes commitment in myeloid leukemia cells.** *J Biol Chem* (2002) **277**(1):218-224.
49. Glasow A, Prodromou N, Xu K, von Lindern M, Zelent A: **Retinoids and myelomonocytic growth factors cooperatively activate rara and induce human myeloid leukemia cell differentiation via map kinase pathways.** *Blood* (2005) **105**(1):341-349.
50. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS: **Model-based analysis of chip-seq (macs).** *Genome Biol* (2008) **9**(9):R137.
51. Meng J, Cui X, Rao MK, Chen Y, Huang Y: **Exome-based analysis for rna epigenome sequencing data.** *Bioinformatics* (2013) **29**(12):1565-1567.
52. Zhang M, Zhang Y, Ma J, Guo F, Cao Q, Zhang Y, Zhou B, Chai J, Zhao W, Zhao R: **The demethylase activity of fto (fat mass and obesity associated protein) is required for preadipocyte differentiation.** *PLoS One* (2015) **10**(7):e0133788.
53. Zhang X, Wei LH, Wang Y, Xiao Y, Liu J, Zhang W, Yan N, Amu G, Tang X, Zhang L, Jia G: **Structural insights into fto's catalytic mechanism for the demethylation of multiple rna substrates.** *Proc Natl Acad Sci U S A* (2019) **116**(8):2919-2924.
54. Masetti R, Vendemini F, Zama D, Biagi C, Gasperini P, Pession A: **All-trans retinoic acid in the treatment of pediatric acute promyelocytic leukemia.** *Expert Rev Anticancer Ther* (2012) **12**(9):1191-1204.
55. Ni X, Hu G, Cai X: **The success and the challenge of all-trans retinoic acid in the treatment of cancer.** *Crit Rev Food Sci Nutr* (2019) **59**(sup1):S71-S80.
56. Rubio V, Calvino E, Garcia-Perez A, Herraiz A, Diez JC: **Human acute promyelocytic leukemia nb4 cells are sensitive to esculetin through induction of an apoptotic mechanism.** *Chem Biol Interact* (2014) **220**:129-139.
57. Kohroki J, Fujita S, Itoh N, Yamada Y, Imai H, Yumoto N, Nakanishi T, Tanaka K: **Atra-regulated asb-2 gene induced in differentiation of hl-60 leukemia cells.** *FEBS Lett* (2001) **505**(2):223-228.
58. Sakamoto K, Imamura T, Yano M, Yoshida H, Fujiki A, Hirashima Y, Hosoi H: **Sensitivity of mll-rearranged aml cells to all-trans retinoic acid is associated with the level of h3k4me2 in the raralpha promoter region.** *Blood Cancer J* (2014) **4**(e205).
59. Wang ZY, Chen Z: **Acute promyelocytic leukemia: From highly fatal to highly curable.** *Blood* (2008) **111**(5):2505-2515.

60. Huang Y, Su R, Sheng Y, Dong L, Dong Z, Xu H, Ni T, Zhang ZS, Zhang T, Li C, Han L *et al*: **Small-molecule targeting of oncogenic fto demethylase in acute myeloid leukemia.** *Cancer Cell* (2019) **35**(4):677-691 e610.
61. Shen C, Sheng Y, Zhu AC, Robinson S, Jiang X, Dong L, Chen H, Su R, Yin Z, Li W, Deng X *et al*: **Rna demethylase alkbn5 selectively promotes tumorigenesis and cancer stem cell self-renewal in acute myeloid leukemia.** *Cell Stem Cell* (2020) **27**(1):64-80 e69.
62. Cancer Genome Atlas Research N, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, Hoadley K, Triche TJ, Jr., Laird PW, Baty JD *et al*: **Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia.** *N Engl J Med* (2013) **368**(22):2059-2074.
63. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR *et al*: **Transformation from committed progenitor to leukaemia stem cell initiated by mll-af9.** *Nature* (2006) **442**(7104):818-822.
64. Zhou DS, Wang HB, Zhou ZG, Zhang YJ, Zhong Q, Xu L, Huang YH, Yeung SC, Chen MS, Zeng MS: **Tacc3 promotes stemness and is a potential therapeutic target in hepatocellular carcinoma.** *Oncotarget* (2015) **6**(27):24163-24177.
65. Song H, Liu C, Shen N, Yi P, Dong F, Li X, Zhang N, Huang T: **Overexpression of tacc3 in breast cancer associates with poor prognosis.** *Appl Immunohistochem Mol Morphol* (2018) **26**(2):113-119.
66. Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, Mapperley C, Lawson H, Wotherspoon DA, Sepulveda C, Vukovic M *et al*: **Targeting the rna m(6)a reader ythdf2 selectively compromises cancer stem cells in acute myeloid leukemia.** *Cell Stem Cell* (2019) **25**(1):137-148 e136.
67. Jacobson EC, Jain L, Vickers MH, Olins AL, Olins DE, Perry JK, O'Sullivan JM: **Tnf-alpha differentially regulates cell cycle genes in promyelocytic and granulocytic hl-60/s4 cells.** *G3 (Bethesda)* (2019) **9**(8):2775-2786.
68. Li Z, Qian P, Shao W, Shi H, He XC, Gogol M, Yu Z, Wang Y, Qi M, Zhu Y, Perry JM *et al*: **Suppression of m(6)a reader ythdf2 promotes hematopoietic stem cell expansion.** *Cell Res* (2018) **28**(9):904-917.
69. Tan SH, Bertulfo FC, Sanda T: **Leukemia-initiating cells in t-cell acute lymphoblastic leukemia.** *Front Oncol* (2017) **7**(218).
70. Raetz EA, Teachey DT: **T-cell acute lymphoblastic leukemia.** *Hematology Am Soc Hematol Educ Program* (2016) **2016**(1):580-588.
71. Mapperley C, van de Lagemaat LN, Lawson H, Tavosanis A, Paris J, Campos J, Wotherspoon D, Durko J, Sarapuu A, Choe J, Ivanova I *et al*: **The mrna m6a reader ythdf2 suppresses proinflammatory pathways and sustains hematopoietic stem cell function.** *J Exp Med* (2021) **218**(3).

72. Eliza Yankova WB, Mark Albertella, Justyna Rak, Etienne De Braekeleer, Georgia Tsagkogeorga, Ewa S. Pilka, Demetrios Aspris, Dan Leggate, Alan G. Hendrick, Natalie A. Webster, Byron Andrews, Richard Fosbeary, Patrick Guest, Nerea Irigoyen, Maria Eleftheriou, Malgorzata Gozdecka, Joao M. L. Dias, Andrew J. Bannister, Binje Vick, Irmela Jeremias, George S. Vassiliou, Oliver Rausch, Konstantinos Tzelepis & Tony Kouzarides **Small molecule inhibition of mettl3 as a strategy against myeloid leukaemia.** *Nature* (26.04.2021).
73. Su R, Dong L, Li Y, Gao M, Han L, Wunderlich M, Deng X, Li H, Huang Y, Gao L, Li C *et al*: **Targeting fto suppresses cancer stem cell maintenance and immune evasion.** *Cancer Cell* (2020) **38**(1):79-96 e11.
74. Olsen SN, Armstrong SA: **It's not what you say but how you say it: Targeting rna methylation in aml.** *Mol Cell* (2020) **78**(6):996-998.
75. Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, Deng X, Wang Y, Weng X, Hu C, Yu M *et al*: **R-2hg exhibits anti-tumor activity by targeting fto/m(6)a/myc/cebpa signaling.** *Cell* (2018) **172**(1-2):90-105 e123.
76. Qing Y, Dong L, Gao L, Li C, Li Y, Han L, Prince E, Tan B, Deng X, Wetzel C, Shen C *et al*: **R-2-hydroxyglutarate attenuates aerobic glycolysis in leukemia by targeting the fto/m(6)a/pfkfb3/ldha axis.** *Mol Cell* (2021) **81**(5):922-939 e929.
77. Bhate A, Sun T, Li JB: **Adar1: A new target for immuno-oncology therapy.** *Mol Cell* (2019) **73**(5):866-868.
78. Shen Q, Zhang Q, Shi Y, Shi Q, Jiang Y, Gu Y, Li Z, Li X, Zhao K, Wang C, Li N *et al*: **Tet2 promotes pathogen infection-induced myelopoiesis through mrna oxidation.** *Nature* (2018) **554**(7690):123-127.
79. Fang ZH, Wang SL, Zhao JT, Lin ZJ, Chen LY, Su R, Xie ST, Carter BZ, Xu B: **Mir-150 exerts antileukemia activity in vitro and in vivo through regulating genes in multiple pathways.** *Cell Death Dis* (2016) **7**(9):e2371.
80. Kumar S, Ashraf MU, Kumar A, Bae YS: **Therapeutic potential of microrna against th2-associated immune disorders.** *Curr Top Med Chem* (2021).
81. Cao Y, Liu Y, Shang L, Wei W, Shen Y, Gu Q, Xie X, Dong W, Lin Y, Yue Y, Wang F *et al*: **Decitabine and all-trans retinoic acid synergistically exhibit cytotoxicity against elderly aml patients via mir-34a/mycn axis.** *Biomed Pharmacother* (2020) **125**(109878).
82. **Epitranscriptomics: The new rna code and the race to drug it.** *C&EN Global Enterprise* (2019) **97**(7):34-39.
83. Liu B, Ma H, Liu Q, Xiao Y, Pan S, Zhou H, Jia L: **Mir-29b/sp1/fut4 axis modulates the malignancy of leukemia stem cells by regulating fucosylation via wnt/beta-catenin pathway in acute myeloid leukemia.** *J Exp Clin Cancer Res* (2019) **38**(1):200.
84. Palmer DC, Guittard GC, Franco Z, Crompton JG, Eil RL, Patel SJ, Ji Y, Van Panhuys N, Klebanoff CA, Sukumar M, Clever D *et al*: **Cish actively silences tcr signaling in cd8+ t cells to maintain tumor tolerance.** *J Exp Med* (2015) **212**(12):2095-2113.

85. Delconte RB, Kolesnik TB, Dagley LF, Rautela J, Shi W, Putz EM, Stannard K, Zhang JG, Teh C, Firth M, Ushiki T *et al*: **Cis is a potent checkpoint in nk cell-mediated tumor immunity.** *Nat Immunol* (2016) **17**(7):816-824.
86. Putz EM, Guillerey C, Kos K, Stannard K, Miles K, Delconte RB, Takeda K, Nicholson SE, Huntington ND, Smyth MJ: **Targeting cytokine signaling checkpoint cis activates nk cells to protect from tumor initiation and metastasis.** *Oncoimmunology* (2017) **6**(2):e1267892.
87. Li HB, Tong J, Zhu S, Batista PJ, Duffy EE, Zhao J, Bailis W, Cao G, Kroehling L, Chen Y, Wang G *et al*: **M(6)a mrna methylation controls t cell homeostasis by targeting the il-7/stat5/socs pathways.** *Nature* (2017) **548**(7667):338-342.
88. Sprent J, Surh CD: **Writer's block: Preventing m(6)a mrna methylation promotes t cell naivety.** *Immunol Cell Biol* (2017) **95**(10):855-856.
89. Shoubao Ma JY, Tasha Barr, Jianying Zhang, Zhenhua Chen, Li-Shu Wang, Joseph C. Sun, Jianjun Chen, Michael A Caligiuria, Jianhua Yua: **The rna m6a reader ythdf2 controls nk cell anti-tumor and anti-viral immunity.** *bioRxiv preprint* (27.04.2021).
90. Zhu H, Blum RH, Bernareggi D, Ask EH, Wu Z, Hoel HJ, Meng Z, Wu C, Guan KL, Malmberg KJ, Kaufman DS: **Metabolic reprogramming via deletion of cish in human ipsc-derived nk cells promotes in vivo persistence and enhances anti-tumor activity.** *Cell Stem Cell* (2020) **27**(2):224-237 e226.
91. Miah MA, Yoon CH, Kim J, Jang J, Seong YR, Bae YS: **Cish is induced during dc development and regulates dc-mediated ctl activation.** *Eur J Immunol* (2012) **42**(1):58-68.
92. Kumar S, Jeong Y, Ashraf MU, Bae YS: **Dendritic cell-mediated th2 immunity and immune disorders.** *Int J Mol Sci* (2019) **20**(9).
93. Hou HA, Lu JW, Lin TY, Tsai CH, Chou WC, Lin CC, Kuo YY, Liu CY, Tseng MH, Chiang YC, Peng YL *et al*: **Clinico-biological significance of suppressor of cytokine signaling 1 expression in acute myeloid leukemia.** *Blood Cancer J* (2017) **7**(7):e588.
94. Wang JY, Chen LJ, Qiang P: **The potential role of n6-methyladenosine (m6a) demethylase fat mass and obesity-associated gene (fto) in human cancers.** *Onco Targets Ther* (2020) **13**(12845-12856).
95. Miah MA, Bae YS: **Regulation of dc development and dc-mediated t-cell immunity via cish.** *Oncoimmunology* (2013) **2**(3):e23404.
96. Levis M: **Midostaurin approved for flt3-mutated aml.** *Blood* (2017) **129**(26):3403-3406.
97. Alfayez M, Kantarjian H, Kadia T, Ravandi-Kashani F, Daver N: **Cpx-351 (vyxeos) in aml.** *Leuk Lymphoma* (2020) **61**(2):288-297.

98. Krauss AC, Gao X, Li L, Manning ML, Patel P, Fu W, Janoria KG, Gieser G, Bateman DA, Przepiorka D, Shen YL *et al*: **Fda approval summary: (daunorubicin and cytarabine) liposome for injection for the treatment of adults with high-risk acute myeloid leukemia.** *Clin Cancer Res* (2019) **25**(9):2685-2690.
99. Dennis M, Russell N, Hills RK, Hemmaway C, Panoskaltsis N, McMullin MF, Kjeldsen L, Dignum H, Thomas IF, Clark RE, Milligan D *et al*: **Vosaroxin and vosaroxin plus low-dose ara-c (ldac) vs low-dose ara-c alone in older patients with acute myeloid leukemia.** *Blood* (2015) **125**(19):2923-2932.
100. Zhao J, Song Y, Liu D: **Correction to: Gilteritinib: A novel flt3 inhibitor for acute myeloid leukemia.** *Biomark Res* (2019) **7**(21).
101. Dhillon S: **Gilteritinib: First global approval.** *Drugs* (2019) **79**(3):331-339.
102. Guerra VA, DiNardo C, Konopleva M: **Venetoclax-based therapies for acute myeloid leukemia.** *Best Pract Res Clin Haematol* (2019) **32**(2):145-153.
103. Kirschbaum MH, Foon KA, Frankel P, Ruel C, Pulone B, Tuscano JM, Newman EM: **A phase 2 study of belinostat (pxd101) in patients with relapsed or refractory acute myeloid leukemia or patients over the age of 60 with newly diagnosed acute myeloid leukemia: A california cancer consortium study.** *Leuk Lymphoma* (2014) **55**(10):2301-2304.
104. Quintas-Cardama A, Santos FP, Garcia-Manero G: **Histone deacetylase inhibitors for the treatment of myelodysplastic syndrome and acute myeloid leukemia.** *Leukemia* (2011) **25**(2):226-235.
105. Wieduwilt MJ, Pawlowska N, Thomas S, Olin R, Logan AC, Damon LE, Martin T, Kang M, Sayre PH, Boyer W, Gaensler KML *et al*: **Histone deacetylase inhibition with panobinostat combined with intensive induction chemotherapy in older patients with acute myeloid leukemia: Phase i study results.** *Clin Cancer Res* (2019) **25**(16):4917-4923.
106. Bug G, Burchert A, Wagner EM, Kroger N, Berg T, Guller S, Metzelder SK, Wolf A, Hunecke S, Bader P, Schetelig J *et al*: **Phase i/ii study of the deacetylase inhibitor panobinostat after allogeneic stem cell transplantation in patients with high-risk mds or aml (panobest trial).** *Leukemia* (2017) **31**(11):2523-2525.
107. DeAngelo DJ, Walker AR, Schlenk RF, Sierra J, Medeiros BC, Ocio EM, Rollig C, Strickland SA, Thol F, Valera SZ, Dasgupta K *et al*: **Safety and efficacy of oral panobinostat plus chemotherapy in patients aged 65 years or younger with high-risk acute myeloid leukemia.** *Leuk Res* (2019) **85**(106197).
108. Kung Sutherland MS, Walter RB, Jeffrey SC, Burke PJ, Yu C, Kostner H, Stone I, Ryan MC, Sussman D, Lyon RP, Zeng W *et al*: **Sgn-cd33a: A novel cd33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant aml.** *Blood* (2013) **122**(8):1455-1463.

109. Vey N, Delaunay J, Martinelli G, Fiedler W, Raffoux E, Prebet T, Gomez-Roca C, Papayannidis C, Kebenko M, Paschka P, Christen R *et al*: **Phase i clinical study of rg7356, an anti-cd44 humanized antibody, in patients with acute myeloid leukemia.** *Oncotarget* (2016) 7(22):32532-32542.
110. Li F, Kennedy S, Hajian T, Gibson E, Seitova A, Xu C, Arrowsmith CH, Vedadi M: **A radioactivity-based assay for screening human m6a-rna methyltransferase, mettl3-mettl14 complex, and demethylase alk5.** *J Biomol Screen* (2016) 21(3):290-297.
111. Moroz-Omori Elena V. HD, Bedi Rajiv Kumar, Cheriyankunel Sherry J., Bochenkova Elena, Dolbois Aymeric, Rzekzkowski Maciej D., Wiedmer Lars, Caflisch Amedeo: **Mettl3 inhibitors for epitranscriptomic modulation of cellular processes.** *bioRxiv* (13.10.2020).
112. Kuipers KJJ, Hellweg S, Verones F: **Potential consequences of regional species loss for global species richness: A quantitative approach for estimating global extinction probabilities.** *Environ Sci Technol* (2019) 53(9):4728-4738.
113. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, Sun G, Lu Z, Huang Y, Yang CG, Riggs AD *et al*: **M(6)a rna methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells.** *Cell Rep* (2017) 18(11):2622-2634.
114. Huang Y, Yan J, Li Q, Li J, Gong S, Zhou H, Gan J, Jiang H, Jia GF, Luo C, Yang CG: **Meclofenamic acid selectively inhibits fto demethylation of m6a over alk5.** *Nucleic Acids Res* (2015) 43(1):373-384.
115. Singh B, Kinne HE, Milligan RD, Washburn LJ, Olsen M, Lucci A: **Important role of fto in the survival of rare panresistant triple-negative inflammatory breast cancer cells facing a severe metabolic challenge.** *PLoS One* (2016) 11(7):e0159072.
116. Zheng G, Cox T, Tribbey L, Wang GZ, Iacoban P, Booher ME, Gabriel GJ, Zhou L, Bae N, Rowles J, He C *et al*: **Synthesis of a fto inhibitor with anticonvulsant activity.** *ACS Chem Neurosci* (2014) 5(8):658-665.
117. Fong CY, Gilan O, Lam EY, Rubin AF, Ftouni S, Tyler D, Stanley K, Sinha D, Yeh P, Morison J, Giotopoulos G *et al*: **Bet inhibitor resistance emerges from leukaemia stem cells.** *Nature* (2015) 525(7570):538-542.
118. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C *et al*: **Inhibition of bet recruitment to chromatin as an effective treatment for mll-fusion leukaemia.** *Nature* (2011) 478(7370):529-533.
119. Dawson MA, Kouzarides T, Huntly BJ: **Targeting epigenetic readers in cancer.** *N Engl J Med* (2012) 367(7):647-657.
120. Tzelepis K, Koike-Yusa H, De Braekeleer E, Li Y, Metzakopian E, Dovey OM, Mupo A, Grinkevich V, Li M, Mazan M, Gozdecka M *et al*: **A crispr dropout screen identifies genetic vulnerabilities and therapeutic targets in acute myeloid leukemia.** *Cell Rep* (2016) 17(4):1193-1205.

121. Biel M, Kretsovali A, Karatzali E, Papamatheakis J, Giannis A: **Design, synthesis, and biological evaluation of a small-molecule inhibitor of the histone acetyltransferase gcn5.** *Angew Chem Int Ed Engl* (2004) **43**(30):3974-3976.