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REVIEW ARTICLE

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KMT2a-rearranged acute leukaemias — insights and potential therapeutic options

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Abstract

The lysine methyltransferase 2A (KMT2A) protein is a histone lysine 4 (H3K4) methyltransferase that is involved in normal development through supporting certain gene transcriptions. On the other hand, it is rather a 'promiscuous' protein that is involved in fusion with a variety of partner proteins creating an altered mixed lineage leukaemia complex that is not expressed in normal cells and is involved in the transformation of normal haematopoietic cells to leukaemic ones. To date, more than 120 fusion partners have been identified. They account for 5–10% of all acute leukaemias. Of these, infants account for more than 70% of the total cases. Another subgroup of acute leukaemia patients with the KMT2A-rearrangement develops it as a result of exposure to certain types of chemotherapeutic agents. The outcome of KMT2A-rearranged acute leukaemia is usually very poor especially when compared to other patients without the KMT2A-rearrangement. They are often chemo-resistant and display cell plasticity. This has prompted a search for better therapies. At the same time, more research has led to elucidation of the KMT2A fusion protein complexes and its involvement in leukaemic transformation.

This article reviews the biology of the KMT2A protein and the role played by some of the partner proteins in the development of acute leukaemias. It also considers cell plasticity in KMT2A-rearranged leukaemia, and finally some promising therapeutics that could impact upon the management landscape of KMT2A-rearranged acute leukaemias.

Keywords: mixed-lineage leukaemia, acute leukaemia, cell plasticity, epigenetics, targeted therapy

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Introduction

Acute leukaemias are a group of heterogeneous haematological malignancies characterised by the abnormal proliferation of clonal immature bone marrow-derived cells (blasts). They sometimes involve peripheral blood and solid organs, and they are characteristically divided into myeloid-lineage or lymphoid (B or T) lineage. The myeloid-lineage is known as acute myeloid leukaemia (AML), while the lymphoid-lineage is known as acute lymphoblastic leukaemia (ALL). These haematological malignancies are the most common childhood cancers, comprising c.30% of all such cancers [1]. Despite significant strides made in therapeutics, especially in ALL, c.10–20% of patients will either relapse or develop resistant disease [2]. In AML, c.30% will develop disease recurrence, which is a major cause of mortality [3, 4]. According to some studies, the 5-year survival rate after relapse in ALL is 40–70% depending on treatment protocol and molecular subtype [5].

Given this statistic, more intensification of the standard chemotherapy, especially for relapsed disease, does not lead to better outcomes. Thus, a deeper understanding of the molecular profiles of the underlying acute leukemia is

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required in order to improve outcomes in treatment and possibly develop more efficacious therapies.

Clonal heterogeneity is a common denominator in acute leukaemia, and especially in relapsed disease. In one of the early studies on clonality using genome-wide SNP genotyping, Mullighan et al. [6] reported clones discovered at ALL relapse were derived from clones that were close relatives of the diagnostic clone. Farrar et al. [7] reported similar findings in paediatric AML patients. Tumour heterogeneity is generally linked to poor prognosis in leukaemias [8, 9], and is believed to be a major cause of treatment failure and drug resistance. Tumours are composed of different cell populations characterised by varying molecular lesions that makes precision targeting a difficult challenge, especially in acute leukaemias. Among these molecular lesions is the lysine (K)-specific methyltransferase 2A (KMT2A; also known as mixed lineage leukemia or MLL) rearrangement. The KMT2A rearrangement is characterised by a poor prognosis in acute leukaemia and occurs in c.10% of both acute lymphoid and myeloid leukaemias [10]. The KMT2A-rearranged mutations in acute leukaemia have since 1999 been recognised as a separate entity in the WHO Classification of Neoplastic Diseases of the Haematopoietic and Lymphoid Tissues [11], and even the latest classification has retained this distinction [12, 13]. KMT2A is known to be involved in leukaemogenesis through its various chromosomal rearrangements that number more than 130 different translocation partners discovered so far [14]. KMT2A-rearrangement can be found in both acute myeloid and in lymphoid leukaemia, but it can also be found in mixed phenotypic acute leukaemia (MPAL) [15]. In a recent study, it was shown that it emerges very early in haematopoiesis, as preexisting lymphomyeloid primed progenitors. However, their surface antigenic expressions have been reported to be potentially targetable [16]. This implies that KMT2A-rearrangement mutations may have druggable targets.

Current technologies such as fluorescence in situ hybridisation (FISH) and high-throughput next-generation sequencing (NGS) used in the investigation of tumour heterogeneity in leukaemias have shown that these cells (including KMT2A-rearrangements) are characterised by different genetic and epigenetic remodelling [17, 18]. While tumour heterogeneity is still obscure in characterisation even with the aforementioned technologies, the advent of single-cell sequencing (SCS) technology has helped our understanding of the molecular basis of intratumoural heterogeneity [16].

The aim of this review was to highlight the current understanding regarding KMT2A-rearranged acute leukaemia, as well as potential therapeutic options.

KMT2A biology

The KMT2A protein (also known as MLL1) is a large, multi-domain protein with c.3,969 amino acid residues, with its gene located on the long arm of chromosome 11 band q23.3 (11q23.3). It is also expressed in many other tissues including the cerebral cortex, kidney, thyroid, and spleen [19]. The KMT2A protein is part of the family of lysine methyltransferases (KMT) known as MLL/KMT2 which includes KMT2D (MLL2), KMT2C (MLL3), KMT2B (MLL4), KMT2E (MLL5), KMT2F (SET1A), and KMT2G (SET1B). The KMT2A protein consists of N-terminal and C-terminal, and has c.2,718 and 1,250 amino acids respectively [20]. The N-terminal is made up of several functional domains which include the menin-binding motifs (MBM); the lens epithelium-derived growth factor (LEDGF)-binding domain (LBD); AT-hooks (ATH); nuclear-localisation signals 1 and 2 (SNL 1 and 2); CxxC domains (a pre-CxxC, post-CxxC and non-methyl-CpG recognising site); plant homology domains 1–4 (PHD 1–4); bromodomain (BRD); and the homodimerisation-facilitating domain (FYRN) [21].

The MBM serves as a link between menin and LEDGF, a chromatin-binding protein that binds KMT2A forming the ternary complex KMT2A-Menin-LEDGF that associates with chromatin leading to the transcriptional upregulation of certain target genes. The ATHs are known to bind to the minor and major grooves of the AT-rich DNA oligomers. The CxxC domains are known to contribute to the unmethylated state of the CpG which is important for the association of KMT2A with chromatin. It is also known to be associated with the Paf1/RNA Polymerase II (pol II) Complex Component (PAF1) transcription elongation complex. The four PHD fingers found in the middle portion of the KMT2A binds histone H3 trimethylated at lysine 4 (H3K4me3) and H3K4me2, a critical function for KMT transcriptional activity. The bromodomains bind to acetylated lysine residues on histone tails, and so are involved in chromatin remodelling and the engagement of some other factors for transcription.

The C-terminal, on the other hand, is made up of transactivation domain (TAD), FYRC-term, Su(var)3–9, enhancer of zeste, trithorax (SET), 4post SET and S-adenosyl-L-methionine binding sites. The TAD stimulates transcriptional activation with the recruitment of co-activators for gene expression activation. The SET domain is a H3K4 methyltransferase that is involved in the mono-, di-, and trimethylation of H3K4. The retinoblastoma binding protein 5 (RbBP5), Set1/Ash2 HMT complex subunit ASH2-like(Ash2L), and WD repeat-containing protein 5 (WDR5) are essential proteins required for the efficient functioning of H3K4. Interactions among these proteins show that the RbBP5-Ash2L heterodimer is involved in the stabilisation of the KMT2A protein, while WDR5 bridges the RbBP5-Ash2L heterodimer and KMT2A interaction. Other proteins such as the histone acetyltransferases CBP/p300, MOZ and MOF (through TAD) for chromatin remodelling and gene expression (Fig. 1).

Upon the translation of KMT2A protein, it is proteolytically cleaved by the enzyme taspase-1 into two parts which later come together to form a protein complex of

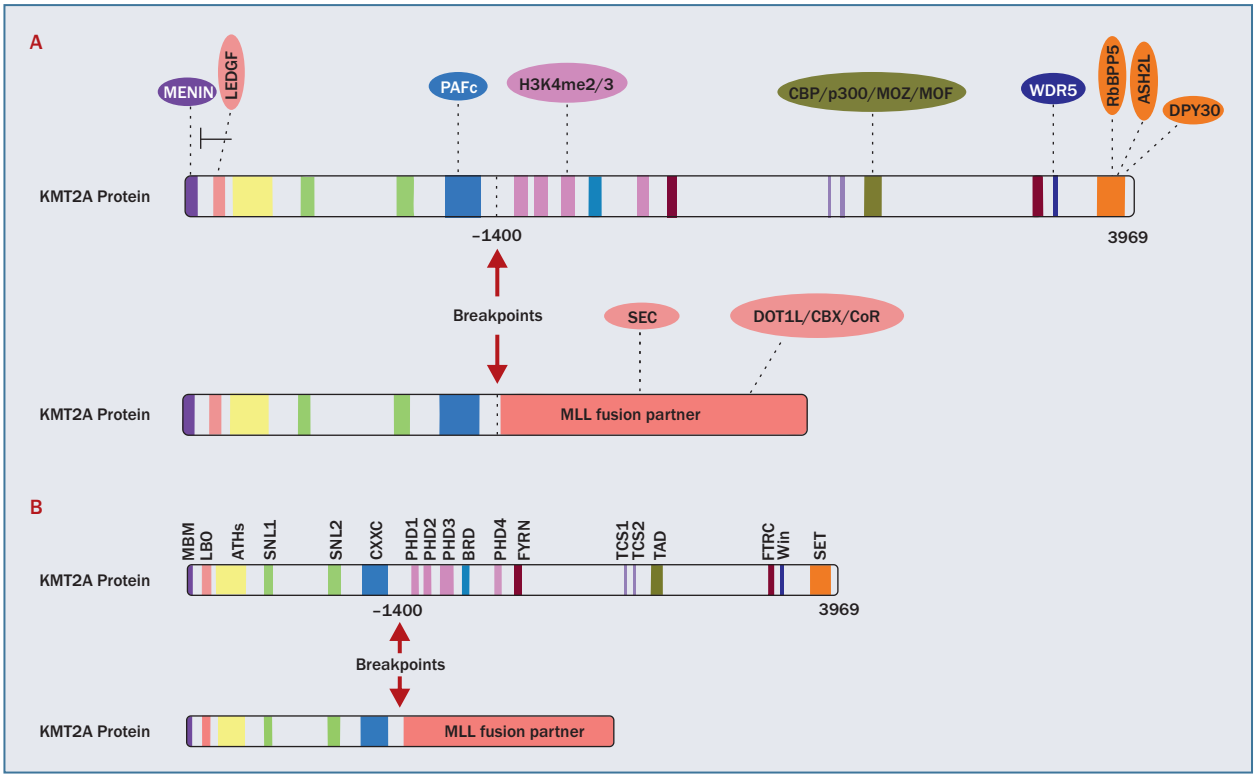

Figure 1. MLL1 protein with oncogenic MLL1 fusion protein. **A.** Potential targetable regions of MLL1 protein along with its fusion partner protein. **B.** MLL1 protein with its functional domains including menin-binding motif (MBM), LEDGF-binding domain (LBD), AT-hooks (ATHs), nuclear-localisation signals 1 and 2 (SNL1 and SNL2), CxxC domain, plant homology domains 1–4 (PHD1, PHD2, PHD3 and PHD4), bromodomain (BRD), FYRN domain, taspase 1 cleavage sites 1 and 2 (TCS1 and TCS2), transactivation domain (TAD), FYRC domain, WDR5 interaction motif (Win), and SET domain

the N-terminal and C-terminal fragments through interaction between FYRN (N-terminal) and FYRC (C-terminal) domains. This interaction takes place in the nucleus. After the KMT2A cleavage, the SET and TAD domains are retained in the C-terminal, but are not part of the N-terminal. The new protein complex that has been formed regulates chromatin modification and gene expression.

The oncogenic forms of KMT2A protein fuse with a variety of partner proteins in both AML and ALL [22]. The oncogenic KMT2A protein is formed by the fusion of the N-terminal with c.130 translocation partners [14], leading to the fusion of the KMT2A gene to different partner genes. Most of these fusions occur in a breakpoint cluster region that extends from exon 8 to exon 14. The breakpoint within intron 11 is frequently observed in infant acute leukaemia and therapy-related leukaemia [23], and carries with it a worse prognosis compared to other breakpoints. The reason for this is the observation of the PHD (1–3) of the KMT2A protein which is encoded between exons 11 and 16. The PHD3 has a dual function that helps make the KMT2A protein to become either a transcriptional activator/maintenance factor or a transcriptional repressor. The breakpoints within intron 11 in this way destroy the dimerisation capacity of the PHD1-3 domain [24]. However, in a small cohort of patients diagnosed with T-cell ALL, the breakpoint in the *KMT2A* gene has been found between intron 19 and exon 24, preferentially within intron 21 and 23 (this breakpoint includes the complete PHD1-4 as well as the bromodomain); a few others within exon 11, and even intron 27 [25].

The KMT2A is involved in epigenetic regulation of developmental genes that play a role in embryogenesis, as well as normal haematopoeisis. It does this through the H3K4 methylation of promoter regions to the target genes and its N-terminal domain's interactions involving menin-LEDGF, ATH, and CxxC domains — a critical involvement for leukaemogenesis which leads to transcriptional activation [26, 27].

Thus, KMT2A is, in a profound sense, a transcription factor [28].

One of the targets for KMT2A transcription is the *Hox* and *Meis1* genes. The *Hox* genes are master transcriptional regulators that play a role in embryogenesis and cancer development, especially with their ability to produce homeotic transformation. The *Hox* genes and Meis1 are also known to be important in the regulation of stem cell differentiation and self-renewal [29, 30]. As haematopoiesis progresses, Hoxa9 and Meis1 are systematically down-regulated from

the progenitor cells to the fully differentiated cells [31, 32]. The inability to repress *Hox* genes can result in the development of leukaemia. It has been shown that Hoxa9 and Meis1 are very critical targets of KMT2A protein, and their overexpression can lead to the induction and maintenance of KMT2A-rearranged leukaemias [33–35].

KMT2A translocations in acute leukaemia

Chromosomal rearrangements that translate to fusion genes are quite common among haematological malignancies, above all acute leukaemias. The *KMT2A* gene which is localised at chromosome 11q23 is frequently associated with chromosomal rearrangements and often portends a poor prognosis [36, 37]. To date, more than 130 KMT2A rearrangements have been identified [14]. These rearrangements involving KMT2A and the different associated genes are found in different acute leukaemias including AML, mixed phenotypic acute leukaemia, precursor B-ALL, T-ALL, Burkitt's lymphoma and even myelodysplastic syndromes (MDS) [23, 38]. Therapy-related acute leukaemias are equally associated with KMT2A rearrangements and, as could be predicted, have a dismal prognosis [39, 40]. KMT2A-mediated leukaemia accounts for c.5% of paediatric ALL and c.15% of adult ALL cases [41, 42]. In infants (<1 year), it accounts for more than 70% of all ALL [16]. In AML, it accounts for c.3% of adult AML [43], c.15–20% of childhood AML, and c.50% of infant AML [44]. Whole-genome sequencing has demonstrated that KMT2A leukaemias (infant KMT2A leukaemias) have one of the lowest frequencies of somatic mutations, averaging 1.3 non-silent mutations in the predominant clone [45]. However, older children are reported to have more somatic mutations than infants (mean of 6.5/case versus 1.3/case) and with more frequent mutations in epigenetic regulators [46]. Though infant KMT2A leukaemia has a low somatic mutation burden, interestingly the tyrosine kinase/PI3K/RAS signalling pathways are active in approximately half of patients [46].

To date, more than 130 partner fusion proteins of the KMT2A protein have been discovered [25]. Meyer et al. have shown that the latest KMT2A recombinome is comprised of 107 direct in-frame KMT2A fusions, 16 direct out-of-frame KMT2A fusions, 18 KMT2A mutations in patients with a translocation with a chromosomal locus where no gene is present, two patients with a deletion of the 5'-KMT2A but with reciprocal fusion genes, one RUNX1::ETV6 patient with an KMT2A insertion, and 16 cases in which no direct KMT2A fusion but only the reciprocal KMT2A fusion could be detected [47]. These fusion proteins highlight the so-called promiscuity of the KMT2A protein complex. This promiscuity of KMT2A is exemplified in such rare fusions as its complex with the casitas B-lineage leukaemia (CBL) protein which functions as an ubiquitin-ligase involved in the regulation of signals mediated by multiple tyrosine kinase receptors. Only four cases of the KMT2A-CBL fusion have been reported [48]. While there are several KMT2A fusion combinations, AF4, AF9, ENL, AF10, and ELL fusion proteins account for more than 85% of MLL-rearranged leukaemias [49]; AF4 and AF9 are found in 66% of ALL and 30% of AML cases respectively (Fig. 2) [50]. However, it is interesting to note that the N-terminus of a pruned KMT2A protein has no known biological function, and so is not able to transform a cell. Its loss from the C-terminus of the KMT2A protein can activate the p53–PAI-1 pathway [51], and also leads to its degradation through a unique mechanism that involves the FYRN domain [52]. The C-terminus is degraded through another pathway which involves its export to the cytoplasm where it is degraded by the proteasome. Thus, the fusion partner proteins may play a role in stabilising the chimeric protein complex.

KMT2A/AFF1 translocation

The KMT2A-AFF1 translocation (also known as MLL-AF4; t(4;11)(q21;q23)) is the most prevalent KMT2A translocation in ALL in both infants and adults; it accounts for c.50% of infant ALL and c.80% of adult ALL with KMT2A rearrangement, but is rare in AML (both infant and adult) [14, 53]. The KMT2A-AFF1 translocation most often is of mixed lineage, presenting morphologically as lymphoblasts, but (immuno) phenotypically present with both lymphoid and myeloid surface markers which are typically CD34+ CD19+ CD10– and CD15+ and CD33+ respectively [54, 55]. While the KMT2A-AFF1 fusion protein is found on chromosome 11 [14], it is interesting to note that reciprocal translocation, where two KMT2A fusion alleles are generated, can equally occur. This event has been noted generally in the KMT2A recombinome [56]; and in the case of KMT2A-AFF1, c.80% of patients have both reciprocal fusion alleles (KMT2A-AFF1 and AFF1-KMT2A), while only 20% encode only the KMT2A-AFF1 fusion allele [57]. Cryptic translocations have sometimes been reported [57, 58]. The KMT2A-AFF1 fusion protein generally consists of the N-terminal part of KMT2A comprising the AT-hook motifs, subnuclear localisation signals, and a DNA methyltransferase homology domain that is linked to the C-terminal part of AFF1 which consists of a transactivation domain, a C-terminal homology domain, and a nuclear localisation signal. This differs from the AFF1-KMT2A fusion protein that has the N-terminal part of AFF1 with the N-terminal homology domain and the ALF homology domain and is combined with the C-terminal part of KMT2A with PHD zinc fingers, a taspase1 cleavage site, a transactivation and a SET domain. These structural differences may be associated with different biological functions. The KMT2A-AFF1 fusion protein can still bind to chromatin through the MEN1/LEDGF region, activating such genes as HOXA4, HOXA5 and HOXA9 as well as MEIS1,

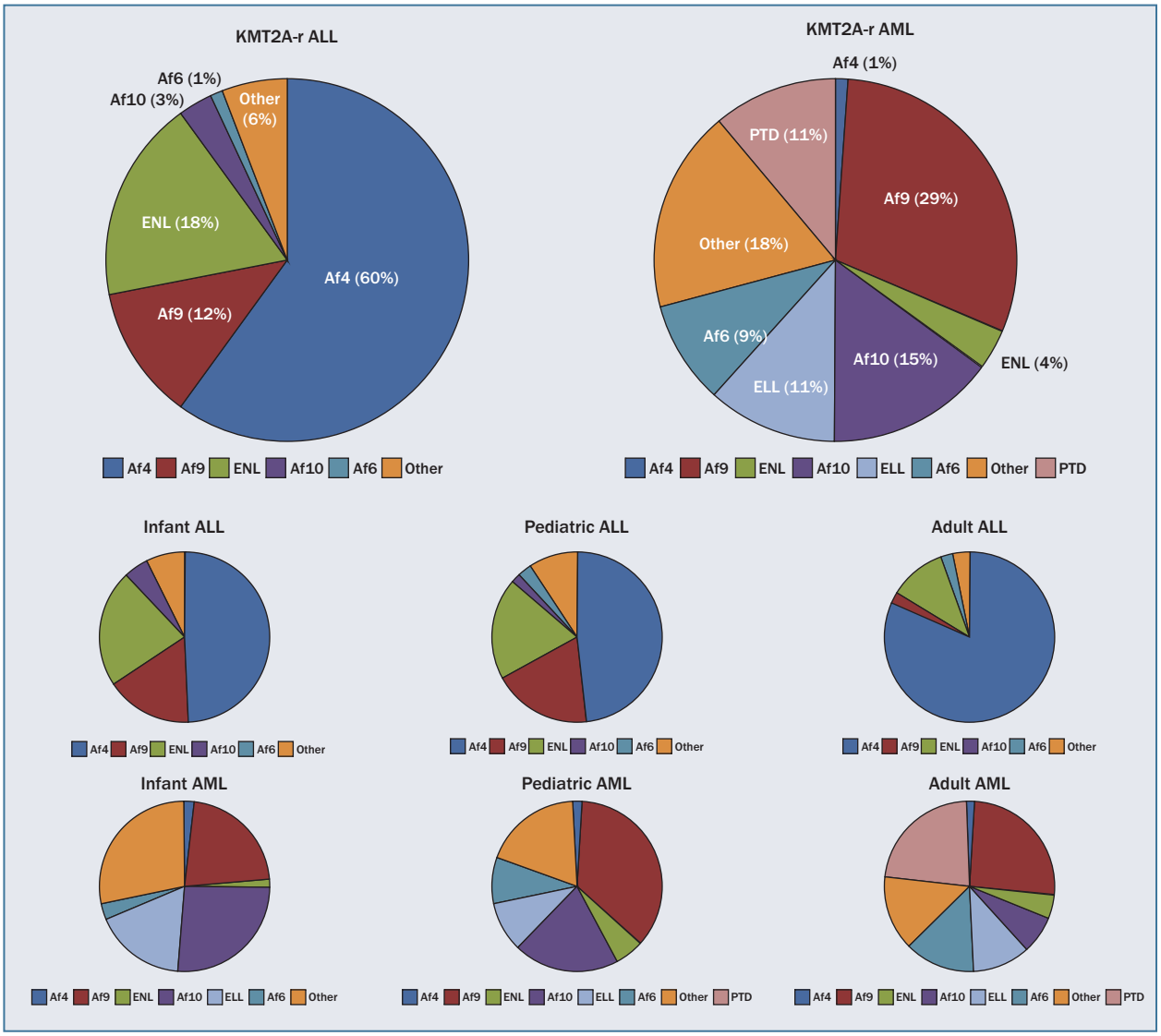

Figure 2. Most frequent partner fusion proteins of MLL1 protein in acute leukaemias (AML and ALL). Lower charts show frequency of fusion partner proteins based on type of leukaemia and age group

but the methylation pattern is affected as a result of the missing SET domain. On the other hand, the AF4-MLL fusion protein retains the PHD3/4 chromatin reader domain, the CREBBP/MOF binding domain, and the SET domain complex, and so is able to read and write chromatin, while the N-terminal portion deriving from AFF1 is able to steer transcriptional elongation [56].

The role played by KMT2A-AFF1 and AFF1-KMT2A in the initiation of leukaemia is unclear. KMT2A-AFF1 has been reported to be involved in the initiation and maintenance of leukaemia, even though on its own it cannot initiate it [59]. However, other studies have shown that KMT2A-AFF1 is the main oncogenic driver through the Lin(–)/Sca1(+) purified cells (LSPCs) [60], but fails to initiate it in human cord blood-derived CD34+ haematopoietic stem/progenitor cells (HSPCs) [61]. KMT2A-AFF1 (alone) has induced a pro B-ALL immunophenotype, and a mixed lineage leukaemia when both alleles are present [56]. Also, interactions between KMT2A-AFF1 and AFF1-KMT2A during haematopoiesis have been shown to increase resistance to apoptosis and to increase the capability of the cell cycle [62]. The role of each allele in leukaemogenesis is still open to debate.

KMT2A-MLLT3 translocation

The KMT2A-MLLT3 [also known as MLL-AF9; t(9,11)(p22,q23)] is the second most common fusion partner of KMT2A and the most common fusion partner protein in both infant and adult AML [63, 64, 65]. It is most often associated with the acute monoblastic leukaemia morphologic type [64, 65]. The MLLT3 protein (including MLLT1) is

a member of the YEATS family (i.e. has the YEATS domain). The YEATS domain is an epigenetic reader that is found in the N-terminal of both the MLLT3 and MLLT1 protein, and it binds to both acetylated and crotonylated lysines of H3K9, H3K18, and H3K27, unlike the bromodomain which can only bind to acetylated lysines. The YEATS family of proteins plays various roles in chromatin remodelling, histone modification, and transcription regulation [66]. In addition, the 84-amino acid of the C-terminal (structurally similar to MLLT1) is important for leukaemic transformation and is intrinsically disordered [through its ANC1 Homology Domain (AHD)]. The C-terminal domain of MLLT3 binds to four proteins of which the functions in transcription elongation include the super elongation complex (SEC) which is a chromatin remodelling complex that consists of AFF1, MLLT3, MLLT10, and MLLT1, the eleven-nineteen Lys-rich leukaemia (ELL) family member ELL1, ELL2, ELL3 and the positive transcription elongation factor b (P-TEFb), disruptor of telomeric silencing-1 (DOT1 Complex; a histone methyltransferase which methylates H3K79) which are transcription activators [67, 68], and CBX8 — which is a part of the PRC1 repressive complex and BCOR, a BCL6 corepressor that is involved in transcription repression [69, 70]. The disruption of the AF4-AHD interaction has been shown to result in cell necrosis of different cell lines that harbour the MLL translocation [71]. Thus, the MLLT3 protein is seen as a signalling hub that regulates transcription through dynamic recruitment of cofactors in normal haematopoiesis and in acute leukaemia [73], even more so in the development of the erythroid/megakaryocyte lineage as well as haematopoietic stem/progenitor cells (HSPC) [56, 73]. Although KMT2A-MLLT3 is most often associated with AML, a few cases of ALL have been reported [74].

KMT2A-MLLT1 translocation

The KMT2A-MLLT1 [MLL-ENL; t(11;19)(q23;p13.3)] is the third most common fusion partner of the KMT2A protein, and also a member of the YEATS family. Unlike its structurally homologous partner KMT2A-MLLT3, it is more prevalent in lymphoid leukaemias than in myeloid leukaemias [63]. Like KMT2A-MLLT3, it is also a component of the super elongation complex (SEC), DOT1L complex (DotCom), as well as the AF4-ENL-P-TEFb complex (AEP) — another important transcriptional regulator that mediates the phosphorylation of RNA Polymerase II (Pol II) as well as leukaemogenesis [56, 75]. Just like MLLT3, it is an epigenetic reader that plays a role in transcriptional regulation and histone binding through the YEATS domain — acetylation, propionylation, butyrylation, and crotonylation [76].

The normal MLLT1 protein is made up of 559 amino acids, with a 75-amino acid YEATS domain found on the N-terminal, while the C-terminal contains the 84-amino acid ANC1 Homology Domain (AHD) (which it shares with AF9). Interestingly, whereas MLLT3 is an important regulator of haematopoiesis, MLLT1 seems to have only a minimal effect on haematopoiesis [77, 78]. MLLT1 plays an important role in leukaemogenesis through the maintenance of a deregulated gene expression in the KMT2A-MLLT1 fusion protein. The AT hooks and MT domain seems to be crucial, as their deletion decreases their ability to initiate leukaemogenesis. Using the Crispr–Cas9 technology, Reimer et al. [79] showed that KMT2A-MLLT1 protein could as a 'first hit' initiate oncogenic transformation. Thus, targeting KMT2A-MLLT1 protein may have therapeutic value. This leukaemia-initiating activity is prominent in the haematopoietic progenitor subsets with granulocytic and monocytic cells, but not with haematopoietic stem cells [80].

The KMT2A-MLLT1 protein is also involved in cellular immunity. Ugale et al. [81] have shown that the induction of KMT2A-MLLT1 can cause inhibition of normal T-cell development both *in vitro* and *in vivo,* which is believed to be related to the inhibition of Notch signalling. Also, NK cells have been shown to be capable of eradication of KMT2A-MLLT1 leukaemia cells *in vivo* [82].

KMT2A-MLLT10

The KMT2A-MLLT10, t(10;11)(p12;q23) translocation accounts for c.8% of all KMT2A-rearranged translocations [14]. It is more commonly found in AML than in ALL. The KMT2A-MLLT10 protein's known target is the aberrant histone 3 lysine 79 (H3K79). It does this through the chromatin-modifying enzyme and histone methyltransferase disruptor of telomeric silencing 1-like (DOT1L) — the only known histone methyltransferase that targets the histone H3K79 for mono-, di-, or trimethylation [83]. The PHD and the leucine-zipper motif of MLLT10 biochemically interact with DOT1L to aid the H3K79 methylation. DOT1L exists as a complex called the DotCom which is made up of some other fusion partners including MLLT10, MLLT1, MLLT3, and MLLT6, and also the known Wnt pathway modifier β-catenin, TRRAP and Skp1 [84]. The leucine zipper motif of the MLLT10 is needed to immortalise myeloid progenitors as well as induce leukaemogenic transformation [85].

The normal MLLT10 gene found on chromosome 10p12 encodes a 109kDa protein. The MLLT10 protein is highly expressed in the testis, peripheral blood, kidney, thymus, and brain [86]. While the translocation t(10;11)(p12;q23) is associated with the KMT2A-MLLT10 translocation, a reciprocal translocation between chromosomes 10 and 11, a t(10; 11)(p13–14; q14–21) in the U937 cell line has also been cytogenetically proven. This fusion partner of MLLT10 at 11q14 is known as CALM (clathrin assembly lymphoid myeloid) leukemia. The CALM-MLLT10 protein can induce leukaemia with a prolonged latency, but will need the collaboration of Zeb2 protein and the ras pathway for

this to happen [87]. The CALM-MLLT10 fusion protein is also known to disrupt differentiation of haematopoietic cells through the upregulation of HoxA cluster genes [88]. The MLLT10 protein is part of the zinc finger/leucine zipper MLLT10/MLLT6 leukaemia fusion genes (ALFs) which include MLLT6, BRL and CEZF proteins.

KMT2A-rearrangements and lineage plasticity

Lineage plasticity can be defined as a process by which cancer cells change from one morphological and functional cell type to another (and back), under the influence of particular environmental pressures [89]. In the context of KMT2A-rearranged acute leukaemias, lineage plasticity would mean a switch in cellular phenotype from a lymphoid cell to myeloid (and vice versa). Although a switch from lymphoid to myeloid is much more common, myeloid to lymphoid switches have been reported in the literature [90, 91]. Switches can also be from a T-cell ALL to a B-cell ALL [92]. These shapeshifters, or 'shadow walkers' as they are aptly called by Iacobucci et al. [93], resemble weird characters from various mythologies that can transform themselves from human to beast in order to elude their adversaries, such as Loki in Norse mythology [93]. This transformative ability is seen in embryonic stem cells and in some physiological responses like tissue repair. They can also be 'hijacked' by cancer cells causing metastasis, relapse and therapeutic resistance. Cancer has therefore been proposed to be a disease of excess plasticity [94]. Lineage plasticity is a complex biological process that involves clonal lineage divergence, with the formation of new populations of cancer cells (with clones and subclones) resulting in tumour heterogeneity.

Two pertinent questions about lineage plasticity are: what regulates lineage plasticity, and how does heterogeneity emerge? Several models have been proposed. These include 'linear evolution', 'branching evolution', 'neutral evolution' and 'punctuated evolution'.

The branching evolution model is one where the clones diverge from a common ancestor, and evolve in parallel in the tumour mass, resulting in multiple clonal lineages [95]. This model has been proven in several sequencing studies including single cell DNA sequencing [96], multi-region sequencing [97], and deep sequencing [98]. The branching model has been reported in several cancer types including leukaemias [95, 99]. A recent study by Ahlgren et al. [100] of KMT2A-rearranged acute leukaemias analysed through whole genome sequencing (WGS), whole exome sequencing (WES) and ultra-deep resequencing, showed that the leukaemias typically evolved through branching evolution. The authors highlighted various facts including that in 76% of the patients studied, a selective sweep occurred at first relapse, and additional subclones were often detected at relapse. In addition, eight pathways were altered, with the signalling pathways the most common, and with c.60% subclonal. In addition, cell cycle, epigenetic, B-cell development, transcription factor, glucocorticoid receptor signalling, purine metabolism and cohesion were also altered. Hyrenius-Wittsten et al. [101] equally showed that signalling mutations of Braf, Cbl, Kras, and Ptpn11 were involved in clonal expansion of KMT2A-MLLT3 leukaemia cells. In the same study, Flt3 mutation was also noted to accelerate onset of AML. Thus, signalling mutations play a major role in lineage plasticity.

Several mechanisms have been suggested for lineage plasticity, especially in KMT2A-rearranged acute leukaemia cells. These include the emergence of a *de novo* unrelated leukaemia, the role of transcription factors in the programming of a lineage-specific blast, and therapy--mediated selection of sub-clones from a common pluripotent progenitor [102]. The molecular basis of lineage plasticity of KMT2A-rearranged acute leukaemia provides some interesting insights. Khabirova et al. [103] carried out cell signal analysis of 1,655 leukaemia transcriptomes of both AML and ALL. Analysis of the transcriptional signatures of the childhood leukaemia transcriptomes showed expected patterns, including those in the Schwann cell precursors used as negative controls.

However, KMT2A-rearranged B-ALL showed similarities to early lymphoid precursors (ELP) — oligopotent lymphoid precursors with the ability to differentiate between different lymphocyte lineages and the cells bearing the immunophenotype CD34+CD127+CD10−CD19− [16, 103]. They also discovered that the ELP signal in later stages of B-cell development was significantly higher in KMT2A--rearranged B-ALL than in other high (cytogenetic)-risk B-ALL subtypes. The ELP signal was present in all types of KMT2A-rearranged B-ALL, irrespective of the fusion partner, but was most pronounced in KMT2A-AFF1. Through the use of single-cell RNA-sequencing, they further discovered that KMT2A-rearranged B-ALL bore very close similarities to ELP cells at diagnosis and relapse and in refractory disease. Phylogenetic analysis has also shown a branching evolution. Thus, KMT2A-rearranged B-ALL starts development very early, before haematopoietic specifications towards a lymphoid or a myeloid lineage. In the same vein, Morris et al. [104], using single cell RNA sequencing, were able to show that KMT2A-rearranged B-ALL leukaemia-initiating cells (LICs) — primitive cells that possess the ability for sustained, deregulated self-renewal and the potential to fully reconstitute fulminant leukaemia at relapse or upon xenotransplantation — were phenotypically and transcriptionally heterogeneous so as to arise from different populations of blast cells [104]. Most of the cells were pro-B cells, with the LICs possessing a primitive phenotype and multipotency programmes. The KMT2A-rearranged B-ALL has a high frequency of LICs with functional heterogeneity

that is driven by c-myc and oxidative phosphorylation. This further supports the theory that KMT2A-rearranged leukaemias originated from a primitive haematopoietic stem/ /progenitor cell.

On the other hand, Tirtakusuma et al. [105], in exploring the molecular basis of lineage switch in KMT2A-AFF1 acute leukaemias (ALL, AML and MPAL), showed through gene expression analysis of lymphoid cells and their corresponding myeloid relapses that lineage switch was associated with low expression of lymphoid transcription factors, surface marker genes (e.g. PAX5, EBF1, CD19, CD20, and CD22), and of immunoglobulin genes, and upregulation of myeloid genes including CSF3R and members of the CEBP TF family. Chromatin-binding motifs were increased for myeloid transcription factors, but reduced for lymphoid transcription factors, indicating an impairment of chromatin accessibility. Impairment of epigenetic regulation was also demonstrated through the loss of chromodomain helicase DNA-binding 4 (CHD4), that encodes the ATPase/helicase subunit of the histone-modifying nucleosome remodelling and deacetylase complex, and was downregulated in AML relapses compared to the lymphoid cell of origin. There was also an increased CD33 expression, which suggests that lineage switching was promoted by disrupted epigenetic regulation in KMT2A-AFF1 leukaemia. The ALL mutational landscape of the KMT2A/AFF1 lineage switch appears rather quiet, as shown from exome sequencing (a median of about 25 non-synonymous somatic single nucleotide variants {SNVs} or insertions/deletions {indels}), while the AML relapse was a mean of 92 SNVs and indels. This significant increase confirms the heterogeneity of the acute leukaemia cells, and shows there is a limited overlap between the original ALL cells and their AML relapses. Since the original subclones at presentation were lost at relapse, this therefore means that alternative subclones must be the origin of the relapse. In pursuit of the origin of lineage switch in KMT2A-AFF1 B-ALL, the original lymphoid samples and their AML relapse were analysed for B-cell receptor (BCR) rearrangements with RNA sequencing and whole exome sequencing. While all the ALL samples showed oligoclonal rearrangements of the BCR loci, their AML relapse revealed three different patterns: I) The AML relapse cells were from a population of primitive precursor cells prior to early immunoglobulin recombination; II) AML relapse from an unrelated BCR rearrangement in a myeloid cell, or B-lymphoid cells committed to undergo rearrangement, or a transdifferentiated minor ALL clone with an alternative rearrangement; and III) The similarity of BCR rearrangement between the original ALL sample and the AML relapse indicated that the myeloid relapse originated from the major ALL clone.

Thus, it has been shown that AML relapse can arise from different stages of lymphoid leukaemogenesis.

While transcriptional factor reprogramming plays a role in clonal evolution of KMT2A-rearranged acute leukaemias, the fusion partner of KMT2A and the cancer microenvironment have also been shown to be important in determining the leukaemia phenotype. KMT2A-AFF1 translocation is most often committed to a lymphoid lineage. Lin et al. [106] showed that mouse models transduced with human CD34+ expressing KMT2A-AFF1 developed a pro-B ALL immunophenotype (CD34+, CD10–) which were reproducible. In a similar experiment, human HSPCs expressing KMT2A-MLLT3 or KMT2A-AFF1 were transplanted into immunodeficient NSGS mice with a known proclivity for myeloid development. The KMT2A-MLLT3 cells seamlessly developed into AML in the mice (CD33+CD19–). On the other hand, the KMT2A-AFF1 cells also developed into AML (CD33+CD19–CD56+CD4+), albeit with a slightly longer latency compared to KMT2A-AFF1. However, the KMT2A-AFF1 cells later displayed CD19+CD33– lymphoid cells with variant expressions of CD34 and CD10, indicating an upregulation of lymphoid committed genes and diminished myeloid committed genes [107]. Lin et al. [107] also showed that while KMT2A-AFF1 can induce a pro-B cell stage, KMT2A-MLLT3 induces a pre-B cell stage. This means that different KMT2A fusion partners can influence gene expression patterns and the ultimate leukaemia phenotype. Recently, Heuts et al. [108] demonstrated that KMT2A-AFF1 deregulates normal hiPSC-derived haematopoiesis by upregulation of important KMT2A-MLLT3 target genes such as MEIS1, HOXA9, and CDK6, and downregulation of genes involved in myelomonocytic differentiation. This and other results show that KMT2A-MLLT3 is highly biased towards AML.

The microenvironment of HSPCs is known to be altered in leukaemias [109, 110]. The tumour microenvironment is known to play a role in the development and progression of cancer [111]. The microenvironment also affects lineage plasticity. Rowe et al. [112] demonstrated that along with age the microenvironment directs lineage commitment in KMT2A-rearranged leukaemias. They discovered that transduced KMT2A-MLLT3 leukaemia cells in the adult microenvironment lead to an expected myeloid phenotype. However, in the neonatal microenvironment, some populations of cells that had agranular features and morphologically appeared lymphoid were interspersed with myeloid cells. Immunophenotyping of the cells showed they expressed B-cell marker B220/CD45R in some leukaemias, with co-expression of the myeloid progenitor marker CD16/32. This result showed that KMT2A-MLLT3 HSPC cells in a neonatal microenvironment can be transformed to the lymphoid lineage. CCL5 from the adult marrow stroma was implicated in the inhibition of lymphoid differentiation. However, KMT2A-MLLT1 HSPC cells that usually develop a mixed-lineage B-lymphoid/myeloid leukaemia in adults when transplanted in secondary recipients of the neonatal

microenvironment, transformed into CD19+ cells that have undergone IgH recombination, reinforcing the fact that the neonatal microenvironment augments lymphoid differentiation. Wei et al. [113] also showed that when KMT2A-MLLT3 transformed cells were grown in an environment with myeloid-differentiating cytokines they developed myeloid surface markers. However, when grown in a milieu of cytokines that promote lymphoid differentiation, they expressed both B cell and myeloid markers. This further buttresses the role of the microenvironment in lineage plasticity. It is important to note that the microenvironment also promotes survival and therapeutic resistance in KMT2A-rearranged acute leukaemia [114].

Extrinsic factors for lineage plasticity can also come in the form of therapy-mediated 'switch'. While a relapse after therapy may be representative of a lineage switch, it is also possible that it is the development of a *de novo* clone unrelated to the original acute leukaemia. Bartsch et al. [115], through RNA sequencing of B-cell precursor ALL, showed that CYB5Aalt was expressed in both the diagnostic and the relapsed samples of the cohorts. Woodward et al. [116], in their study of clonal origin of hyperdiploidy type ALL, showed that chromosome 8 recurrently differed between diagnostic and relapse samples. However, some of these mutations may be subclonal. Sayyab et al [117], in their study on the clonal evolution of ALL from diagnosis to relapse, observed that patients with persistent clones that are resistant to treatment at diagnosis will still present with resistant disease from first to second relapse. Patients with rising clones that have subclonal mutations, and are pre-resistant to therapy, did not acquire additional driver mutations at relapse. However, the founding clones had a three-fold increase in driver mutations from diagnosis to relapse [117].

None of the aforementioned cases made reference to the role of therapy in relapse (though there was no lineage switch). Pandit et al. [118] reported the case of a 19-year-old male with an initial diagnosis of B-ALL who received the augmented Berlin-Frankfurt-Munster (aBFM)-90 regimen for induction, but relapsed to AML with monocytic differentiation and this time loss of TP 53(17p13). Qing et al. [119] reported the case of a 13-year-old who was initially diagnosed with B-ALL and given induction therapy with vincristine, daunorubicin, prednisone, and PEG-asparaginase as well as intrathecal cytrabine and methrotrexate, who achieved initial remission followed by relapse treated by blinatumomab, and relapsed again with KMT2A-rearranged AML which was therapy-related and not present at the initial diagnosis. These cases represent the development of a lineage switch unrelated to the initial leukaemia type. Park et al. [120] also reported four cases of ALL lineage switch to AML after therapy. In three of these, the original karyotype had been replaced by an entirely different abnormal karyotype. C.5-10% of KMT2A-rearranged acute leukaemias are therapy-related [121]. Generally, alkylating agents and topoisomerases are implicated, although topoisomerases, such as etoposide, can cause a *de novo* leukaemia which can be unrelated to the initial cancer, and in most cases is KMT2A-rearranged. Thiopurine has also been implicated in therapy-related ALL as well as some chemo-resistant mutations [122, 123].

In recent years, lineage switch has been frequently reported in newer immunotherapeutic agents including the bi-specific T-cell engager (BiTE), blinatumumab. Blinatumumab is a monoclonal antibody with bi-specificity for both CD19 on B cells and CD3 on a cytotoxic T cell. The patient's cytotoxic T cell is directed against CD 19-bearing leukaemia cells after it has been activated. Blinatumumab has shown significant benefits in the management of ALL [124–127] including in KMT2A-rearranged ALL [128, 129]. However, some refractory ALL express CD19 negative blast, while some still with CD19 positivity develop some resistance as a result of antigen downregulation; in some reported cases, there is a lineage switch to AML [130–133] especially in KMT2A-rearranged ALL [134, 135]. This also applies to CAR-T cell therapy [136]. In a retrospective review of 420 refractory/relapsed B-ALL patients treated with a murine-based CD19-CAR construct by Lamble et al. [137], 166 (39.5%) relapsed, including 83 (50%) CD19+, 68 (41%) CD19–, and 12 (7.2%) had lineage switch relapses with c.75% having KMT2A-rearrangement. Jacoby et al. [138] reported that CAR-T therapy could inadvertently 'pressurise' a lineage switch.

Clinical presentation of KMT2A-rearranged acute leukaemia

KMT2A-rearranged acute leukaemia is more common in infants than in adults, where it accounts for more than 70% of acute leukaemias [16]. It is reported to have a slightly higher occurrence in males than in females [139]. KMT2A-rearranged acute leukaemia has certain features that distinguish it from other forms of leukaemias, most especially in infants. Some of these features include hyperleukocytosis (WBC > 30 × 10^9/L) and central nervous system involvement (CNS). Leukaemia cutis (skin infiltration) is another feature that is associated with KMT2A-rearranged leukaemia [140]. In the ELAM02 study, KMT2A-gene rearrangements were significantly more associated in patients with leukaemia cutis than in those without [141]. Though more common in AML than ALL, the KMT2A-AFF1 fusion is the most common KMT2A type in leukaemia cutis; KMT2A-AFF1 is very rare [142–144]. Other features seen in them include hepatomegaly and splenomegaly. Jaundice, lymphadenopathy and pleural effusion are less common. Immunophenotypic expression of KMT2A-rearranged ALL is usually characterised by CD19 and CD34 positivity in the pro-B immunophenotype as well as the lack of CD10.

The co-expression of CD15, CD33, CD65, and CD68 has also been reported [145]. Chiaretti et al. [146] reported that CD19, CD22 (membrane and cytoplasm) and CD79a are the earliest B-cell markers, and the identification of any two without further differentiation markers identifies pro-B ALL. The presence of CD22-, CD34-, CD 19-, TdT-, cytoplasmic (Cy) CD79a-, CD10-, and Cy mμ-positive, and cortical/thymic T-ALL, where lymphoblasts are cyCD3-, CD7-, TdT-, CD5-, and CD1a-positive is identified as pre-B ALL. Higher frequency of CD7 and neuron-glial antigen-2 (NG2), as well as exclusivity of expression of CD15 and 133 in KMT2A-AFF1, have been reported by Gao et al. [147] in Chinese children. The NG-2 protein is frequently expressed in KMT2A-rearranged leukaemia, and has been reported to be involved in leukaemia invasiveness and CNS infiltration which is associated with lower event-free survival (EFS) and higher occurrence of CNS relapse [148, 149].

The immunophenotype of AML with KMT2A-rearrangement is quite diverse, without any clues. In the immunophenotypic study of AML with MLL-rearrangement by Konoplev et al. [150], the blasts were classified into five categories and were varied, with many being HLA-DR-, CD117-, CD13-, CD33-, CD123-, CD15-, CD38-, CD64-, and CD14-positive. A few others were not fully expressed including CD34, CD56, CD7, CD2, CD4, CD19, CD22, CD25, and TdT [150]. In analysing immunophenotypic expression in KMT2A-MLLT3 fusion, Bain et al. [151] reported the expression of monocytic markers without expression of CD19. Some were CD33 and CD4 positive with dim co-expression of CD15 and CD65. CD13 and CD34 were somewhat lacking [151]. Generally, there were no distinct immunophenotypic markers for KMT2A-rearranged AML.

Management of KMT2A-rearranged acute leukaemias

The management of KMT2A-rearranged acute leukaemias is often based on chemotherapy. It usually involves intensive chemotherapy for induction followed by additional chemotherapy for consolidation. Prognosis of KMT2A-rearranged acute leukaemias is often poor [145], and current treatment regimens are generally ineffective, with shorter overall survival (OS). In infant ALL, which is often dominated by the KMT2A-AFF1 translocation, 4-year event-free survival (EFS) is 20–50%. While 80–90% of patients achieve remission, about two-thirds of them will eventually relapse within a year while still on treatment [152]. The standard regimen for paediatric ALL does include a steroid and vincristine, in combination with asparaginase and an anthracycline according to the risk group for induction therapy. After induction therapy, a consolidation and maintenance therapy including CNS prophylaxis is given to all patients. Popular regimens used include the interfant-06, interfant-99 and Berlin-Frankfurt-Munster (BFM) regimens [153]. These intensification therapies can reduce the risk of relapse. However, complications such as infections are usually increased, and in most cases survival is not increased [154, 155]. More recently, blinatumumab, the CD3/CD19 bi-specific T-cell engager, has been shown to be effective in the management of relapsed/refractory precursor B-cell ALL [126, 156, 157]. It has equally been shown to be effective in KMT2A-rearranged infant ALL [158]. Van der Sluis et al. [128] found that the addition of blinatumumab to the interfant-06 protocol resulted in markedly improved outcomes among infants with KMT2A-rearranged acute lymphoblastic leukaemia (ALL) compared to historical controls; when comparing the blinatumumab regimen to historical controls in interfant-06, 2-year disease-free survival was 81.6% *vs.* 49.4% and 2-year overall survival was 93.3% *vs.* 65.8% [128]. In other cases, blinatumumab has been reported to be good in adults with mixed phenotypic acute leukaemia (MPAL) and CD19-positive AML, especially as a bridge to transplant or consolidation chemotherapy [159, 160]. In a phase III clinical trial, Locatelli et al. [160] showed that blinatumumab was superior to high-risk third course consolidation chemotherapy (HC3) in prolonging event-free survival (EFS) in children with high-risk first relapse BCP-ALL [161]. Bartram et al. [162] reported three cases of MPAL that were successfully treated with blinatumumab prior to transplant. In a recent phase III clinical trial of children, adolescents, and young adults with low-risk B-Cell ALL in first relapse treated with blinatumumab + chemotherapy or chemotherapy alone (NCT02101853), Hogan et al. [163] reported that blinatumumab significantly improved DFS and OS for two thirds of patients with bone marrow (BM) ± extramedullary (EM) relapse.

Given these outstanding findings, especially those by Van der Sluis et al. [102], blinatumumab may become the new standard of care in the management of KMT2A-rearranged acute leukaemia, although the issue of lineage switch may still need to be addressed [164].

In KMT2A-rearranged AML, the outcome is usually less straightforward compared to ALL. The standard therapy is usually '7 + 3' *i.v.* cytarabine and an anthracycline. Complete remission (CR) is achieved in 60-80% of younger adults and in 40–60% of older adults (aged 60 or over) [165]. More often, those patients who are high risk (e.g. KMT2A-AFF1) and eligible go for an HSCT after a CR [166]. In patients with a therapy-associated AML (KMT2A-rearrangement are often present), CPX-351, a liposomal encapsulation of cytarabine/daunorubicin in a 5:1 molar ratio is approved for its management [167, 168]. The KMT2A-MLLT3 translocation is the most common MLL fusion in AML, but it is classed as being associated with an intermediate prognosis, and thus may benefit from HSCT or intermediate dose cytarabine [166, 169]. Recently, Lo et al. [170] re-categorised AML patients with t(7;11)(p15;p15)/NUP98::HOXA9 into the adverse risk subset. Thus, their line of management may slightly alter.

The role of HSCT in the management of KMT2A-rearranged acute leukaemia is still somewhat controversial, most especially in terms of efficacy and its toxicities. According to Hunger et al. [171], there is no basis for the use of HSCT as first-line therapy for infant ALL in first remission. Also, according to the interfant-99 international ALL trial, the majority of infant KMT2A-r patients did not benefit from HSCT over standard consolidation chemotherapy, but a small subset with high risk infant ALL were reported to benefit from HSCT [172, 155]. According to Pieters et al. [173], the interfant-06 study was not designed to compare HSCT to chemotherapy, although from their findings the use of HSCT should be restricted to the high risk group. An analysis of the North American CCG 1953 and POG 9407 infant ALL trials by Dreyer et al. [174] showed that routine use of HSCT for infants with KMT2A-rearranged ALL is not indicated: the 5-year EFS rate was 48.8% (95% CI, 33.9% to 63.7%) in patients who received HSCT and 48.7% (95% CI, 33.8% to 63.6%) in patients treated with chemotherapy alone ($p = 0.60$). In 62 patients treated with the MLL03 protocol of the Japanese Paediatric Leukaemia/Lymphoma Study Group: short-course intensive chemotherapy followed by early allogeneic haematopoietic stem cell transplantation (HSCT) within four months of the initial induction, Koh et al. [175] reported that although early use of HSCT effectively prevented early relapse, and was feasible for infants with KMT2A-rearranged ALL, a considerable proportion still relapsed, and that therefore HSCT should be reserved only for the subgroup with poor risk factors. On the other hand, the Japanese Paediatric Leukaemia/Lymphoma Study Group trial MLL-10 stratified infants with ALL into three risk groups (low risk [LR], intermediate risk [IR], and high risk [HR]) according to their KMT2A status, age, and presence of central nervous system leukaemia. In the Children's Oncology Group AALL0631, modified chemotherapy with the addition of high-dose cytarabine in early intensification was introduced to KMT2A-r patients and the option of HSCT was restricted to HR patients only. Of the 56 HR patients recruited, 49 achieved complete remission. The 3-year event-free survival (EFS) rate for patients with KMT2A-r ALL (IR + + HR) was 66.2%. For those with germline KMT2A (KMT2A-g) ALL (LR), the 3-year EFS rate was 93.3%, and the 3-year EFS rate was 94.4% for IR patients and 56.6% for HR patients [176–178]. Although they mentioned the issue of life-threatening toxicities and that HSCT should be further limited in use, their trial showed some benefits in infant ALL. The use of more efficacious targeted therapies has been suggested as the way forward instead [179].

In non-infant (paediatric) KMT2A-rearranged ALL, Bai et al. [180] showed that HSCT at first CR had significantly better overall survival (OS), event-free survival (EFS), and cumulative incidence of relapse (CIR) than those who received consolidation therapy only. Their study was further validated in another cohort of non-infant KMT2A-rearranged AML where pre-HSCT MRD + status was reported to be an independent risk factor [181]. Pollard et al. [182] in their study (NCT01407757) showed that gemtuzumab ozogamicin (GO) when added to chemotherapy showed superior EFS compared to chemotherapy alone (EFS 48% with GO v 29% without GO, $p = 0.003$) in KMT2A-rearranged AML, and for patients who subsequently received HSCT, prior GO therapy was associated with a decreased risk of relapse (5-year RR: 28% GO and HSCT v 73% No GO and HSCT, $p = 0.006$).

The benefit of HSCT in adults with KMT2A-rearranged ALL is also inconclusive. Yu et al. [183] reported an improvement in OS and 3-year relapse when compared to chemotherapy. This was different from what was reported by Marks et al. [184] in the UKALLXII/ECOG2993 trial (NCT00002514) that sought to determine the outcome of KMT2A-AFF1 translocation treated via HSCT. Marks et al. determined that age was a predictor of outcome, and that HSCT did not show any advantage over chemotherapy. HSCT showed a favourable outcome in adults with MPAL in another study, but the same could not be said of the paediatric group [185]. However, Munker et al. [186] reported that OS was best in those aged under 20 in their own study of MPAL patients. Recently, Tong et al. [187] reported that umbilical cord blood transplantation (UCBT) was superior to chemotherapy in KMT2A-MLLT3 patients, with 3-year overall survival (OS) of the UCBT group of 71.3% (95% CI, 34.4–89.8%) *vs.* 10% (95% CI, 5.89–37.3%) in the chemotherapy group, ($p = 0.003$). Disease-free survival (DFS) was 60.8% (95% CI, 25.0–83.6%), *vs.* 10% (95% CI, 5.72–35.8%) ($p = 0.003$), and the relapse rate was 23.6% (95% CI, 0–46.8%), *vs.* 85.4% (95% CI, 35.8–98.4%) ($p < 0.001$). In summary, HSCT appears to be beneficial in adults and some non-infant KMT2A-rearranged AML.

Prognosis of KMT2A-rearranged acute leukaemias

As earlier stated, while different subtypes of KMT2A-rearranged acute leukaemia can be managed, the prognosis is generally poor, with long-term survival rates of less than 60% across all age groups. However, a number of factors determine the prognosis. Children aged >1 often have a better prognosis than infants [140]. This is because they usually have a more favourable genetic profile. Infant B-ALL with KMT2A rearrangement has been noted to possess a more immature phenotype (pro-B cell) with the frequent aberrant co-expression of myeloid markers that makes it more aggressive [103]. Among adults, KMT2A-rearranged AML has been shown to be associated with inferior outcomes compared to AML with a normal karyotype. Age at diagnosis <60 is equally seen as a better prognostic indicator in adults.

Different studies have also shown that fusion partners of the KMT2A protein have prognostic value. For example, the KMT2A-MLLT3 fusion protein has been observed to have a better PFS and OS than other fusion proteins, both in children and adults. On the other hand, KMT2A-MLLT10 and KMT2A-AFDN have a very poor prognosis among children and adults alike. Interestingly, the KMT2A-MLLT11 has been observed to have a significantly favourable outcome, with a PFS of 92% and OS of 100%. This demonstrates that not all KMT2A rearrangements in acute leukaemia are poor prognostic indicators. The European LeukaemiaNet (ELN) recommendations for diagnosis and management of AML also place KMT2A-MLLT3 as an intermediate prognosis [169]. The germline KMT2A rearrangement, KMT2A-NUTM1 in infants, also has a favourable prognosis.

Associated mutations like the ras mutation, SETD2, TP53 and FLT3 have also been shown to be poor prognostic indicators with poor EFS and OS, especially in the paediatric age group. Increasingly, MRD is being seen as a marker of inferior disease outcome in KMT2A-rearranged acute leukaemia. MRD negativity has been shown to have a superior EFS and OS with a lower cumulative incidence of relapse. Also in adults undergoing allo-HSCT, achieving a first complete remission was seen as a good prognostic indicator in a study among KMT2A-rearranged acute leukaemia.

Other factors that affect prognosis in KMT2A-rearranged acute leukaemia include hyperleukocytosis and extramedullary disease with CNS involvement especially in infants, therapy-related disease, and lineage switch.

Other treatment options

CAR-T cell therapy

Chimeric antigen receptor T cell (CAR-T) therapy is an immunotherapy whereby the body's own immune system is harnessed to target and destroy cancer cells. CAR-T involves the use of synthetically engineered autologous CD4+ and CD8+ T lymphocytes receptors against a specific antigen present on the malignant cell [188].

CAR-T therapy is one of the most exciting therapies in the management of ALL, with high rates of CR achieved in patients who have relapsed following other treatments [189]. The number of KMT2A-rearranged acute leukaemia patients enrolled in CAR-T therapy studies is reported to be limited, although good responses have been observed. Gardner et al. [190], in their study of seven patients with KMT2A-rearranged B-ALL treated with CD19-targeted CAR-T cells, showed that all seven patients achieved CR in the blood and bone marrow; however, two of the patients with KMT2A-AFF1 translocation relapsed into AML a month after treatment. This lineage plasticity and immune escape appears to be one of the drawbacks of CAR-T therapy in the management of KMT2A-rearranged acute leukaemia [136, 191]. Most CAR-T cell clinical trials do not include infants in their studies, as FDA-approved age restrictions of >3 and <25 apply to CAR-T therapies, which is rather discouraging as infants are the highest risk group for KMT2A-rearranged acute leukaemia [192]. These restrictions are due to concerns regarding the feasibility of T-cell collection and expansion. However, a few case reports have shown the feasibility of CAR-T in infants, and it is hoped more infants could be enrolled in future trials [193, 194]. Consortia studies have equally validated the use of CAR-T in KMT2A-rearranged infant leukaemia [195, 196].

Finally, while CAR-T cell therapy is seen as a viable option in the management of ALL, to date there has been no CAR option for the management of AML. This is due to different factors including a high heterogeneity at the genetic and molecular levels of AML, an immunosuppressive tumour microenvironment that dampens CAR T-cell responses, and a lack of unique tumour-specific antigens in AML [197, 198]. Overcoming some of these challenges may be daunting, but it is hoped that in future years CAR therapies could be available, especially for high risk AML patients such as the KMT2A-rearranged type [199, 200].

Hypomethylating agents

Hypomethylating agents have been shown to be viable treatment options in the management of AML patients who are transplant-ineligible. Hypomethylating agents are nucleoside analogues the main activity of which is the inhibition of DNA methyltransferases that are involved in the epigenetic regulation of some genes, especially the tumour suppressor genes. KMT2A-rearranged acute leukaemias are characterised by an extensive promoter hypermethylation which leads to silencing of a subset of tumour suppressor genes. Stumpel et al. [201] demonstrated that KMT2A-AFF1 and KMT2A-ELL were extensively hypermethylated, and display a higher risk of relapse. They were equally able to show that the demethylating agent zebularine reverses aberrant DNA methylation and effectively induces apoptosis in KMT2A-rearranged ALL cells. Zebularine was also able to re-activate some miRNAs that were downregulated in KMT2A-AFF1-positive infant ALL [202]. These activities of zebularine in KMT2A-rearranged acute leukaemia mean hypomethylating agents can be used in their management. Decitabine and 5-azacitidine are FDA-approved for the management of AML and MDS. In their 2018 study, Roolf et al. [203] showed that decitabine has antileukaemic activity on KMT2A-rearranged BCP-ALL cell lines, and its strongest antiproliferative effects were observed when combined with a cytostatic drug. However, the *in vivo* combination of decitabine and cytarabine did not enhance the antiproliferative effect compared to decitabine alone. Schneider et al. [204] also discovered that decitabine prolonged survival in xenograft mice of KMT2A-rearranged ALL by 8.5 days, but was insufficient to halt progression. However, it had additive and moderately

synergistic effects with the histone deacetylase inhibitor panobinostat, the BCL2 inhibitor venetoclax, the MEK inhibitor pimasertib, and the receptor tyrosine kinase foretinib when combined *in vitro* [204]. Ball et al. [205] also reported 18 patients with MLL-rearranged AML treated with hypomethylating agents and venetoclax, a BCL-2 inhibitor. 9/18 achieved an overall response (ORR 50%), including eight with complete remission (CR/CRi 44%) and one (6%) with a morphologic leukaemia-free state.

Combination therapy of a hypomethylating agent and venetoclax has been shown to be a viable option both in pre-clinical studies and clinical studies of KMT2A-rearranged acute leukaemias and other high-risk leukaemias [206–211]. Currently, a clinical trial of azacitidine in combination with chemotherapy in infants with KMT2A-rearranged ALL is being conducted by the National Cancer Institute (NCT02828358), which hopefully might lead to a new standard in the management of KMT2A-rearranged acute leukaemias [212].

HDAC inhibitors

Histone deacetylase inhibitors (HDACi) have been reported to show some activity in KMT2A-rearranged acute leukaemias. HDACi are enzymes that remove acetyl groups from histones and other proteins, thereby regulating chromatin accessibility and target gene expression. Many studies have shown the overexpression of HDAC classes in various leukaemias, especially acute leukaemias, and studies have shown that HDACi such as panobinostat, vorinostat, and tricostatin A can induce cell death through apoptosis and autophagy, and equally cause growth arrest [213–218]. Early studies have shown the potential of HDACi in the management of cancers: Tonelli et al. [219, 220] reported that an HDACi (valproic acid) caused G1 growth arrest and apoptosis in a KMT2A-MLLT3 cell line through the upregulation of p21 [221]. Panobinostat, a pan-HDAC inhibitor from cinnamic hydroxamic acid, was shown to induce cell death in KMT2A-AFF1 xenograft mice by Castro et al [222, 223]. In a related study, they showed it reduced disease burden and increased OS through the depletion of H2B ubiquitination via suppression of the RNF20/RNF40/WAC E3 ligase complex. It has also been shown to work well in combination with some other chemotherapies. Panobinostat in combination with curaxin CBL0137 (CBL0137), a carbazole derivative with chromatin-modulatory effects and antineoplastic activity on different cancers including KMT2A-rearranged acute leukaemia [224–228], was shown to be potentiated by the latter and induced apoptosis with extended survival in an aggressive model of KMT2A-MLLT3 [229]. It equally showed synergistic effect with bortezomib, although in a preclinical study of xenografted ALL mice in combination with methotrexate and 6-mercaptopurine it did not have any synergistic effect [229, 230]. Similarly, romidepsin has been found to enhance the activity of cytarabine *in vitro* and in xenografted mice [231, 232]. Although a clinical case report described a complete cytogenetic response (CCR) in a 60-year-old man with therapy-related KMT2A-rearranged AML treated with single agent panobinostat, a number of early clinical trials did not show such efficacy [233–236]. However, the Mayo Clinic has an ongoing phase I clinical trial, the PAVE study (NCT04172844), combining a hypomethylating agent (azacitidine/decitabine) and an HDACi (pevonedistat) in combination with venetoclax for the treatment of adults with AML. A recent phase II clinical trial, a substudy under the Beat AML umbrella Master trial (NCT03013998), showed that treatment with pevonedistat and azacitidine did not induce CCR responses in older patients with TP53-mutated AML, which stands in sharp contrast to what was reported by Swords et al. [237, 238] (NCT01814826). Whether pevonedistat or any other HDACi in a combination therapy can achieve sustained objective responses remains to be seen, as we await the results from PAVE . The very recent phase I/II study of azacitidine, venetoclax and pevonedistat in newly-diagnosed secondary AML and in MDS or CMML (NCT03862157) by Short et al. [239] showed encouraging activity of the triplet regimen.

Proteasome inhibitors

Proteasome inhibitors (PIs) have shown significant efficacy in the management of different haematological cancers. Their efficacy in cancer management is believed to be a result of a high dependence of cancer cells on the proteasome machinery as a result of a high cell turnover compared to normal cells. However, the use of proteasome inhibitors has come with mixed results. Various preclinical and clinical studies have suggested that proteasome inhibitors may have some good activity against KMT2A-rearranged acute leukaemia. Bortezomib has been noted to have activity against AML cell lines, especially in synergy with some other chemotherapies [240–241]. Szczepanek et al. [242] also showed that bortezomib displayed more activity in T-ALL patient samples compared to common/pre-B ALL. PIs have also been shown to have activity in KMT2A-rearranged leukaemia cells. Liu et al. [243] showed that PIs increased the protein levels of both wild-type KMT2A and KMT2A fusion proteins, and also triggered apoptosis and cell cycle arrest which involved the cleavage of BID by Caspase-8 and upregulation of p27. Recently, Ge et al. [245] equally demonstrated the mechanism of bortezomib resistance in KMT2A-AFF1 cell xenografts, and how to overcome it. Bortezomib has also been reported to selectively target leukaemia stem cells and inhibit its progression, and to extend overall survival in KMT2A-MLLT3 transformed leukaemic mice [245]. Another PI, carfilzomib, has been shown to be more sensitive to acute leukaemia cells than bortezomib [246, 247]. However, in a recent study, while carfilzomib could also synergise with chemotherapies *in vitro*, it did not translate to an *in vivo* benefit, nor was there any survival

advantage in KMT2A-rearranged ALL; the same is true with bortezomib [248–250].

Bortezomib has shown some promise both in clinical trials and case reports especially in ALL. The clinical trials NCT00440726 and NCT00873093 have shown the clinical benefits of bortezomib in combination therapy for ALL [251, 252]. In a recent phase III trial by Teachey et al. [253], patients with T-ALL had significantly improved EFS and OS with bortezomib (NCT02112916). The incorporation of bortezomib and vorinostat into an ALL chemotherapy backbone for newly diagnosed infants with ALL is currently being investigated by St Jude Children's Research Hospital in a single arm phase II study, with unpublished data showing some good results in KMT2A-rearranged ALL (NCT02553460) [254]. An earlier study of bortezomib and vorinostat in KMT2A-rearranged haematological cancers was terminated early (NCT02419755). If these good results are sustained, there is the prospect of PIs in the management of KMT2A-rearranged ALL. Ixazomib is also being investigated in acute leukaemia [255].

CDK4/6 inhibitors

CDK6 is a member of the cyclin dependent kinase family of proteins. It plays a key role in the regulation of the cell cycle. It promotes cell cycle progression through phosphorylation and inhibition of target genes such as RB1 after binding to D cyclins, and so it is a D cyclin-activated kinase. Different studies have shown that CDK6 is indeed a direct target of KMT2A fusion proteins, playing an important role in myeloid leukaemogenesis as well as conferring proliferation advantages of KMT2A-rearranged ALL cells [256, 257]. While KMT2A-rearranged fusion proteins are exceptionally dependent on CDK6, the same cannot be said of its functional homologue CDK4; CDK6 is thus seen as a target for KMT2A-rearranged acute leukaemias [256, 258]. Pre-clinical studies show that CDK6 inhibitors are active against acute leukaemia especially in combination therapy [259–263]. Abemaciclib, a CDK4/6 inhibitor approved for treating refractory hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2−) metastatic breast cancer has been shown to significantly suppress the proliferation of, and induce differentiation of, leukaemic stem cells *in vitro* as well as to enhance the anti-neoplastic effects of venetoclax [264]. It is also reported to have efficacy in KMT2A-rearranged AML cell lines and xenografts [265]. Ribociclib has demonstrated significant anti-proliferative effects in AML and B-ALL cell lines and primary samples including a KMT2A-MLLT10 cell line [266]. Palbociclib also demonstrated activity in KMT2A-rearranged acute leukaemias [267, 268]. In a recent pre-clinical study, Wang et al. [269] reported that a combination of palbociclib, venetoclax and azacitidine performed better than venetoclax and azacitidine both *in vitro* and *in vivo* by enhancing the down-regulation of MCL-1 and BCL-XL, anti-apoptotic proteins [269]. Clinical studies have shown mixed results. A phase I/II trial of CPX-351 + palbociclib in patients with AML with poor prognosis showed good tolerance and high efficacy with no reported deaths; phase II results are expected (NCT03844997) [270]. Raetz et al. [271] very recently published their findings of the Children's Oncology Group AINV18P1 phase I trial of palbociclib in combination with standard four-drug re-induction chemotherapy in children and young adults with relapsed/refractory ALL. Five CRs were observed, and palbociclib was well tolerated. The phase I/IIa AMLSG 23–14 trial of palbociclib in the management of KMT2A-rearranged acute leukaemias, which initially showed good tolerability and clinical efficacy, however achieved only 2/16 responses [272, 273].

Novel therapies under development

Menin inhibitors

The menin protein as discussed earlier plays an important role in the oncopathogenesis of the MLL fusion proteins, including KMT2A-MLLT1, KMT2A-AF1, KMT2A-AFDN, and KMT2A-MLLT3, which can later transform into acute leukaemia. Paradoxically, menin, which is a product of the MEN1 gene found in chromosome 11q13, is a tumour suppressor in endocrine organs [275]. Menin is therefore a scaffold protein that is involved in the regulation of gene expression and cell signalling. Several studies have revealed the crystal structure of menin in complex with the N-terminus of KMT2A and in a ternary complex with it (KMT2A) and lens epithelium-derived growth factor (LEDGF) which is important for the upregulation of Hoxa9 and Meis1 [276, 277]. Targeting menin-KMT2A interaction has been postulated as a possible therapeutic approach for the development of novel therapeutics for KMT2A-rearranged leukaemias. This has led to research into the development of small molecule inhibitors that target and inhibit interaction between menin and KMT2A [278]. Some of these molecules include M1-3454, MI-538, MI-1481, and BAY-155 [279]. Recently, two molecules have been pushed into clinical trials, with good results: KO-539 (ziftomenib) and SNDX-5613 (revumenib). Ziftomenib, an orally active menin-KMT2A interaction inhibitor being developed by Kura Oncology Inc. with good pre-clinical results, had a phase I clinical trial reported at the American Society of Haematology (ASH) meeting in December 2022, the KOMET-001 (NCT04067336) [280–282]. Phase II of KOMET-001 is still ongoing [283]. Revumenib, another potent, oral, selective inhibitor of menin–KMT2A interaction has been reported to have good activity in KMT2A-rearranged acute leukaemia. In the AUGMENT-101 phase I trial of R/R AML treated with revumenib, the ORR was 53% with the rate of CR+CRh+CRp of 38% (23/60) and CR/CRh rate of 30% (18/60); 12 patients were able to proceed to allogeneic stem cell transplantation [284, 285]. Based on the phase I/II AUGMENT-101 trial (NCT04065399), the US FDA

Table I. Current clinical trials of menin inhibitors in adult and paediatric KMT2A-rearranged acute leukaemias

Trial identifier	Phase	Condition	Drug	Status
NCT04065399	I/II	R/R AML and ALL	Revumenib monotherapy	Recruiting
NCT05326516	I	R/R AML and ALL/ /MPAL	Revumenib/Pred/VCR/ASP/DNR Revumenib/Flu ± Ara-C	Completed
NCT05761171	II	R/R KMT2A ALL	Revumenib/Flu + Ara-C, MTX	Recruiting
NCT05360160	I/II	R/R AML/MPAL	Revumenib/VEN/ASTX727	Recruiting
NCT06177067	I	R/R AML/ ALAL	Revumenib/VEN/AZA	Recruiting
NCT04067336	I/II	R/R AML	Ziftomenib monotherapy	Recruiting
NCT05735184	I	R/R AML/Newly diagnosed	Ziftomenib/7 + 3 Ziftomenib/VEN/AZA	Recruiting
NCT05848687	I/II		Ziftomenib/multiagent	Recruiting
NCT04811560	I/II I	Infant ALL R/R AML and ALL	Bleximenib monotherapy Bleximenib/VEN/AZA	Recruiting Recruiting
NCT05453903	I/II	R/R AML/Newly diagnosed	DSP-5336 monotherapy	Recruiting
NCT04988555	I/II	R/R AML and ALL	DS-1594 ± AZA, VEN or mini-HCVD	Completed
NCT04752163	I	R/R AML and ALL	BMF-219 monotherapy	Recruiting
NCT05153330		R/R AML and ALL		

granted a breakthrough therapy designation to revumenib [286]. Phase II of AUGMENT-101 has been recruiting, but recently Syndax Pharmaceuticals announced that it has met its primary endpoint in the trial [287]. Recently, the NUP98 fusion protein which is dependent on menin–KMT2A interaction, and characterised by elevated expression of HOXA and MEIS1 genes, has also been shown to be inhibited by menin inhibitors [288, 289]. Another novel menin inhibitor, DS-1594a, is also being investigated. Menin inhibitors have also been shown to have good activity in combination therapy [290–293].

Thus, menin inhibitors are potential therapeutic agents against KMT2A-rearranged acute leukaemias (Tab. I).

Dot1L inhibitors

Preclinical studies have shown that the histone 3 lysine 79 methyltransferase disruptor of telomeric silencing 1-like (Dot1L) is necessary for the neoplastic transformation of KMT2A-rearranged fusion proteins. DOT1L is involved in the transfer of S-methyl group of (S)-adenosyl-l-methionine (SAM) to the amino group of H3K79, producing a methylated substrate and (S)-adenosyl-l-homocysteine. DOT1L is the only known histone methyltransferase that specifically targets nucleosomal histone H3 lysine 79 (H3K79) for mono-, di-, or trimethylation (H3K79me1, me2, or me3) and it is involved in gene transcription and DNA damage response [294, 295]. Two Dot1L inhibitors have been studied thoroughly, EPZ004777 and EPZ-5676 (pinometostat), but due to its poor pharmacokinetic properties, EPZ004777 is limited in its clinical development [296]. On the other hand, xenograft model of KMT2A-rearranged acute leukaemia has been shown to cause tumour regression when treated with EPZ-5676 (pinometostat). Furthermore, pinometostat has been shown to have synergy with either cytarabine or daunorubicin against MOLM-13 and MV4-11 leukaemia cell lines; likewise with azacitidine [297]. It also showed a synergistic effect when combined with some chemotherapy agents against ALL cell line RS4;11 [298].

Pinometostat has been in some clinical trials. In a phase I dose escalation clinical trial (NCT01684150), it was found to be safe and tolerable with modest efficacy as a single dose. Although its maximal tolerable dose was not reached in the trial, two patients, both with t(11;19) translocation, entered complete remission at 54 mg/m^2 per day by continuous *i.v.* infusion. The most common adverse events of any cause were fatigue (39%), nausea (39%), constipation (35%), and febrile neutropenia (35%) [299]. Another phase I trial in children with relapsed/refractory KMT2A-rearranged acute leukaemia (NCT02141828) showed that pinometostat has a good safety profile with a recommended phase II dose of 70 mg/m^2 continuous *i.v.* in children aged over 1 [300]. A phase Ib/II study of pinometostat with azacitidine in adult KMT2A-rearranged AML has completed enrollment (NCT03701295), but is yet to publish any findings. However, a phase Ib/II trial of pinometostat with standard chemotherapy in the treatment of patients with newly diagnosed acute myeloid leukaemia and KMT2A-rearrangement (NCT03724084) has been terminated [301]. Newer, orally available Dot1L inhibitors, with improved pharmacokinetics, are being developed for better efficacy [302–304].

Table II. Potential treatment options for MLL1-rearranged acute leukaemias

Investigational drug	Therapeutic target
FLT-3 inhibitors (e.g. quizartinib, gliteritinib)	FLT3 protein
Hypomethylating agents (e.g. azacitidine)	Inhibition of DNA methyltransferases
HDAC inhibitors (vorinostat)	HDAC enzymes
Proteasome inhibitors (e.g. bortezomib)	Proteasome machinery; NF-KB
CDK4/6 inhibitors (e.g. palbociclib)	Cyclin dependent kinases
BCL-2 inhibitors (e.g. venetoclax)	BCL-2 proteins
MCL-1 inhibitors (e.g. maritoclax)	MCL-1 proteins
MEK inhibitors (e.g. trametinib)	Ras mutations
Menin inhibitors (e.g. revumenib)	Menin protein
DOT1L inhibitors (e.g. pinometostat)	DOT1L protein
BET inhibitors (e.g. I-BET151)	BET proteins
LSD1 inhibitors (e.g. tranylcypromine)	LSD1 enzyme
Integrin inhibitors (e.g. OS2966)	Integrin beta-1 (ITGB1) protein
Polycomb protein inhibitors (e.g. PRT4165)SYK inhibitors (e.g. entospletinib)	Histone (H2A) ubiquitination SYK protein
CAR-T cells (e.g. brexucabtagene autoleucel)	CD19
Bi-specific antibodies (e.g. blinatumumab)	CD3/19

Bromodomain inhibitors

The bromodomain and extra-terminal domain (BET) family of proteins consists of four conserved members (Brd2, Brd3, Brd4, and Brdt) that recognise and bind to acetyl--lysine residues. BET proteins have been linked to different cancers because of their role in post-translational modifications, which is generally associated with transcription activation that can lead to the aberrant expression of some oncogenes. While acetyltransferases, and deacetylases and sirtuins, are considered the 'writers', and 'erasers' of histone acetylation, BET proteins are considered the 'readers' of the same process [305]. Brd4 is known to be involved in the SEC/PTEF-b complex for the phosphorylation of RNA polymerase II. Some other transcriptional activators, including NSD3, JMJD6, and CHD4, are recruited to the ET domain of Brd4 to further promote gene transcription [306]. Brd3, on the other hand, helps in the upregulation of GATA-1 associated genes [307]. Brd4 under normal circumstances is involved in chromatin stability and cell cycle control from the M phase to the G1 phase [308]. Brd4 is seen as a pivotal pharmacological target in cancer because of its inhibition of the MYC oncogene which is associated with many cancers, but has no known inhibitor as yet [309, 310].

Preclinical studies have shown that BET proteins play a role in KMT2A-rearranged leukaemia. Using the KMT2A-MLLT3/NrasG12D AML cell line and an AML mouse model, Zuber et al. [311] were able to demonstrate that Brd4 was critically required for disease maintenance, and the inhibition of Brd4 using a small molecule inhibitor led to a significant cell cycle arrest and apoptosis as well as terminal myeloid differentiation. BET inhibitors have also been shown to have activity against KMT2A-AFF1, KMT2A-MLLT3 and RS4;11 cell lines [312, 313]. In their preclinical study of a mouse model of KMT2A-AFF1 + infant ALL, Bardini et al. [314] showed that a BET inhibitor (I-BET151) impaired leukaemic engraftment and lowered disease burden *in vivo*. It was also able to cause cell cycle arrest and induce apoptosis as well as sensitise glucocorticoid-resistant MLL-rearranged cells to prednisolone *in vitro*; there was a deregulation of BRD4, HOXA7/HOXA9, and RUNX1 gene [314]. Several other BET inhibitors have been studied in preclinical studies with good efficacy [315–317]. A few clinical trials have also been initiated across different cancers. Most of the studies, especially in acute leukaemias, have been in phase I/II and with modest results [318, 319]. A recent report of a phase I trial of a BET inhibitor in R/R AML patients (NCT02308761) showed that the BET inhibitor tested lacked efficacy as a monotherapy. Therefore, acombination with other therapeutic agents is being considered [320].

Other investigational treatments

Besides the aforementioned, other investigational treatment options include FLT-3 inhibitors, MEK inhibitors, integrin inhibitors, lysine-specific demethylase-1 (LSD1) inhibitors, SYK inhibitors, BCL-2 inhibitors, polycomb protein inhibitors, MCL-1 inhibitors and bi-specific antibodies. Some of these agents are undergoing clinical trials, and it is to be hoped that they will form part of the combination therapy for the management of KMT2A-rearranged acute leukaemias (see Tab. II).

Conclusions

KMT2A-rearranged acute leukaemias have been shown to be a very complex neoplasm with a variable prognosis, but usually poor. The use of standard chemotherapies and allogeneic haematopoietic stem cell transplantation has not yielded many results, especially in the infants who are most affected. However, extensive research efforts in terms of its biology and plasticity have provided more insights into druggable targets.

With precision medicine, more targeted therapies are being evaluated, although with mixed results. There is optimism that with the array of exciting therapies under development, we may be getting closer to a combination that could improve outcomes in this subset of patients with aggressive disease.

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