EPIGENETICS AND GENETICS

Leukaemogenesis: more than mutant genes

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Abstract | Acute leukaemias are characterized by recurring chromosomal aberrations and gene mutations that are crucial to disease pathogenesis. It is now evident that epigenetic modifications, including DNA methylation and histone modifications, substantially contribute to the phenotype of leukaemia cells. An additional layer of epigenetic complexity is the pathogenetic role of microRNAs in leukaemias, and their key role in the transcriptional regulation of tumour suppressor genes and oncogenes. The genetic heterogeneity of acute leukaemias poses therapeutic challenges, but pharmacological agents that target components of the epigenetic machinery are promising as a component of the therapeutic arsenal for this group of diseases.

Chromosome translocation
A structural abnormality resulting from the rearrangement of pieces generally between two non-homologous chromosomes.

Acute leukaemia
A type of malignancy that results in the rapid growth of abnormal immature white blood cells (myeloid or lymphoid leukaemic blasts) in the bone marrow and blood and inhibition of normal haematopoiesis.

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Our understanding of carcinogenesis in general, and of leukaemogenesis and lymphomagenesis in particular, has been substantially aided by the discovery of chromosome translocations that transform cells. Identifying the genes at translocation breakpoints was the first step[1,2] to understanding why chromosome translocations can be oncogenic. Indeed, the first genes identified were oncogenes, *MYC* and *ABL1*. Importantly, for those who questioned the role of chromosome translocations in malignancy, these findings resolved any doubts because one of the partner genes in the 8;14 translocation (Burkitt's lymphoma)[3,4] and the 9;22 translocation (chronic myeloid leukaemia (CML))[5] was a bona fide oncogene[6,7]. Moreover, for those who had doubts about the relevance of oncogenes to human cancer (they were, after all, generally cloned from experimental mouse tumours), these discoveries showed their relevance to human disease.

However, as translocation breakpoints in acute leukaemia (TABLE 1), especially acute myeloid leukaemia (AML), were cloned the oncogenic function of the translocated genes became less obvious. For example, the 8;21 translocation (generally found in acute myeloblastic leukaemia)[8] and the 15;17 translocation[9] (found in a rare subtype of AML, acute promyelocytic leukaemia (APL)), each involved a gene crucial for myeloid cell self-renewal, proliferation and/or differentiation, but the partner gene was generally not active in myeloid cells. For the t(8;21) translocation the active gene was *AML1* (also known as *RUNX1*), the DNA binding portion of the α-subunit of core binding factor (CBF) and the inactive gene was *ETO* (also known as *RUNX1T1*), a homologue of *Drosophila melonogaster nervy* that is active in neurons[10]. For the t(15;17) translocation, the active gene was retinoic acid receptor-α (*RARA*)[11,12], the protein product of which is centrally involved in cell differentiation; its partner was a newly identified gene called *PML* for promyelocytic leukaemia[12]. The fusion proteins AML1–ETO and PML–RARA were found to repress the transcription of wild-type *AML1* and *RARA* target genes, respectively, by recruiting co-repressor complexes containing histone deacetylases (HDACs) (FIG. 1)[13–17]. This was a revelation and immediately pointed to a potential therapeutic strategy, namely transcriptional derepression using pharmacological inhibitors of these co-repressor complex components.

In reality, the prototype for the successful therapeutic targeting of transcriptional repression by fusion proteins present in leukaemia cells has been the use of all-*trans* retinoic acid (ATRA) for leukaemias with the PML–RARA fusion[18,19], whereas HDAC inhibitors have been less successful in treating other acute leukaemias. We now know that histone alterations are not so simple. In fact, the histone code is remarkably complex, with a pattern that includes acetylation and methylation (monomethylation, dimethylation and trimethylation) that seems to proceed in an ordered, complex and incompletely understood fashion[20]. In addition, there is a complex interplay of histone modifications and methylation that determines the state of chromatin structure and is crucial to the regulation of gene transcription[21]. Clearly, to develop effective treatment that reverses transcriptional deregulation in leukaemia, we must understand more about the precise details of these epigenetic marks that are part of the epigenetic code.

At a glance

- Acute leukaemias, arising from neoplastic transformation of uncommitted or partially committed haematopoietic stem cells, are characterized by recurring chromosomal aberrations and gene mutations that are crucial to disease pathogenesis.
- The recurring chromosomal translocations in acute myeloid leukaemia (AML) result in the generation of chimeric fusion proteins that in many cases function as transcriptional regulators. These include AML1–ETO (generated by a translocation between chromosomes 8 and 21, t(8;21)); CBFB–MYH11 (generated by an inversion of chromosome 16, inv(16) or t(16;16)); PML–RARA (generated by t(15;17)); MOZ–CBP (generated by t(8;16)); MORF–CBP (generated by t(10;16)); MOZ–TIF2 (generated by inv(8)); and MLL fused with various partners (generated by t(11q23)). They contribute to leukaemogenesis, at least in part by causing transcriptional deregulation through epigenetic modifications.
- Epigenetic modifications, including DNA methylation, DNA demethylation and histone changes, lead to the activation or repression of gene expression. Aberrant epigenetic changes occur frequently in acute leukaemias. Fusion genes resulting from chromosome translocations can be regulators or mediators of the epigenetic machinery.
- MicroRNA (miRNA) regulation may also considerably contribute to leukaemogenesis. Some miRNAs function as oncogenes or tumour suppressor genes in acute leukaemias. miRNA signatures correlate with cytogenetic and molecular subtypes of acute leukaemias, and some miRNA signatures are associated with outcome or survival of acute leukaemias.
- Not only do miRNAs function in an epigenetic manner by post-transcriptional regulation of target genes, but they can also be targets of the epigenetic machinery and effectors of DNA methylation and histone modifications. These functions might be involved in leukaemogenesis.
- Although the genetic heterogeneity of acute leukaemias poses therapeutic challenges, drugs or small molecules that target components of the epigenetic machinery hold great promise in the treatment of leukaemias. The use of all-*trans* retinoic acid in the therapy of acute promyelocytic leukaemia is one of the best known and most successful examples of targeted therapy involved in epigenetic changes; progress has also been made in the clinical trials of histone deacetylase inhibitors and DNA methyltransferase inhibitors. However, more effective treatment strategies are needed.

Histone deacetylase
An enzyme that regulates chromatin structure and function through the removal of the acetyl group from the lysine residues of core nucleosomal histones.

Histones
The chief protein components of chromatin, which have an important role in DNA packaging, chromosome stabilization and gene expression. Histones form the core component of nucleosomes.

Histone code
The 'rules' governing the pattern of covalent histone tail modifications. Histone tail modifications have an important role in the chromatin structure, and thereby in the regulation of gene expression.

To complicate matters, microRNAs (miRNAs) have been discovered[22]. These small (~22 nucleotides in length) non-protein coding RNAs pair to target mRNAs, usually at the 3′ untranslated regions, leading to the degradation of mRNA or interference with its translation[22–24]. miRNAs seem to have powerful regulatory effects on many genes in various cancers[24–27], including leukaemia[28–31]. Although our understanding of the regulation of miRNA expression is in its infancy, it is clear that mechanisms such as deletion, amplification and methylation (or other forms of epigenetic repression) are likely to play a part.

Besides the transcriptional and epigenetic deregulation conferred by oncogenic fusion proteins resulting from chromosomal translocations in acute leukaemia, mutations involving specific genes that mediate crucial signalling pathways and mutations in key transcription factors also have a crucial role in leukaemogenesis. For example FMS-related tyrosine kinase 3 (*FLT3*), *KIT*, *NRAS*, *KRAS*, C/EBP-α (*CEBPA*) and nucleophosmin (*NPM1*) mutations have been described in AML, and *PAX5*, transcription factor 3 (*TCF3*; also known as *E2A*), *EBF1*, *LEF1* and *IKZF1* (also known as *IKAROS*) mutations in acute lymphoblastic lukaemia (ALL). The role of these gene mutations in leukaemogenesis is outside the scope of this Review, and has been addressed recently in several other reports[32–34]. This Review describes the current understanding of epigenetic changes (including miRNA regulation) in acute leukaemias, with a particular focus on acute leukaemias characterized by balanced chromosomal aberrations.

Epigenetic changes in leukaemogenesis

The term epigenetics is generally used to refer to mitotically and meiotically heritable changes in gene expression that occur without alteration of the DNA coding sequence[35]. Epigenetic changes that underlie the development of leukaemia can be in one of two major categories: changes in the DNA methylation state (BOX 1) and alterations in the histone modification pattern (BOX 2). Recent insights suggest that these two major pathways of epigenetic modification function in concert to regulate gene transcription[21]. In comparison to normal cells, cancer cells exhibit global DNA hypomethylation accompanied by aberrant methylation of cytosine residues that precede guanosine (CpG) islands in gene promoters or coding regions[36]. In the context of leukaemogenesis, aberrant CpG island methylation in promoter regions in genes such as cyclin-dependent kinase inhibitor 2B (*CDKN2B*), which encodes the tumour suppressor INK4B, and *CDKN2A*, which encodes the tumour suppressors INK4A and ARF, is a well-described phenomenon. It is associated with transcriptional silencing, which also involves the recruitment of methyl-binding proteins and HDACs to regions near the transcriptional initiation sites[37,38].

In addition, the recurring chromosomal translocations in AML result in the generation of chimeric fusion genes, which in many cases have been identified as transcriptional regulators (TABLE 1). Several of these fusion proteins result in the development of leukaemia, partly by causing transcriptional deregulation through mechanisms linked to chromatin alterations (FIG. 1).

Core binding factor leukaemias. The fusion proteins resulting from the chromosomal translocations t(8;21)(q22;q22) (AML1–ETO) and inv(16)(p13q22) (CBFB–MYH11) (TABLE 1) have been identified as transcriptional repressors[15,39,40]. These fusion proteins are characterized by the disruption of CBF, a heterodimeric transcription factor that is important in haematopoietic differentiation. CBF consists of an α-unit, AML1 (the DNA binding component), and a β-unit, CBFB, which stabilizes AML1. In mouse models, homozygous loss of *Aml1* or *Cbfb* is characterized by a lack of definitive haematopoiesis and embryonic lethality[41,42]. Wild-type AML1 functions as a transcriptional activator. By contrast, the fusion proteins repress the transcription of AML1 target genes by either recruiting HDACs directly or cooperating with co-repressors, including nuclear receptor co-repressor 1 (NCOR1), NCOR2 and SIN3A[15–17,43]. Recently, AML1–ETO has also been shown to recruit DNA methyltransferase 1 (DNMT1)[44]; this finding implies that transcriptional silencing of AML1 target genes occurs at least partly through an interplay between histone deacetylation and promoter DNA methylation. AML1–ETO also

Table 1 | **Acute leukaemia and chromosome abnormalities***

Malignancy (FAB subtype)	Chromosomal abnormality	Molecular alterations (genes involved)	Frequency in AML	Frequency in ALL	Risk group assignment
AML (M2)	t(8;21)(q22;q22)	*AML1–ETO*	5–12%	NA	Favourable
AML (M4eo)	inv(16)(p13q22)/ t(16;16)(p13;q22)	*CBFB–MYH11*	3–10%	NA	Favourable
AML (M3)	t(15;17)(q22;q21)	*PML–RARA*	6–15%	NA	Favourable
AML (M4 and M5) or ALL	t(11q23)	*MLL*–various	5–8%	7–10%	Unfavourable or intermediate
AML	–5/del(5q)	NA	1–11%	NA	Unfavourable
AML	–7/del(7q)	NA	1–7%	NA	Unfavourable
AML	+8	NA	3–10%	NA	Unfavourable
AML or ALL	Normal karyotype	NA	30–50%	20–45%	Favourable or intermediate
AML or ALL	t(9;22)(q34;q11.2)	*BCR–ABL1*	1–2%	5–20%	Unfavourable
ALL (L1 or L2)	t(12;21)(p13;q22)	*TEL–AML1*	NA	10–25%	Favourable
ALL (L1 or L2)	t(1;19)(q23;p13.3)	*TCF3–PBX1*	NA	2–5%	Favourable or intermediate
ALL	t(17;19)(q22;p13)	*TCF3–HLF*	NA	1%	Unfavourable
ALL	t(8;14); t(2;8); t(8;22)	*MYC*–various	NA	1–2%	Unfavourable
ALL (L1 or L2)	Hyperdiploidy (>50 chromosomes)	NA	NA	10–25%	Favourable
ALL	Hypodiplody (<45 chromosomes)	NA	NA	1–5%	Unfavourable or intermediate

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; *AML1*, also known as *RUNX1*; *BCR*, breakpoint cluster region; *ETO*, also known as *RUNX1T1*; FAB, French-American-British classification; *HLF*, hepatic leukaemia factor; L, subtype of ALL; M, subtype of AML; M4eo, M4 with eosinophilia; *MLL*, mixed lineage leukaemia; NA, not applicable; *PBX1*, pre-B cell leukaemia homeobox1; *PML*, promyelocytic leukaemia; *RARA*, retinoic acid receptor-α; *TCF3*, transcription factor 3 (also known as *E2A*); *TEL*, ets variant 6 (also known as *ETV6*). *Acquired (somatic) clonal karyotype abnormalities are detected in 55–80% of patients with acute leukaemia, and the remaining 20–45% have a normal karyotype[177–182]. Recurrent genetic abnormalities have prognostic and therapeutic implications and also provide insights into the mechanisms of leukaemogenesis[178,179,182,183].

directly represses transcription of tumour suppressor genes such as *ARF* and *NF1* through the AML1 DNA binding domain[45,46].

Acute promyelocytic leukaemia. All patients with APL have the t(15;17) translocation or one of its variants, which results in a fusion protein comprised of all but the first 30 amino acids of RARA[47] fused to a variable partner at its amino terminus[12,48–52]. Wild-type RARA functions as a transcriptional activator, whereas the fusion protein functions as a transcriptional repressor through the recruitment of the HDAC, NCOR1 and NCOR2 (N-CoR) complex, DNMT1, DNMT3A, repressive histone methyltransferases and polycomb group proteins[53,54]. In cells with *PML–RARA* translocations, treatment with pharmacological doses of ATRA[18,19] relieves this repression by allowing the release of the N-CoR complex and the recruitment of a co-activator complex, which contains proteins with histone acetyltransferase (HAT) activity[55–57]. This results in the activation of RARA target genes as well as transcription factors crucial for normal haematopoiesis such as *SPI1* (also known as *PU.1*) and C/EBPβ (*CEBPB*), with subsequent differentiation of leukaemia cells[55–57]. Patients with APL have a high complete response rate to ATRA used in conjunction with chemotherapy, and APL has served as a paradigm for the successful therapeutic targeting of epigenetic changes in acute leukaemia. Although the effects of ATRA on cellular differentiation are crucial to its success in APL, ATRA also results in the degradation of PML–RARA[58], leading to growth arrest and a decline in leukaemia-initiating cells or leukaemia stem cells. Recently, the use of arsenic trioxide has also been found to result in the degradation of the PML–RARA fusion protein and apoptosis of APL cells. It also has significant clinical efficacy in the therapy of APL and can complement the use of ATRA in the treatment of patients with APL. In addition, treatment with arsenic trioxide potentially obviates the need for chemotherapy in some patients with this disease[55,57,59]. Gene expression and proteomic profiling experiments following treatment with both of these compounds reveal effects on multiple genes, including a pattern of upregulation of genes associated with myeloid differentiation and the downregulation of genes increasing cellular proliferation[55,57,60]. The synergistic effect observed with the combination of ATRA and arsenic trioxide in producing durable remissions in patients with APL has also been linked to the eradication of leukaemia stem cells[58].

Leukaemias that disrupt HATs. Besides the recruitment of HDACs, DNMTs and co-repressor complexes, the scope of epigenetic deregulation by chromosomal translocations in acute leukaemias also includes a disruption of the actual enzymes that are involved in chromatin modification[61]. For example, HATs such as CREB binding

Normal haematopoiesis
A developmental process by which all types of blood cells are continuously produced by rare pluripotent self-renewing haematopoietic stem cells. In normal adults, haematopoiesis occurs primarily in the bone marrow and lymphatic tissues.

protein (CBP; also known as CREBBP) and the closely related p300, as well as the monocytic leukaemia zinc finger (MOZ) and the related monocytic leukaemia zinc finger protein-related factor (MORF; also known as MYST4) are rearranged in chromosomal translocations in leukaemia. MOZ and MORF belong to the MYST family of HATs. The t(8;16) and t(10;16) translocations result in the fusion of two proteins with HAT activity: MOZ–CBP and MORF–CBP, respectively[62,63]. The MOZ–CBP fusion inhibits AML1-mediated transcription, resulting in a differentiation block, and the HAT domain of CBP was found to be indispensable in this regard[64]. In addition, the HAT domain of MOZ also has a crucial role in haematopoiesis, with abrogation of the HAT activity in embryonic stem cell lines and mouse cell lines leading to a significant reduction in the proliferation potential of haematopoietic precursors[65]. Therefore, it is plausible that deregulation of CBP and MOZ-mediated acetylation by chromosomal rearrangements could lead to a disruption in the balance between proliferation and differentiation during haematopoiesis, and so contribute to the leukaemogenic phenotype.

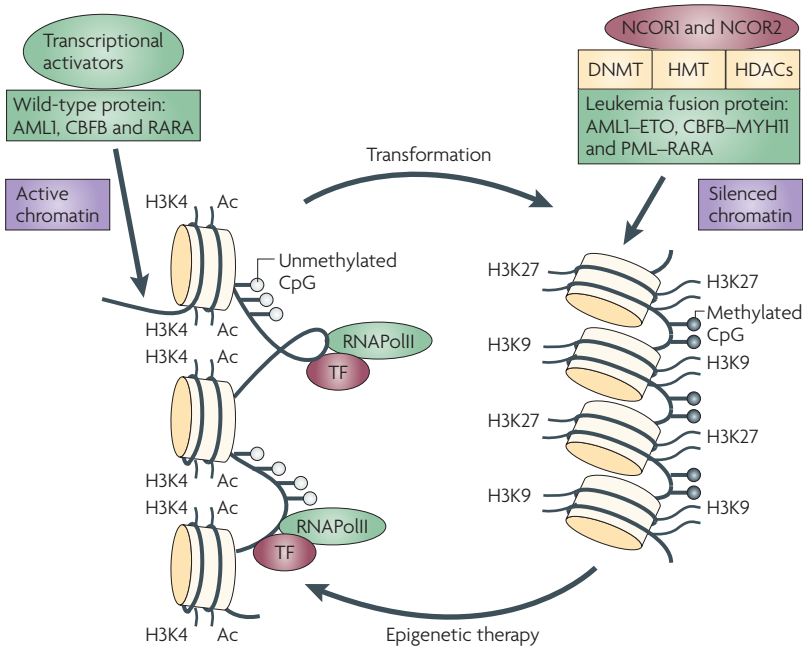

Figure 1 | **Leukaemia fusion proteins and epigenetic deregulation.** Oncogeneic fusion proteins such as AML1–ETO, CBFB–MYH11 and PML–RARA recruit transcriptional co-repressor complexes (including nuclear receptor co-repressor 1 (NCOR1) and NCOR2) that result in the loss of histone acetylation and the acquisition of repressive histone modification marks, such as histone H3 lysine 9 (H3K9) methylation and H3K27 trimethylation, as well as DNA methylation, and thereby a closed chromatin structure. This leads to the transcriptional silencing of various target genes, including genes that are crucial for haematopoietic differentiation. Epigenetic or transcriptional therapy (targeting the fusion proteins, components of the co-repressor complexes and downstream effectors such as microRNAs) has the potential to reverse these changes, leading to histone acetylation, acquisition of active marks such as H3K4 methylation, an open chromatin structure with subsequent transcriptional activation and differentiation of the leukaemic clone. Ac, histone acetylation; AML1, acute myeloid leukaemia 1; CBFB, core binding factor-β; CpG, cytosine residues that precede guanosine; DNMT, DNA methyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; PML, promyelocytic leukaemia; RARA, retinoic acid receptor-α; RNApolII, RNA polymerase II; TF, transcription factor.

An inversion on chromosome 8 (inv(8)) fuses the HAT domain of MOZ to the transcription factor TIF2 (also known as NCOA2)[66]. The MOZ HAT domain consists of a nucleosomal binding motif and an acetyltransferase catalytic domain (acetyl-CoA binding domain). Mouse models of the inv(8) fusion suggest that the nucleosomal binding domain of MOZ and the CBP interaction domain of TIF2 are essential for leukaemogenesis, whereas the N-terminal PHD domain and acetyl-CoA binding domain of MOZ are dispensable[67]. This implies that the MOZ–TIF2 fusion results in the deregulation of transcription through the aberrant recruitment of CBP to nucleosomal regions targeted by MOZ — the HAT activity of CBP might at least partly contribute to leukaemogenesis.

Translocation of mixed lineage leukaemia. The t(11;16) translocation, which fuses mixed lineage leukaemia (*MLL*) and CBP, is another example of a chromosomal translocation involving a protein with HAT activity[68]. *MLL* is located on chromosome band 11q23, has homology to the *D. melanogaster trithorax* gene (especially in the SET domain) and is involved in both myeloid and lymphoid leukaemias, as well as biphenotypic or mixed lineage leukaemias[69,70]. *MLL* is involved in chromosomal translocations with more than 60 different partner genes in acute leukaemias, and the mechanism of leukaemogenesis by MLL fusion proteins remains perplexing given the disparate nature of the multiple known partner genes that have nuclear or cytoplasmic functions[71,72]. However, because CBP had long been recognized to be a HAT, the MLL–CBP fusion protein provided an initial insight into potential mechanisms of leukaemogenesis induced by MLL fusions, and suggested that transcriptional deregulation through mechanisms linked to histone modifications and altered chromatin structure was important in disease pathogenesis[68].

Subsequently, MLL was demonstrated to possess histone (H3K4) methyltransferase activity (and so transcriptional activation properties) through its carboxy-terminal SET domain[73,74]. MLL and the tumour suppressor protein menin (encoded by multiple endocrine neoplasia type 1 (*MEN1*)), which binds MLL at its N terminus, have been shown to associate with the homeobox A9 (*HOXA9*) promoter[75,76]; recently, the chromatin-associated protein PSIP1 (also known as LEDGF) has been shown to be a crucial cofactor for this interaction[77]. MLL H3K4 methyltransferase activity is associated with the activation of MLL target genes including *Hoxa9* (REF. 74), which is important in the survival of *MLL*-rearranged leukaemias[78]. The absence of menin and/or PSIP1 results in a failure of MLL and MLL fusions to regulate *Hoxa9* transcription, illustrating the importance of MLL–menin interaction in MLL fusion protein-induced leukaemogenesis[71,77].

Despite the association of the SET domain with H3K4 methyltransferase activity and *Hoxa9* activation, it is consistently lost in the MLL fusions, except for the partial tandem duplication of MLL (MLL-PTD), in which the SET domain and thereby the histone H3 lysine 4 (H3K4) methyltransferase activity is maintained[79].

Box 1 | **DNA methylation and associated enzymes**

DNA methylation is mediated by DNA methyltransferases (DNMTs), which catalyse the conversion of cytosine residues that precede guanosine (CpG) to 5-methylcytosine, by the covalent addition of a methyl group at the 5-carbon position of the cytosine[175]. These CpG residues are under-represented in the genome as a whole and occur at only 5–10% of the predicted frequency. This under-representation is probably linked to the propensity of methylated cytosine to undergo spontaneous deamination to thymidine, leading to the progressive depletion of CpG dinucleotides over time. CpG residues cluster particularly in the promoter regions of genes, in so-called CpG islands, and are generally unmethylated in normal cells. They are associated with hyperacetylated histones and an open chromatin configuration, which facilitates accessibility to transcription factors and transcriptional activation. CpG island methylation in the promoter regions of genes is associated with transcriptional repression, gene silencing and a condensed chromatin state and is seen physiologically, for example, in the context of genes silenced on the inactive X chromosome and imprinted genes.

The known enzymatically active DNMTs include DNMT3A and DNMT3B, which are *de novo* methylases and bind to both unmethylated and hemimethylated CpG sites. DNMT1 is responsible for maintaining DNA methylation patterns and binds preferentially to hemimethylated DNA.

However, MLL fusion-mediated leukaemogenesis is not as simple a process as perturbed MLL-dependent H3K4 methylation[74]. Indeed, for several of the MLL fusion proteins, the loss of the SET domain and H3K4 methyltransferase activity may potentially be compensated for by the acquisition of an alternative unique histone methyltransferase activity conferred by the partner proteins[71,80]. For example, recent studies have shown that MLL fusion partners such as AF10, AF9, AF4 and ENL associate with the H3K79 histone methyltransferase DOT1L[81–83]. H3K79 methylation is also associated with transcriptional activation, and the acquisition of H3K79 methyltransferase activity has been demonstrated to be important for transformation by a subset of MLL fusion proteins[81,84]. In a recently developed mouse model of *MLL–AF4* leukaemia, genome-wide assessment of H3K79 methylation was carried out using a chromatin immunoprecipitation (ChIP)–chip technique, and approximately 1,000 promoters were found to be associated with increased H3K79 methylation compared with normal B cells[85]. Interestingly, short interfering RNA (siRNA)-mediated suppression of DOT1L decreased the expression of genes crucial for MLL fusion-mediated leukaemogenesis[85], suggesting that the modification of H3K79 methylation may be a potential therapeutic strategy in leukaemias involving MLL fusions.

miRNAs in leukaemogenesis

Besides the two classic epigenetic modifications (that is, DNA methylation and histone modifications), a third epigenetic mechanism has recently gained attention, namely miRNA regulation. miRNAs are crucial regulators of many physiological processes such as development, cell apoptosis, differentiation and proliferation. Emerging evidence shows that altered miRNA expression is associated with various types of cancers[24–26]. In addition, miRNAs function in complex regulatory networks to regulate haematopoietic differentiation (Supplementary information S1,S2 (box, table)) and contribute to leukaemogenesis[28–31] (FIG. 2; TABLE 2; see Supplementary information S2 (table)).

French-American-British (FAB) classification of AML
The classification system divides AML into eight subtypes, M0 to M7, on the basis of the type of cells from which the leukaemia developed and the maturity of the cells.

miRNAs as oncogenes in acute leukaemia. Several miRNAs have been shown to function as oncogenes in acute leukaemia. Those that have been most extensively studied are discussed in this section and included in TABLE 2.

The *mir-17-92* polycistron located at 13q31, which contains seven individual miRNAs (miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1), functions as an oncogene in various cancers including lymphoma, and lung, colon, pancreas and prostate tumours, medulloblastoma and multiple myeloma[86–88]. Recently, Li *et al.*[89,90] showed that miRNAs in the *mir-17-92* cluster are particularly overexpressed in acute leukaemia cells with *MLL* rearrangements, which is at least partly owing to the genomic DNA amplification of the locus[91]. Retroviral transduction of the *mir-17-92* cluster significantly increased proliferation as well as the colony-forming and replating capacity of mouse normal bone marrow progenitor cells alone and particularly, in cooperation with the *MLL–ELL* fusion[91]. These data suggest that the *mir-17-92* cluster may have an important role in the development of *MLL*-associated leukaemia. In normal haematopoiesis, *mir-17-92* has an essential role in monocytopoiesis[92] and megakaryocytopoiesis[93], and in B cell development[94–96]. *mir-17-92* is downregulated during monocytopoiesis and megakaryocytopoiesis and its forced expression represses monocytopoiesis (through targeting *AML1*)[92] and megakaryocytopoiesis[93]. *mir-17-92* inhibits B cell development at the pro-B to pre-B transition probably through targeting *PTEN* and *BIM*[94–96]. Therefore, aberrant overexpresion of *mir-17-92* in leukaemia inhibits normal haematopoiesis and thereby contributes to leukaemogenesis. In addition to the targets described above, E2F family transcription factors have also been suggested as both functional targets and regulators of *mir-17-92* (REFS 97–99). Moreover, Li *et al.*[90] reported that 19 predicted targets of *mir-17-92*, including *RASSF2* and *RB1*, were significantly downregulated in *MLL*-rearranged leukaemia and exhibit a significant inverse correlation with expression of the miRNAs. The direct regulation of *RASSF2* and *APP* has been confirmed by using a luciferase reporter assay[90]. Therefore, a group of target genes of *mir-17-92* has been identified and it is essential to determine which ones are crucial in leukaemogenesis.

miR-155 has an important role in megakaryopoiesis, erythropoiesis[93,100,101] and lymphopoiesis[102]. Transgenic mice with forced expression of miR-155 initially exhibit a preleukaemic pre-B cell proliferation evident in spleen and bone marrow, followed by a frank B cell malignancy, indicating that miR-155 can induce polyclonal expansion, favouring the occurrence of secondary genetic changes for full transformation[103]. In AML, miR-155 is specifically overexpressed in leukaemia with internal tandem duplication of the receptor tyrosine kinase *FLT3* (*FLT3*-ITD)[104,105], but the upregulation of miR-155 is independent from FLT3 signalling[106]. miR-155 was reported to be overexpressed in a subset of AML (particularly M4 and M5 according to the French-American-British (FAB) classification of AML), and sustained expression of miR-155 in haematopoietic stem cells (HSCs) caused a myeloproliferative disorder[107].

Short-term repopulating HSCs
Haematopoietic stem cells (HSCs) are composed of short-term repopulating (STR) and long-term repopulating (LTR) stem cells. STR HSCs can sustain the haematopoietic system for only a short term, whereas LTR HSCs can reconstitute haematopoiesis for life.

Antagomir oligos
A class of chemically engineered antisense oligonucleotides that are complementary to either the mature miRNAs or their precursors and are used to specifically inhibit the activity of endogenous miRNAs, probably through irreversibly binding them. Antagomirs are used experimentally to constitutively inhibit specific miRNAs.

Nucleosomes
The basic units of chromatin that consist of approximately 146 base pairs of DNA wound around an octameric core of histone proteins: an H3-H4 tetramer and two H2A-H2B dimers.

miR-196a and miR-196b are significantly upregulated in AMLs with *NPM1* mutations[104] and in *MLL*-associated paediatric ALL[108], as well as in *MLL*-associated AML[89]. Popovic *et al.*[109] showed that during mouse embryonic stem cell differentiation, *Mll* normally regulates the expression of miR-196b in a pattern similar to that of the surrounding Hox genes, *Hoxa9* and *Hoxa10*. In the haematopoietic lineage, the expression level of miR-196b reached a peak in short-term repopulating HSCs and then decreased as cells became more differentiated. Leukaemogenic MLL fusion proteins caused overexpression of miR-196b, and treatment of *MLL–AF9*-transformed bone marrow cells with miR-196-specific antagomir oligos abrogated their replating potential in methylcellulose. Forced expression of miR-196b in bone marrow progenitor cells led to increased proliferative capacity and survival, as well as a partial block in differentiation[109]. Consistently, miR-196 (and miR-21) is significantly downregulated by the transcriptional repressor GFI1 during the transition from common myeloid progenitors to granulocyte–macrophage progenitors, and forced expression of miR-196b (particularly when co-expressed with miR-21) significantly blocks granulopoiesis[110]. Therefore, miR-196 probably contributes to leukaemogenesis through increasing proliferation while blocking differentiation of haematopoietic progenitor cells.

miRNAs as tumour suppressor genes in acute leukaemia. miRNAs have also been shown to function as tumour suppressors in acute leukaemia (TABLE 2), although data in this area are more limited than for those miRNAs that function as oncogenes. The let-7 family is a well-known tumour suppressor gene family, and functions as a negative regulator of a set of oncogenes including *NRAS*, *KRAS* and *HMGA2* (REFS 111,112). In acute leukaemia, let-7b and let-7c were downregulated in CBF leukaemia cases[104]. On treatment of APL primary leukaemia samples and cell lines with ATRA, let-7a-3, let-7c and let-7d were upregulated, whereas their target Ras genes were downregulated[113]. The tumour suppressor property of miR-15a and miR-16-1 was first highlighted by the findings that they were deleted or downregulated in 68% of chronic lymphocytic leukaemias and targeted *BCL2*, an anti-apoptotic gene[114,115]. In AML, miR-15a, miR-15b and miR-16-1 are upregulated while their target *BCL2* is downregulated in APL cells after treatment with ATRA[113]. In addition, a MYB–miR-15a autoregulatory feedback loop was reported in which miR-15a targeted MYB and blocked the cells in the G1 phase of the cell cycle, while MYB bound the promoter region of miR-15a and was required for miR-15a expression; moreover, MYB and miR-15a expression was inversely correlated in cells undergoing erythroid differentiation[116].

miRNA expression profiling in acute leukaemia. Li *et al.*[89] observed distinct miRNA expression patterns for t(15;17) translocations, *MLL* translocations and t(8;21) and inv(16) CBF fusions using large-scale, genome-wide miRNA profiling in AMLs. Expression signatures of a minimum of two (that is, miR-126 and miR-126*), three (miR-224, miR-376c or miR-368 and miR-382), and seven (miR-17-5p and miR-20a, plus the previous five) miRNAs could accurately discriminate between CBF, t(15;17) and *MLL*-rearranged AMLs, respectively[89]. Similarly, Jongen-Lavrencic *et al.*[104] showed that miRNA signatures correlated with cytogenetic and molecular subtypes of AML (that is, AMLs with t(8;21), t(15;17), inv(16), *NPM1* and *CEBPA* mutations). For example, all six AML cases with t(15;17) aggregated in a cluster; a significant upregulation of miR-10a, miR-10b, miR-196a and miR-196b was identified in AMLs with *NPM1* mutations, and a significant upregulation of miR-155 was found in AMLs carrying *FLT3*-ITD. Notably, they also observed a significant upregulation of miR-126 in CBF leukaemia and a significant upregulation of miR-224 and miR-382 in t(15;17) AML[104]. The specific miRNA signature of t(15;17) (APL) cases and the upregulation of miR-10a and miR-10b in AMLs with *NPM1* mutations and of miR-155 in AMLs with *FLT3*-ITD were also reported by others[105,106,117]. Marcucci *et al.* reported that miR-181a, miR-181a*, miR-181b, miR-181c, miR-181d, miR-128, miR-192, miR-219-1-3p, miR-224, miR-335 and miR-340 were upregulated whereas miR-34a and miR194 were downregulated in cytogenetically normal AMLs with *CEBPA* mutations[118].

In addition, the expression signature of some miRNAs was associated with the outcome and survival of patients with leukaemia. Garzon *et al.*[105] showed that patients with high expression of miR-191 and miR-199a had significantly worse overall and event-free survival than patients with AML who had low expression. In cytogenetically normal AMLs, Marcucci *et al.*[119] found that the expression signature of

Box 2 | **Histone modifications and associated enzymes**

Structural studies have revealed that the amino-terminal tails of histones protrude outwards from the nucleosome and are subject to various post-translational modifications, including acetylation, methylation, ubiquitylation, phosphorylation, sumoylation and ADP-ribosylation[176].

Histone acetylation is associated with transcriptionally active chromatin (euchromatin) and is catalysed by histone acetyltransferases (HATs). Several transcriptional co-activators, including CREB binding protein (CBP) and p300, PCAF and NCOA1, have been shown to possess HAT activity. Conversely, transcriptional co-repressor complexes such as nuclear co-repressor 1 (NCOR1), NCOR2 (also known as SMRT) and SIN3A have been shown to contain subunits with histone deacetylase (HDAC) activity.

Histone methylation is catalysed by histone methyltransferases and can occur on lysine and/or arginine residues. In contrast to histone acetylation, which results in a transcriptionally active state, histone methylation can result in the activation or repression of transcription depending on the residue that is affected. For example, histone H3 lysine 4 (H3K4) methylation is recognized as an active mark associated with actively transcribed genes, and H3K20 trimethylation or H3K9 methylation are inactive marks associated with transcriptional repression and heterochromatic states. Histone methyltransferases also tend to be more specific with regard to their histone substrates, in contrast to HATs for example, and contain a conserved SET domain. Some of the histone methyltransferases identified in mammalians so far include SETD7, SMYD3 and mixed lineage leukaemia (MLL), which catalyse H3K4 methylation; SUV39H1, euchromatic histone-lysine *N*-methyltransferase 2 (EHMT2), EHMT1 and SETDB1, which catalyse H3K9 methylation; EZH2, which catalyses H3K27 methylation; and DOT1L, which catalyses H3K79 methylation.

Histone demethylases have recently been identified and include amine oxidases such as LSD1, which can demethylate H3K4 or H3K9 depending on the associated protein, and JmjC family members, which demethylate monomethylated, dimethylated and trimethylated lysine.

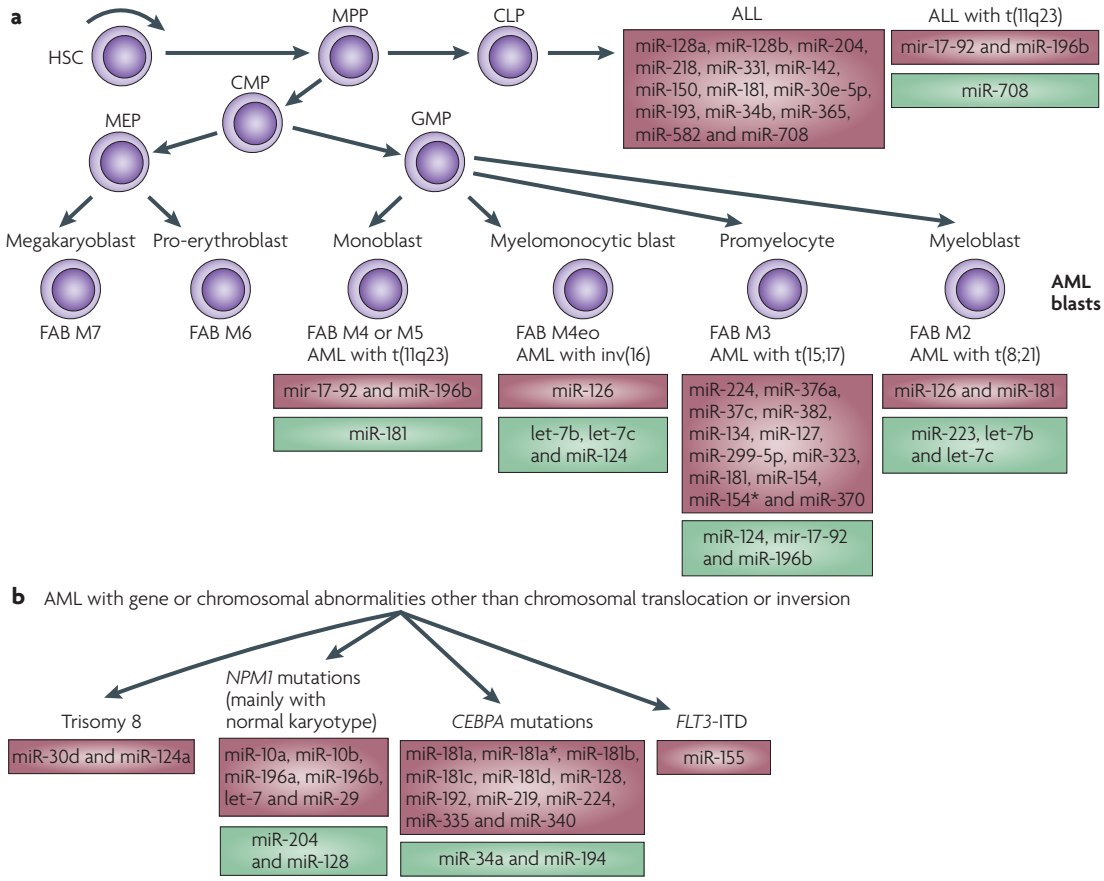

Figure 2 | **Involvement of miRNAs in acute leukaemia.** MicroRNAs (miRNAs) that are upregulated (in red boxes) or downregulated (in green boxes) in a subtype of acute leukaemia are shown. Some miRNAs are associated with specific leukaemia subtypes and might be serve as biomarkers for the classification and diagnosis of these subtypes. **a** | miRNAs upregulated or downregulated in acute leukaemias with chromosomal translocations or inversions that are derived from specific classes of haematopoietic progenitor cells. Examples of possible biomarkers in these leukaemias are miR-126 in core binding factor (CBF) leukaemias (leukaemias with t(8;21) or inv(16))[89,104], *mir-17-92* (REFS 89,90) and miR-196b[89,108,109] in mixed lineage leukaemia (*MLL*)-associated leukaemia (those with t(11q23)), and miR-224, miR-382 and the miR-376 family in acute promyelocytic leukaemia (APL) (t(15;17))[89,104,117]. **b** | miRNAs upregulated or downregulated in acute myeloid leukaemia (AML) with gene or chromosomal abnormalities other than chromosomal translocation or inversion. These leukaemias can fall under any of the categories of AMLs in **a**, although with different distribution among them. In these leukaemias, possible biomarkers for classification and diagnosis include miR-196a and miR-196b[104], and miR-10a and miR-10b[104,106] in AML with nucleophosmin (*NPM1*) mutations, and miR-155 in AML with FMS-related tyrosine kinase 3 (*FLT3*)-internal tandem duplications (ITD)[104,105]. ALL, acute lymphoblastic leukaemia; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; FAB, French-American-British classification; GMP, granulocyte-macrophage progenitor; HSC, haematopoietic stem cell; M2, acute myeloid leukaemia with maturation; M3, acute promyelocytic leukaemia; M4, acute myelomonocytic leukaemia; M4eo, M4 with eosinophilia; M5, acute monoblastic leukaemia; M6, acute erythroleukaemia; M7, acute megakaryoblastic leukaemia; MEP, megakaryocyte-erythrocyte progenitor; MPP, multipotent progenitor cell.

miR-181a (using two probes) and miR-181b (using three probes) was associated with good outcome whereas that of miR-124, miR-128, miR-194, miR-219-5p, miR-220a and miR-320 (using two probes) was associated with poor outcome.

In a study of 17 ALL and 52 AML cases, Mi *et al.*[120] identified 27 miRNAs that were differentially expressed between ALL and AML. Among them, miR-128a and miR-128b were significantly overexpressed, whereas let-7b and miR-223 were significantly downregulated in ALL compared with AML. Using the expression signatures of a minimum of two of these four miRNAs could distinguish ALL and AML with greater than 95% accuracy, indicating that expression signatures of as few as two miRNAs could accurately discriminate ALL from AML[120]. Notably, significant overexpression of miR-128 in ALL has also been reported elsewhere[108,121].

Therefore, miRNA signatures correlate with cytogenetic and molecular subtypes of acute leukaemia, as well as the outcome of patients with leukaemia. Further large-scale miRNA expression profiling assays conducted by different groups are crucial to identify the miRNAs that can be reliable biomarkers for diagnosis and prognosis and/or therapeutic targets of leukaemia.

Leukaemic blasts
Abnormal immature white blood cells that are malignant (neoplastic). Typically found in the bone marrow and peripheral blood of patients with acute leukaemia.

miRNAs as targets and effectors of the epigenetic machinery. It is now becoming clear that not only do miRNAs themselves function in an epigenetic manner by post-transcriptional regulation of expression of target genes, but they can also be targets of the epigenetic machinery, as well as effectors of DNA methylation and histone modifications. These functions may all have crucial roles in leukaemogenesis.

As examples of miRNAs as targets of the epigenetic machinery, their aberrant expression in acute leukaemia is directly associated with DNA methylation. For example, the increased expression of miR-126 and miR-126* in CBF AMLs and of miR-128 in ALL was associated with promoter demethylation[89,120]. In addition, the fusion oncoproteins that arise from chromosomal translocations have been associated with epigenetic silencing of miRNAs. For example, expression of miR-223 is downregulated by the AML1–ETO fusion resulting from t(8;21) in AML[122] owing to a heterochromatic silencing of the miR-223 genomic region directly triggered by AML1–ETO. Increasing miR-223 expression through demethylation restores differentiation of leukaemic blasts[122]. Similarly, transcriptional repression of miR-210, miR-23 and miR-24 by PML–RARA was reported in APL with the t(15;17) translocation[123].

Histone modifications may also have a role in the regulation of miRNA expression in acute leukaemia. Roman-Gomez *et al.*[124] observed high levels of dimethylation of H3 lysine 9 (H3K9me2) and/or low levels of trimethylation of H3 lysine 4 (H3K4me3) (these are patterns of histone modifications underlying a closed chromatin structure that is associated with repressive gene expression) in CpG islands around 13 miRNAs. Their analysis of 353 patients with primary ALL showed that 65% of the ALL samples had at least 1 methylated miRNA. Notably, patients with miRNA methylation had a significantly poorer disease-free survival (DFS; 24%) than patients with unmethylated miRNAs (78%) and overall survival (OS; 28% compared with 71%). Multivariate analysis demonstrated that the patient's methylation profile was an independent prognostic factor for predicting DFS and OS. Their results suggest that aberrant miRNA methylation is a common phenomenon in ALL and that miRNA methylation profiles might be important in predicting the clinical outcome of patients with ALL[124].

Conversely, miRNAs might also function as effectors of the epigenetic machinery. Two recent studies[125,126] show that miR-290, a mouse embryonic stem cell-specific miRNA, controls DNA methylation and telomere recombination through retinoblastoma-like 2 (*Rbl2*)-dependent regulation of DNMTs. In leukaemia, forced expression of miR-29b in AML cells induced global DNA hypomethylation and re-expression of tumour suppressor genes including *INK4B* and oestrogen receptor 1 (*ESR1*) by targeting DNMT3A and DNMT3B directly and DNMT1 indirectly[127].

Epigenetic therapy in acute leukaemia

Unlike gene deletions that lead to an irreversible loss of function, transcriptional repression by epigenetic mechanisms such as histone deacetylation and promoter DNA

Table 2 | **Examples of oncogenic and tumor suppressor miRNAs in leukaemogenesis**

miRNA	Function in normal haematopoiesis	Function in acute leukaemia	Regulator	Known targets	Refs
let-7	Represses megakaryocytopoiesis	Lower in ALL than in AML, downregulated in CBF leukaemias and upregulated in AMLs with *NPM1* mutations	LIN28	*NRAS*, *KRAS* and *HMGA2*	93,104,106, 111,112, 120,184
miR-15 and miR16	Promote erythropoiesis	Upregulated in APL cells after ATRA treatment	ND	*BCL2* and *MYB*	100,113,115, 116,185
mir-17-92 cluster	Downregulated during monocytopoiesis and megakaryocytopoiesis. Represses monocytopoiesis and megakaryocytopoiesis, and promotes the transition from pro-B to pre-B cell stage	Overexpressed in *MLL*-rearranged leukaemia, significantly downregulated in APL, enhances cell proliferation and blocks cell differentiation and promotes leukaemogenesis	MYC, E2F1, E2F2 and E2F3	*BIM* (also known as *BCL2L11*), *PTEN*, *E2F1*, *E2F2*, *E2F3*, *RASSF2*, *APP*, *CDKN1A* (which encodes p21) and *AML1* (also known as *RUNX1*)	89–94, 96–99
miR-155	Represses both megakaryopoiesis and erythropoiesis. Important in lymphopoiesis and immune response (for both B and T cells)	Significantly upregulated in AMLs carrying *FLT3*-ITD, overexpressed in a subset of AML (particularly AML M4 and M5), and sustained expression in HSCs caused a myeloproliferative disorder	FOXP3	*MAP3K7IP2* (also known as *TAB2*), INPP5D (also known as *SHIP*) and *CEBPB*	93,101–106, 186–189
miR-196a and miR-196b	In the haematopoietic lineage, reach a peak in STR-HSCs and then decrease as cells become more differentiated. Significantly downregulated during the transition from CMPs to GMPs	Upregulated in AMLs with *NPM1* mutations, upregulated in *MLL*-rearranged leukaemia, significantly downregulated in APL, and enhance cell proliferation and block differentiation	GFI1	*HOXB8*, *HOXC8*, *HOXD8* and *HOXA7*	89,104, 108–110

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; APL, acute promyelocytic leukaemia; *APP*, amyloid-β (A4) precursor protein; *ATRA*, all-*trans* retinoic acid; *CDKN1A*, cyclin-dependent kinase inhibitor 1A; *CEBPB*, C/EBPβ; CMP, common myeloid progenitor; *FLT3*, FMS-related tyrosine kinase 3; *FOXP3*, forkhead box P3; GMP, granulocyte-macrophage progenitor; Hox, homeobox; HSCs, haematopoietic stem cells; *INPP5D*, inositol polyphosphate-5-phosphatase; ITD, internal tandem duplication; *MAP3K7IP2*, MAPK kinase kinase 7 interacting protein 2; miRNA, microRNA; *MLL*, mixed lineage leukaemia; ND, not determined; *NPM1*, nucleophosmin; *PTEN*, phosphatase and tensin homologue; STR-HSCs, short-term repopulating HSCs.

Myelodysplastic syndrome
A group of clonal haematopoietic stem cell disorders characterized by cytopenias (low blood counts) and ineffective haematopoiesis, dysplasia in one or more myeloid cell lines, and an increased risk of transformation to AML.

methylation can be reversed by pharmacological inhibitors of such processes. One of the best known and most successful examples of targeted therapy that can induce epigenetic changes is the use of ATRA in the therapy of APL, which has been described above. Because epigenetic mechanisms are crucial to the pathogenesis of acute leukaemias, there has been considerable interest in the investigation of agents that target the epigenome in these diseases.

Histone deacetylase inhibitors (HDACIs). HDACIs have been associated with effects on various genes, including those involved with cell cycle regulation, apoptosis and angiogenesis. HDACIs exert anti-tumour effects *in vitro* and *in vivo*, and are now in clinical trials for acute leukaemias, as well as related neoplastic disorders of the bone marrow such as myelodysplastic syndromes (MDS) and myeloproliferative neoplasms. In preclinical studies, HDACIs have been shown to induce differentiation of APL cell lines, including those resistant to standard differentiating therapy with ATRA[128–130]. They abolish tumours with t(15;17) translocations in nude mice, and induce remissions in transgenic mouse models of ATRA-resistant APL[130,131]. HDACIs also induce differentiation and apoptosis of t(8;21) leukaemia cell lines and primary leukaemia blasts[39,132–134]. In the clinical setting, therapy with the HDACI sodium phenylbutyrate has been shown to restore ATRA responsiveness in a patient with APL who had experienced multiple relapses and was clinically resistant to therapy with ATRA alone[135]. Treatment with phenylbutyrate induced a complete clinical and cytogenetic remission and a time-dependent histone acetylation in peripheral blood and bone marrow mononuclear cells[135]. However, butyrates are short-chain fatty acids and generally not very potent in inhibiting HDACs[136]. More potent HDACIs, including hydroxamic acids, cyclic tetrapeptides and benzamides, have been developed and are under clinical investigation[137]. Currently, advanced primary cutaneous T cell lymphoma is the only tumour in which significant efficacy of an HDACI, SAHA (vorinostat), has been demonstrated in the clinic. This resulted in its approval by the US Food and Drug Administration (FDA) for the treatment of this disease[138].

Most clinical trials using HDACIs as single agents in patients with advanced AML or MDS have demonstrated limited clinical activity[139–147] (TABLE 3). Recently, published preclinical studies have also focused on distinct cytogenetic subsets of AML such as CBF AML, a cytogenetic subset that is thought to be particularly amenable to therapy with this class of drugs, on the basis of transcriptional repression through HDAC recruitment by the oncogenic fusion proteins[132,133]. In a recent clinical trial, transient anti-leukaemia activity was demonstrated in patients with advanced CBF leukaemia treated with the HDACI romidepsin, and this was associated with the upregulation of AML1–ETO target genes[146]. Given the limited single agent activity of HDACIs in acute leukaemias, ongoing trials are investigating the combination of HDACIs with other agents, including DNMT inhibitors (TABLE 3).

DNMT inhibitors. Although DNMT inhibitors have existed for several decades, they were originally used at high doses, which resulted in substantial cytotoxicity and an unacceptable toxicity profile[148]. At lower doses, however, the DNA demethylating and differentiating effects of these drugs predominate, and they are active in a broad range of myeloid neoplasms, including MDS, myeloproliferative neoplasms and AML[149]. The DNMT inhibitors in clinical and/or preclinical development fall into two broad categories: nucleoside analogues and non-nucleoside demethylating agents[137,149]. The prototypic nucleoside analogue DNMT inhibitors 5-azacytidine (also known as 5-Aza or azacitidine) and 5-aza-2′deoxycytidine (also known as decitabine), are both incorporated into DNA (5-Aza is also incorporated into RNA) and form a covalent complex with the DNMT enzyme resulting in the trapping and degradation of the enzyme and progressive loss of DNMT activity in cells. Both compounds have recently been approved by the US FDA for the treatment of MDS[150–152]. Objective response rates (complete and partial responses) in these trials ranged from 20% to 30%, but an additional 20–30% of patients derive clinical benefit in terms of improvement in blood counts or the number of blood transfusions required (haematological improvement), despite evidence of the persistence of significant disease in the bone marrow[150,152,153]. Several of the trials conducted in MDS have included patients who have a myeloblast count of 20–30% in the bone marrow (and are therefore considered to have AML), and in these patients the overall response rates, including haematological improvement, have ranged from 35% to 48%[154]. There are now several early-phase trials using DNMT inhibitors, as either single agents or in combination with other agents, which confirm the clinical activity of this group of drugs in AML[155–162], including elderly patients with AML who are unable to tolerate standard cytotoxic chemotherapy[155–157].

It is important to note that DNMT inhibitors can require several cycles of administration for activity to be demonstrated. This might be because repetitive administration is necessary for progressive demethylation and epigenetic modulation of crucial genes, such as cell cycle regulatory and pro-apoptotic genes. In addition, the effects of these drugs on methylation are not permanent, and therefore chronic exposure is required to maintain the effects. The clinical activity of these agents is thought to be mediated through the reversal of epigenetic silencing and there are some published clinical trials that support this hypothesis[158,163]. However, in general this has been challenging to prove conclusively in the clinical setting[156,160,164] (TABLE 3), and it is likely that alternative mechanisms of action, such as the induction of DNA damage[159,165–167], might contribute to the pleiotropic effects of these drugs.

Efforts are ongoing to develop DNMT inhibitors that have greater selectivity for cancer cells and that are suitable for chronic oral administration in the clinical setting. In addition, given the inter-relationship between DNA methylation and histone modifications in the regulation of gene expression[168], it is not surprising that HDACIs and DNMT inhibitors are being combined in clinical trials in AML and MDS (TABLE 3) in an effort to optimize the

Table 3 | **Selected trials of agents targeting the epigenome in AML**

Structural class	Agent	Target	Phase of study	Comments
Single agent HDACI trials				
Short-chain fatty acids	Phenylbutyrate	HDAC I/II	I	Safety of PB established and haematological improvement in platelet counts documented in the occasional patient with AML[190,191]
	Valproate (with or without ATRA)	HDAC I/II	I/II	Haematological improvement noted in one-third of patients with MDS, including one patient with sAML and MDS[145]. Of 11 patients with AML, 3 had CR and CRi[147] with a 5% response rate in AML[144]. Histone hyperacetylation demonstrated at therapeutic levels of VPA and two of eight patients had haematological improvement associated with differentiation of the leukaemia clone[142]
Cyclic tetrapeptides	Romidepsin (Depsipeptide)	HDAC I/II	I/II	No objective responses in AML but histone acetylation demonstrated in mononuclear cells[139]. Response in one of nine patients with AML[143]. Anti-leukaemia activity limited to CBF AML and associated with upregulation of AML1–ETO target genes[146]
Hydroxamic acids	Vorinostat (SAHA)	HDAC I/II	I	CR and CRi in 4 of 31 patients with AML, and antioxidant gene expression signature correlated with vorinostat resistance[140]
	Panobinstat (LBH589)*	HDAC I/II	I	Transient reductions in peripheral blasts, and histone acetylation demonstrated in blast cells[192]
Benzamides	Entinostat (MS275)	HDAC I	I	No objective clinical responses, histone acetylation, *CDKN1A* induction or caspase 3 activation demonstrated in leukaemia blasts[141]
	MGCD0103	HDAC I/IV	I	Of 22 patients with AML, 2 had a decline in bone marrow blasts to <5%[193]
Single agent DNMT inhibitor trials				
Nucleoside analogues	Azacitidine	DNMT	II/III	Up to 48% of patients with clinical benefit[154]. Overall response rate including haematological improvement was 60%[162]
	Decitabine	DNMT	I	Of 35 patients with AML, 8 had a response, with no correlation of baseline *CDKN2B* methylation with clinical activity[160]
Combination DNMT and HDACI trials				
Nucleoside analogue and benzamide	Azacitidine and MS275	DNMT and HDAC	I	Responses were seen in 46% of patients with MDS or AML, but no correlation of clinical response with reversal of methylation or gene expression[159]
Nucleoside analogue and hydroxamic acid	Azacitidine and SAHA	DNMT and HDAC	I/II	Responses occurred in 18 of 21 (86%) patients[194]
	Azacitidine and PXD101	DNMT and HDAC	I	Responses in 7 of 21 patients, and the study is now in randomized phase[195]
Nucleoside analogue and short-chain fatty acid	Azacitidine and PB	DNMT and HDAC	Pilot/I	Anti-leukaemia effect in two of eight patients with AML, with no correlation of response with histone acetylation[161]. Responses in 11 of 36 patients with MDS or AML, with significant correlation of response with *CDKN2B* or *CDH1* methylation reversal[158]
	Azacitidine, VPA and ATRA	DNMT and HDAC	I/II	Response rate was 42%. Induction of histone acetylation, global DNA methylation and upregulation of *CDKN2B* and *CDKN1A* expression was observed, which did not correlate with clinical response. Correlation of VPA levels with response[157]
	Decitabine and VPA	DNMT and HDAC	I/II	Of 48 patients with AML, 9 responded. Patients with lower *CDKN2B* methylation had a significantly higher response rate, but *CDKN2B* gene reactivation was not associated with clinical response. Correlation of VPA levels with response[156]. Response rate was 44%, induction of ER expression was associated with response. Addition of VPA did not seem to increase the response rate[155]
Histone methyltransferase antagonist				
Nucleoside analogue	DZNep	HMT	Preclinical	Inhibits *S*-adenosyl-L-methionine-dependent methyltransferases and leads to degradation of PRC2 HMTs and decrease in H3K27 methylation. Being investigated in leukaemia cell lines and primary leukaemia cells[174]
Hydroxamic acid	LAQ824	HDAC and HMT*	Preclinical	Degradation of HMTs such as EZH2 leading to a decrease in H3K27 methylation and apoptosis in human primary leukaemia cells[196]

AML, acute myeloid leukaemia; CBF, core binding factor; *CDH1*, E-cadherin; *CDKN1A*, cyclin-dependent kinase inhibitor 1A (which encodes p21); *CDKN2B*, CDK inhibitor 2B (which encodes INK4B); CR, complete response; CRi, CR with incomplete blood count recovery; DNMT, DNA methyltransferase; DZNep, 3-deazaneplanocin A; ER, oestrogen receptor; *EZH2*, enhancer of zeste homologue 2; HDACI, histone deacetylase inhibitor; HMT, histone methyltransferase; MDS, myelodysplastic syndrome; PB, phenylbutyrate; PRC2, polycomb repressive complex 2; sAML, secondary AML; VPA, valproic acid. *HDACI with putative HMT inhibitory activity.

anti-tumour activities of these agents and recapitulate the synergistic interaction that has been demonstrated in the preclinical setting. Several of these published trials have been conducted using low potency HDACIs[155–158] and have shown the feasibility of this approach, but have not demonstrated a clear contribution of the HDACIs to the clinical or biological activity of the combination[155]. The results from ongoing randomized trials with newer, more potent HDACIs will be necessary to validate the synergy between these two classes of epigenetic modulators in patients with AML and MDS.

miRNAs as potential therapeutic targets and tools. Because they can function as oncogenes or tumour suppressor genes in leukaemogenesis, miRNAs also have the potential to be therapeutic targets or tools. miRNA-based cancer gene therapy offers the chance of targeting multiple gene networks that are controlled by a single, aberrantly expressed miRNA[169]. Reconstitution of a tumour-suppressive miRNA, or sequence-specific knockdown of oncogenic miRNAs by antagomir oligos, has produced favourable anti-tumour outcomes in experimental models[169]. In addition, the efficacy of some existing clinical therapeutic approaches may be mediated through the modulation of miRNA expression. For example, tumour suppressor miRNA upregulation has been demonstrated with the use of ATRA in APL cell lines and primary leukaemia samples[113,123], and the DNMT inhibitor 5-azacytidine has been associated with the reversal of epigenetic silencing of an miRNA linked to the differentiation block in AML1–ETO AML blasts[122].

However, there are still many issues to be resolved before consideration of conducting miRNA-based clinical therapy, including dosage, efficacy, functionality, delivery, nonspecific toxicity and immune activation[24,169]. In addition, because of the redundancy of some miRNA families or functional redundancy of a set of miRNAs that are not in a family, targeting a single member might not be sufficient in terms of gene therapy. In such cases, simultaneously targeting several miRNAs would be crucial. Furthermore, some miRNAs can have a different role (as oncogenes or tumour suppressors) depending on the cellular context. For example, the *mir-17-92* cluster is a well-known oncogene in various types of cancers[86,87,89,170,171], including leukaemia[89,104,108,109], but can function as a tumour suppressor gene in breast cancer[172,173]. Therefore, before considering a potential clinical application, it is important to understand the expression pattern and potential role of the candidate miRNA(s) in other tissues to avoid causing undesirable side effects.

Conclusions and perspectives

The complexity and biological heterogeneity of acute leukaemias poses considerable challenges to therapeutic advances. It is clear, however, that an in-depth understanding of the biology of acute leukaemias is essential to make meaningful progress. The oncogenic fusion proteins in acute leukaemia associate in macromolecular complexes, lack intrinsic enzymatic activity and are therefore not easily druggable. This is in contrast to the clinical development of ABL kinase inhibitors in CML, for example. Epigenetic and transcriptional therapeutic strategies that focus on the disruption of the association of oncoproteins with substrate DNA or interference with key molecules that oncoproteins may associate with to promote leukaemia (such as the disruption of the MLL–MEN1 interaction) hold promise for the future. In addition, besides the ongoing clinical studies of HDACIs and DNMT inhibitors, other components of the epigenetic regulatory machinery, such as histone methyltransferases, histone demethylases, HATs and sirtuins, are potential targets for future anticancer and anti-leukaemia therapy[174]. The importance of miRNAs in increasing our basic knowledge about the pathobiology of leukaemias as well as their therapeutic potential and some of the potential pitfalls that could be encountered in clinical translation have been mentioned above. Given the importance of cooperating gene mutations in signalling pathways in the generation of the acute leukaemia phenotype, it is likely that the most effective treatment strategies in the future will involve a combination of rationally designed transcriptional treatment approaches with those that inhibit relevant activated signal transduction molecules.

1. Rowley, J. D. Chromosomal translocations: revisited yet again. *Blood* **112**, 2183–2189 (2008).
2. Heim, S. & Mitelman, F. *Cancer Cytogenetics* (Wiley-Blackwell, Hoboken, NJ, 2009).
A current, most comprehensive reference on chromosome abnormalities in cancer.
3. Dalla-Favera, R. *et al.* Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7824–7827 (1982).
4. Taub, R. *et al.* Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7837–7841 (1982).
Cloning of the first translocation breakpoint revealed that one of the genes involved was a known oncogene.
5. Rowley, J. D. Letter, A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243**, 290–293 (1973).
This paper showed that the Philadelphia chromosome, a specific chromosomal abnormality associated with CML, was the result of a reciprocal translocation between chromosomes 9 and 22.
6. Heisterkamp, N. *et al.* Localization of the c-ab1 oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature* **306**, 239–242 (1983).
7. Groffen, J. *et al.* Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* **36**, 93–99 (1984).
8. Rowley, J. D. Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukaemia. *Ann. Genet.* **16**, 109–112 (1973).
9. Rowley, J. D., Golomb, H. M. & Dougherty, C. 15/17 translocation, a consistent chromosomal change in acute promyelocytic leukaemia. *Lancet* **1**, 549–550 (1977).
10. Erickson, P. *et al.* Identification of breakpoints in t(8;21) acute myelogenous leukaemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. *Blood* **80**, 1825–1831 (1992).
11. Longo, L. *et al.* Rearrangements and aberrant expression of the retinoic acid receptor alpha gene in acute promyelocytic leukaemias. *J. Exp. Med.* **172**, 1571–1575 (1990).
12. de Thé, H. *et al.* The PML-RARα fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukaemia encodes a functionally altered RAR. *Cell* **66**, 675–684 (1991).
13. Grignani, F. *et al.* Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase in promyelocytic leukaemia. *Nature* **391**, 815–818 (1998).
14. Lin, R. J. *et al.* Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature* **391**, 811–814 (1998).
This paper shows that the PML–RARA and PLZF–RARA fusion oncoproteins in APL result in transcriptional repression of retinoic acid target genes through recruitment of the N-CoR histone deacetylase complex. A molecular explanation for the clinical efficacy of ATRA in APL with the PML–RARA fusion was also provided.

15. Wang, J., Hoshino, T., Redner, R. L., Kajigaya, S. & Liu, J. M. ETO, fusion partner in t(8;21) acute myeloid leukaemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. *Proc. Natl Acad. Sci. USA* **95**, 10860–10865 (1998).
This paper highlights the fact that the mechanism of transcriptional repression by the AML1–ETO fusion protein in AML is through recruitment of the N-CoR complex. This implies that effective inhibitors of such repressor complexes might provide therapeutic benefit in this subset of AML
16. Lutterbach, B. *et al.* ETO, a target of t(8;21) in acute leukaemia, interacts with the N-CoR and mSin3 co-repressors. *Mol. Cell. Biol.* **18**, 7176–7184 (1998).
17. Amann, J. M. *et al.* ETO, a target of t(8;21) in acute leukaemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. *Mol. Cell. Biol.* **21**, 6470–6483 (2001).
18. Huang, M. E. *et al.* Use of all-trans retinoic acid in the treatment of acute promyelocytic leukaemia. *Blood* **72**, 567–572 (1988).
This paper showed the efficacy of ATRA in the treatment of patients with APL. It was the first example of genotype-specific treatment of translocation-associated AML.
19. Warrell, R. P. Jr *et al.* Differentiation therapy of acute promyelocytic leukaemia with tretinoin (all-trans-retinoic acid). *N. Engl. J. Med.* **324**, 1385–1393 (1991).
20. Ruthenburg, A. J., Li, H., Patel, D. J. & Allis, C. D. Multivalent engagement of chromatin modifications by linked binding modules. *Nature Rev. Mol. Cell Biol.* **8**, 983–994 (2007).
21. Vaissiere, T., Sawan, C. & Herceg, Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat. Res.* **659**, 40–48 (2008).
22. Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281–297 (2004).
23. He, L. & Hannon, G. J. MicroRNAs: small RNAs with a big role in gene regulation. *Nature Rev. Genet.* **5**, 522–531 (2004).
24. Wu, W., Sun, M., Zou, G. M. & Chen, J. MicroRNA and cancer: Current status and prospective. *Int. J. Cancer* **120**, 953–960 (2007).
25. Esquela-Kerscher, A. & Slack, F. J. Oncomirs - microRNAs with a role in cancer. *Nature Rev. Cancer* **6**, 259–269 (2006).
26. Calin, G. A. & Croce, C. M. MicroRNA signatures in human cancers. *Nature Rev. Cancer* **6**, 857–866 (2006).
27. Lu, J. *et al.* MicroRNA expression profiles classify human cancers. *Nature* **435**, 834–838 (2005).
28. Fabbri, M. *et al.* MicroRNAs and noncoding RNAs in haematological malignancies: molecular, clinical and therapeutic implications. *Leukaemia* **22**, 1095–1105 (2008).
29. Garzon, R. & Croce, C. M. MicroRNAs in normal and malignant haematopoiesis. *Curr. Opin. Haematol.* **15**, 352–358 (2008).
30. Kluiver, J., Kroesen, B. J., Poppema, S. & van den Berg, A. The role of microRNAs in normal haematopoiesis and haematopoietic malignancies. *Leukaemia* **20**, 1931–1936 (2006).
31. Yendamuri, S. & Calin, G. A. The role of microRNA in human leukaemia: a review. *Leukaemia* **23**, 1257–1263 (2009).
32. Frohling, S., Scholl, C., Gilliland, D. G. & Levine, R. L. Genetics of myeloid malignancies: pathogenetic and clinical implications. *J. Clin. Oncol.* **23**, 6285–6295 (2005).
33. Renneville, A. *et al.* Cooperating gene mutations in acute myeloid leukaemia: a review of the literature. *Leukaemia* **22**, 915–931 (2008).
34. Mullighan, C. G. *et al.* Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* **446**, 758–764 (2007).
35. The American Association for Cancer Research Human Epigenome Task Force and the European Union, Network of Excellence, Scientific Advisory Board. Moving AHEAD with an international human epigenome project. *Nature* **454**, 711–715 (2008).
36. Feinberg, A. P. & Tycko, B. The history of cancer epigenetics. *Nature Rev. Cancer* **4**, 143–153 (2004).
37. Melki, J. R., Vincent, P. C. & Clark, S. J. Concurrent DNA hypermethylation of multiple genes in acute myeloid leukaemia. *Cancer Res.* **59**, 3730–3740 (1999).
38. Garcia-Manero, G. *et al.* Epigenetics of acute lymphocytic leukaemia. *Semin. Haematol.* **46**, 24–32 (2009).
39. Wang, J., Saunthararajah, Y., Redner, R. L. & Liu, J. M. Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukaemia cells. *Cancer Res.* **59**, 2766–2769 (1999).
40. Lutterbach, B., Hou, Y., Durst, K. L. & Hiebert, S. W. The inv(16) encodes an acute myeloid leukaemia 1 transcriptional co-repressor. *Proc. Natl Acad. Sci. USA* **96**, 12822–12827 (1999).
41. Okuda, T., van Deursen, J., Hiebert, S. W., Grosveld, G. & Downing, J. R. AML1, the target of multiple chromosomal translocations in human leukaemia, is essential for normal fetal liver haematopoiesis. *Cell* **84**, 321–330 (1996).
42. Wang, Q. *et al.* Disruption of the *Cbfa2* gene causes necrosis and haemorrhaging in the central nervous system and blocks definitive haematopoiesis. *Proc. Natl Acad. Sci. USA* **93**, 3444–3449 (1996).
43. Gelmetti, V. *et al.* Aberrant recruitment of the nuclear receptor co-repressor-histone deacetylase complex by the acute myeloid leukaemia fusion partner ETO. *Mol. Cell. Biol.* **18**, 7185–7191 (1998).
44. Liu, S. *et al.* Interplay of RUNX1/MTG8 and DNA methyltransferase 1 in acute myeloid leukaemia. *Cancer Res.* **65**, 1277–1284 (2005).
45. Linggi, B. *et al.* The t(8;21) fusion protein, AML1 ETO, specifically represses the transcription of the p14(ARF) tumour suppressor in acute myeloid leukaemia. *Nature Med.* **8**, 743–750 (2002).
46. Yang, G. *et al.* Transcriptional repression of the Neurofibromatosis-1 tumour suppressor by the t(8;21) fusion protein. *Mol. Cell. Biol.* **25**, 5869–5879 (2005).
47. Borrow, J., Goddard, A. D., Sheer, D. & Solomon, E. Molecular analysis of acute promyelocytic leukaemia breakpoint cluster region on chromosome 17. *Science* **249**, 1577–1580 (1990).
48. de The, H., Chomienne, C., Lanotte, M., Degos, L. & Dejean, A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. *Nature* **347**, 558–561 (1990).
49. Kakizuka, A. *et al.* Chromosomal translocation t(15;17) in human acute promyelocytic leukaemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell* **66**, 663–674 (1991).
50. Chen, Z. *et al.* Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. *EMBO J.* **12**, 1161–1167 (1993).
51. Licht, J. D. *et al.* Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukaemia associated with translocation (11;17). *Blood* **85**, 1083–1094 (1995).
52. Redner, R. L., Rush, E. A., Faas, S., Rudert, W. A. & Corey, S. J. The t(5;17) variant of acute promyelocytic leukaemia expresses a nucleophosmin-retinoic acid receptor fusion. *Blood* **87**, 882–886 (1996).
53. Licht, J. D. Reconstructing a disease: What essential features of the retinoic acid receptor fusion oncoproteins generate acute promyelocytic leukaemia? *Cancer Cell* **9**, 73–74 (2006).
54. Villa, R. *et al.* Role of the polycomb repressive complex 2 in acute promyelocytic leukaemia. *Cancer Cell* **11**, 513–525 (2007).
55. Zheng, P. Z. *et al.* Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukaemia. *Proc. Natl Acad. Sci. USA* **102**, 7653–7658 (2005).
56. Mueller, B. U. *et al.* ATRA resolves the differentiation block in t(15;17) acute myeloid leukaemia by restoring PU.1 expression. *Blood* **107**, 3330–3338 (2006).
57. Wang, Z. Y. & Chen, Z. Acute promyelocytic leukaemia: from highly fatal to highly curable. *Blood* **111**, 2505–2515 (2008).
58. Nasr, R. *et al.* Eradication of acute promyelocytic leukaemia-initiating cells through PML-RARA degradation. *Nature Med.* **14**, 1333–1342 (2008).
59. Niu, C. *et al.* Studies on treatment of acute promyelocytic leukaemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukaemia patients. *Blood* **94**, 3315–3324 (1999).
60. Nowak, D., Stewart, D. & Koeffler, H. P. Differentiation therapy of leukaemia: 3 decades of development. *Blood* **113**, 3655–3665 (2009).
61. Linggi, B. E., Brandt, S. J., Sun, Z. W. & Hiebert, S. W. Translating the histone code into leukaemia. *J. Cell Biochem.* **96**, 938–950 (2005).
62. Borrow, J. *et al.* The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nature Genet.* **14**, 33–41 (1996).
This paper highlights the involvement of a HAT in chromosomal translocations in AML, and provides evidence that disruption of chromatin-modifying enzymes is associated with leukaemogenesis.
63. Panagopoulos, I. *et al.* Fusion of the MORF and CBP genes in acute myeloid leukaemia with the t(10;16)(q22;p13). *Hum. Mol. Genet.* **10**, 395–404 (2001).
64. Kitabayashi, I., Aikawa, Y., Nguyen, L. A., Yokoyama, A. & Ohki, M. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *EMBO J.* **20**, 7184–7196 (2001).
65. Perez-Campo, F. M., Borrow, J., Kouskoff, V. & Lacaud, G. The histone acetyl transferase activity of monocytic leukaemia zinc finger is critical for the proliferation of haematopoietic precursors. *Blood* **113**, 4866–4874 (2009).
66. Liang, J., Prouty, L., Williams, B. J., Dayton, M. A. & Blanchard, K. L. Acute mixed lineage leukaemia with an inv(8)(p11q13) resulting in fusion of the genes for MOZ and TIF2. *Blood* **92**, 2118–2122 (1998).
67. Deguchi, K. *et al.* MOZ-TIF2-induced acute myeloid leukaemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell* **3**, 259–271 (2003).
68. Sobulo, O. M. *et al.* MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukaemia with a t(11;16)(q23;p13.3). *Proc. Natl Acad. Sci. USA* **94**, 8732–8737 (1997).
This paper highlights the involvement of a HAT in an *MLL*-associated leukaemia, and provided an early insight that alluded to transcriptional deregulation through histone and chromatin modifications as being important in *MLL*-mediated leukaemogenesis.
69. Ziemin-van der Poel, S. *et al.* Identification of a gene, MLL, that spans the breakpoint in 11q23 translocations associated with human leukaemias. *Proc. Natl Acad. Sci. USA* **88**, 10735–10739 (1991).
70. Thirman, M. J. *et al.* Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukaemias with 11q23 chromosomal translocations. *N. Engl. J. Med.* **329**, 909–914 (1993).
71. Krivtsov, A. V. & Armstrong, S. A. MLL translocations, histone modifications and leukaemia stem-cell development. *Nature Rev. Cancer* **7**, 823–833 (2007).
72. Dou, Y. & Hess, J. L. Mechanisms of transcriptional regulation by MLL and its disruption in acute leukaemia. *Int. J. Haematol.* **87**, 10–18 (2008).
73. Nakamura, T. *et al.* ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol. Cell* **10**, 1119–1128 (2002).
This paper shows that MLL is found in a large multiprotein complex, that the SET domain has histone methyltransferase (H3K4) activity and that this multiprotein complex associates with the promoter of target genes such as *HOXA9*.
74. Milne, T. A. *et al.* MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol. Cell* **10**, 1107–1117 (2002).
This paper shows that although the SET domain of MLL is an H3K4 methyltransferase associated with Hox gene activation, a leukaemogenic MLL fusion protein (MLL–AF9) that activates Hox expression had no effect on H3K4 methylation. This implies that the mechanism for *MLL*-fusion gene activation and leukaemogenesis is not merely through perturbation of H3K4 methylation.
75. Yokoyama, A. *et al.* The menin tumour suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell* **123**, 207–218 (2005).
76. Milne, T. A. *et al.* Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. *Proc. Natl Acad. Sci. USA* **102**, 749–754 (2005).
77. Yokoyama, A. & Cleary, M. L. Menin critically links MLL proteins with LEDGF on cancer-associated target genes. *Cancer Cell* **14**, 36–46 (2008).
78. Faber, J. *et al.* HOXA9 is required for survival in human MLL-rearranged acute leukaemias. *Blood* **113**, 2375–2385 (2009).

79. Dorrance, A. M. *et al.* Mll partial tandem duplication induces aberrant Hox expression *in vivo* via specific epigenetic alterations. *J. Clin. Invest.* **116**, 2707–2716 (2006).
80. Popovic, R. & Zeleznik-Le, N. J. MLL: how complex does it get? *J. Cell Biochem.* **95**, 234–242 (2005).
81. Okada, Y. *et al.* hDOT1L links histone methylation to leukemogenesis. *Cell* **121**, 167–178 (2005).
82. Zhang, W., Xia, X., Reisenauer, M. R., Haemnway, C. S. & Kone, B. C. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaCalpha in an aldosterone-sensitive manner. *J. Biol. Chem.* **281**, 18059–18068 (2006).
83. Bitoun, E., Oliver, P. L. & Davies, K. E. The mixed-lineage leukaemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. *Hum. Mol. Genet.* **16**, 92–106 (2007).
84. Mueller, D. *et al.* A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. *Blood* **110**, 4445–4454 (2007).
85. Krivtsov, A. V. *et al.* H3K79 methylation profiles define murine and human MLL-AF4 leukaemias. *Cancer Cell* **14**, 355–368 (2008).
This paper highlights increased H3K79 methylation in a genome-wide analysis in a mouse model of *MLL*-fusion leukaemia (*MLL–AF4*), and provides some evidence for the inhibition of H3K79 as a therapeutic strategy in leukaemias involving MLL fusions.
86. He, L. *et al.* A microRNA polycistron as a potential human oncogene. *Nature* **435**, 828–833 (2005).
87. Hayashita, Y. *et al.* A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res.* **65**, 9628–9632 (2005).
88. Uziel, T. *et al.* The miR-17 ~ 92 cluster collaborates with the Sonic Hedgehog pathway in medulloblastoma. *Proc. Natl Acad. Sci. USA* **106**, 2812–2817 (2009).
89. Li, Z. *et al.* Distinct microRNA expression profiles in acute myeloid leukaemia with common translocations. *Proc. Natl Acad. Sci. USA* **105**, 15535–15540 (2008).
This paper shows that specific alterations in miRNA expression distinguish AMLs with common translocations and implies that the deregulation of specific miRNAs can have a role in the development of leukaemia with these associated genetic rearrangements.
90. Li, Z. *et al.* Consistent deregulation of gene expression between human and murine MLL rearrangement leukaemias. *Cancer Res.* **69**, 1109–1116 (2009).
91. Mi, S. *et al.* Aberrant overexpression and function of the mir-17-92 cluster in MLL-rearranged acute leukaemia. *Proc. Natl Acad. Sci. USA* (in the press).
92. Fontana, L. *et al.* MicroRNAs 17-5p-20a-106a control monocytopoiesis through AML1 targeting and M-CSF receptor upregulation. *Nature Cell Biol.* **9**, 775–787 (2007).
This paper shows that miRNA 17-5p-20a–106a functions as a master gene complex interlinked with AML1 in a mutual negative feedback loop in the control of monocytopoiesis.
93. Garzon, R. *et al.* MicroRNA fingerprints during human megakaryocytopoiesis. *Proc. Natl Acad. Sci. USA* **103**, 5078–5083 (2006).
94. Ventura, A. *et al.* Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell* **132**, 875–886 (2008).
95. Koralov, S. B. *et al.* Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. *Cell* **132**, 860–874 (2008).
96. Xiao, C. *et al.* Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nature Immunol.* **9**, 405–414 (2008).
97. Sylvestre, Y. *et al.* An E2F/miR-20a autoregulatory feedback loop. *J. Biol. Chem.* **282**, 2135–2143 (2007).
98. O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V. & Mendell, J. T. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **435**, 839–843 (2005).
99. Woods, K., Thomson, J. M. & Hammond, S. M. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. *J. Biol. Chem.* **282**, 2130–2134 (2007).
100. Bruchova, H., Yoon, D., Agarwal, A. M., Mendell, J. & Prchal, J. T. Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. *Exp. Haematol.* **35**, 1657–1667 (2007).
101. Masaki, S., Ohtsuka, R., Abe, Y., Muta, K. & Umemura, T. Expression patterns of microRNAs 155 and 451 during normal human erythropoiesis. *Biochem. Biophys. Res. Commun.* **364**, 509–514 (2007).
102. Georgantas, R. W., 3rd. *et al.* CD34+ haematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. *Proc. Natl Acad. Sci. USA* **104**, 2750–2755 (2007).
103. Costinean, S. *et al.* Pre-B cell proliferation and lymphoblastic leukaemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc. Natl Acad. Sci. USA* **103**, 7024–7029 (2006).
104. Jongen-Lavrencic, M., Sun, S. M., Dijkstra, M. K., Valk, P. J. & Lowenberg, B. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukaemia. *Blood* **111**, 5078–5085 (2008).
This paper revealed distinctive miRNA signatures that correlate with cytogenetic and molecular subtypes of AMLs.
105. Garzon, R. *et al.* MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukaemia. *Blood* **111**, 3183–3189 (2008).
This paper showed that miRNA expression was closely associated with selected cytogenetic and molecular abnormalities (for example, t(11q23), isolated trisomy 8, and *FLT3*-ITD) and expression of some miRNAs (for example, miR-191 and miR-199a) was associated with survival of patients with AML.
106. Garzon, R. *et al.* Distinctive microRNA signature of acute myeloid leukaemia bearing cytoplasmic mutated nucleophosmin. *Proc. Natl Acad. Sci. USA* **105**, 3945–3950 (2008).
107. O'Connell, R. M. *et al.* Sustained expression of microRNA-155 in haematopoietic stem cells causes a myeloproliferative disorder. *J. Exp. Med.* **205**, 585–594 (2008).
108. Schotte, D. *et al.* Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukaemia. *Leukaemia* **23**, 313–322 (2009).
109. Popovic, R. *et al.* Regulation of mir-196b by MLL and its overexpression by MLL fusions contributes to immortalization. *Blood* **113**, 3314–3322 (2009).
This paper suggests a mechanism whereby increased expression of miR-196b by MLL fusion proteins significantly contributes to leukaemia development.
110. Velu, C. S., Baktula, A. M. & Grimes, H. L. Gfi1 regulates miR-21 and miR-196b to control myelopoiesis. *Blood* **113**, 4720–4728 (2009).
111. Johnson, S. M. *et al.* RAS is regulated by the let-7 microRNA family. *Cell* **120**, 635–647 (2005).
112. Mayr, C., Haemann, M. T. & Bartel, D. P. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science* **315**, 1576–1579 (2007).
113. Garzon, R. *et al.* MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukaemia. *Oncogene* **26**, 4148–4157 (2007).
114. Calin, G. A. *et al.* Frequent deletions and downregulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukaemia. *Proc. Natl Acad. Sci. USA* **99**, 15524–15529 (2002).
115. Cimmino, A. *et al.* miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl Acad. Sci. USA* **102**, 13944–13949 (2005).
116. Zhao, H., Kalota, A., Jin, S. & Gewirtz, A. M. The c-myb proto-oncogene and microRNA-15a comprise an active autoregulatory feedback loop in human haematopoietic cells. *Blood* **113**, 505–516 (2009).
117. Dixon-McIver, A. *et al.* Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. *PLoS ONE* **3**, e2141 (2008).
118. Marcucci, G. *et al.* Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukaemia with high-risk molecular features: a Cancer and Leukaemia Group B Study. *J. Clin. Oncol.* **26**, 5078–5087 (2008).
119. Marcucci, G. *et al.* MicroRNA expression in cytogenetically normal acute myeloid leukaemia. *N. Engl. J. Med.* **358**, 1919–1928 (2008).
This paper reports that a miRNA signature is associated with the clinical outcome of adults under the age of 60 years who have cytogenetically normal AML and high-risk molecular features.
120. Mi, S. *et al.* MicroRNA expression signatures accurately discriminate acute lymphoblastic leukaemia from acute myeloid leukaemia. *Proc. Natl Acad. Sci. USA* **104**, 19971–19976 (2007).
This paper shows that expression signatures of as few as two miRNAs can accurately discriminate ALL from AML.
121. Zanette, D. L. *et al.* miRNA expression profiles in chronic lymphocytic and acute lymphocytic leukaemia. *Br. J. Med. Biol. Res.* **40**, 1435–1440 (2007).
122. Fazi, F. *et al.* Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. *Cancer Cell* **12**, 457–466 (2007).
123. Saumet, A. *et al.* Transcriptional repression of microRNA genes by PML-RARA increases expression of key cancer proteins in acute promyelocytic leukaemia. *Blood* **113**, 412–421 (2009).
124. Roman-Gomez, J. *et al.* Epigenetic regulation of microRNAs in acute lymphoblastic leukaemia. *J. Clin. Oncol.* **27**, 1316–1322 (2009).
This paper highlights that aberrant methylation affecting miRNA genes is a common phenomenon in ALL that affects the clinical outcome of these patients.
125. Sinkkonen, L. *et al.* MicroRNAs control *de novo* DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nature Struct. Mol. Biol.* **15**, 259–267 (2008).
126. Benetti, R. *et al.* A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nature Struct. Mol. Biol.* **15**, 268–279 (2008).
127. Garzon, R. *et al.* MicroRNA-29b induces global DNA hypomethylation and tumour suppressor gene reexpression in acute myeloid leukaemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood* **113**, 6411–6418 (2009).
This paper shows that miR-29b targets DNMTs, thereby resulting in global DNA hypomethylation and reexpression of hypermethylated, silenced genes in AML.
128. Kitamura, K. *et al.* Histone deacetylase inhibitor but not arsenic trioxide differentiates acute promyelocytic leukaemia cells with t(11;17) in combination with all-*trans* retinoic acid. *Br. J. Haematol.* **108**, 696–702 (2000).
129. Amin, H. M., Saeed, S. & Alkan, S. Histone deacetylase inhibitors induce caspase-dependent apoptosis and downregulation of daxx in acute promyelocytic leukaemia with t(15;17). *Br. J. Haematol.* **115**, 287–297 (2001).
130. He, L. Z. *et al.* Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukaemia. *J. Clin. Invest.* **108**, 1321–1330 (2001).
131. He, L. Z.e.a. Distinct interactions of PML-RAR-alpha and PLZF-RAR-alpha with co-repressors determine differential responses to RA in APL. *Nature Genet.* **18**, 126 (1998).
132. Gozzini, A., Rovida, E., Dello Sbarba, P., Galimberti, S. & Santini, V. Butyrates, as a single drug, induce histone acetylation and granulocytic maturation: possible selectivity on core binding factor-acute myeloid leukaemia blasts. *Cancer Res.* **63**, 8955–8961 (2003).
133. Klisovic, M. I. *et al.* Depsipeptide (FR 901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML1/ETO-positive leukemic cells. *Leukaemia* **17**, 350–358 (2003).
134. Yang, G., Thompson, M. A., Brandt, S. J. & Hiebert, S. W. Histone deacetylase inhibitors induce the degradation of the t(8;21) fusion oncoprotein. *Oncogene* **26**, 91–101 (2007).
135. Warrell, R. P., Jr., He, L. Z., Richon, V., Calleja, E. & Pandolfi, P. P. Therapeutic targeting of transcription in acute promyelocytic leukaemia by use of an inhibitor of histone deacetylase. *J. Natl Cancer Inst.* **90**, 1621–1625 (1998).
136. Gore, S. D. & Carducci, M. A. Modifying histones to tame cancer: clinical development of sodium phenylbutyrate and other histone deacetylase inhibitors. *Expert Opin. Investig. Drugs* **9**, 2923–2934 (2000).
137. Yoo, C. B. & Jones, P. A. Epigenetic therapy of cancer: past, present and future. *Nature Rev. Drug Discov.* **5**, 37–50 (2006).
138. Mann, B. S., Johnson, J. R., Cohen, M. H., Justice, R. & Pazdur, R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *Oncologist* **12**, 1247–1252 (2007).
139. Byrd, J. C. *et al.* A phase 1 and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukaemia and acute myeloid leukaemia. *Blood* **105**, 959–967 (2005).
140. Garcia-Manero, G. *et al.* Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukaemias and myelodysplastic syndromes. *Blood* **111**, 1060–1066 (2008).

141. Gojo, I. *et al.* Phase 1 and pharmacologic study of MS-275, a histone deacetylase inhibitor, in adults with refractory and relapsed acute leukaemias. *Blood* **109**, 2781–2790 (2007).
142. Cimino, G. *et al.* Sequential valproic acid/all-*trans* retinoic acid treatment reprograms differentiation in refractory and high-risk acute myeloid leukaemia. *Cancer Res.* **66**, 8903–8911 (2006).
143. Klimek, V. M. *et al.* Tolerability, pharmacodynamics, and pharmacokinetics studies of depsipeptide (Romidepsin) in patients with acute myelogenous leukaemia or advanced myelodysplastic syndromes. *Clin. Cancer Res.* **14**, 826–832 (2008).
144. Kuendgen, A. *et al.* The histone deacetylase (HDAC) inhibitor valproic acid as monotherapy or in combination with all-*trans* retinoic acid in patients with acute myeloid leukaemia. *Cancer* **106**, 112–119 (2006).
145. Kuendgen, A. *et al.* Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. *Blood* **104**, 1266–1269 (2004).
146. Odenike, O. M. *et al.* Histone deacetylase inhibitor romidepsin has differential activity in core binding factor acute myeloid leukaemia. *Clin. Cancer Res.* **14**, 7095–7101 (2008).
This paper showed that patients with CBF AML were particularly susceptible to the anti-leukaemic effects of the HDACI romidepsin, and this was associated with upregulation of AML1–ETO target genes. This work provided evidence to support the hypothesis that reversal of transcriptional repression mediated by *AML1* fusion genes can be achieved *in vivo* with the use of a HDACI.
147. Raffoux, E., Chaibi, P., Dombret, H. & Degos, L. Valproic acid and all-trans retinoic acid for the treatment of elderly patients with acute myeloid leukaemia. *Haematologica* **90**, 986–988 (2005).
148. Karon, M. *et al.* 5-Azacytidine: a new active agent for the treatment of acute leukaemia. *Blood* **42**, 359–365 (1973).
149. Issa, J. P. & Kantarjian, H. M. Targeting DNA methylation. *Clin. Cancer Res.* **15**, 3938–3946 (2009).
150. Silverman, L. R. *et al.* Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukaemia group B. *J. Clin. Oncol.* **20**, 2429–2440 (2002).
151. Kornblith, A. B. *et al.* Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukaemia Group B study. *J. Clin. Oncol.* **20**, 2441–2452 (2002).
152. Kantarjian, H. *et al.* Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer* **106**, 1794–1803 (2006).
153. Steensma, D. P. *et al.* Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: the alternative dosing for outpatient treatment (ADOPT) trial. *J. Clin. Oncol.* **27**, 3842–3848 (2009).
154. Silverman, L. R. *et al.* Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukaemia Group B. *J. Clin. Oncol.* **24**, 3895–3903 (2006).
155. Blum, W. *et al.* Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukaemia. *J. Clin. Oncol.* **25**, 3884–3891 (2007).
156. Garcia-Manero, G. *et al.* Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukaemia. *Blood* **108**, 3271–3279 (2006).
157. Soriano, A. O. *et al.* Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-*trans* retinoic acid in acute myeloid leukaemia and myelodysplastic syndrome. *Blood* **110**, 2302–2308 (2007).
158. Gore, S. D. *et al.* Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res.* **66**, 6361–6369 (2006).
This paper showed that clinical response to combined DNMT and HDAC inhibition was associated with reversal of *CDKN2B* or *CDH1* promoter methylation and provided some evidence to support the hypothesis that the clinical activity of DNMT inhibitors and HDACIs is based on the reversal of epigenetic silencing of tumour suppressor genes.
159. Fandy, T. E. *et al.* Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. *Blood* **114**, 2764–2773 (2009).
160. Issa, J. P. *et al.* Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2′-deoxycytidine (decitabine) in haematopoietic malignancies. *Blood* **103**, 1635–1640 (2004).
161. Maslak, P. *et al.* Pilot study of combination transcriptional modulation therapy with sodium phenylbutyrate and 5-azacytidine in patients with acute myeloid leukaemia or myelodysplastic syndrome. *Leukaemia* **20**, 212–217 (2006).
162. Sudan, N. *et al.* Treatment of acute myelogenous leukaemia with outpatient azacitidine. *Cancer* **107**, 1839–1843 (2006).
163. Daskalakis, M. *et al.* Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. *Blood* **100**, 2957–2964 (2002).
164. Yang, A. S. *et al.* DNA methylation changes after 5-aza-2′-deoxycytidine therapy in patients with leukaemia. *Cancer Res.* **66**, 5495–5503 (2006).
165. Jiemjit, A. *et al.* p21(WAF1/CIP1) induction by 5-azacytosine nucleosides requires DNA damage. *Oncogene* **27**, 3615–3623 (2008).
166. Link, P. A., Baer, M. R., James, S. R., Jones, D. A. & Karpf, A. R. p53-inducible ribonucleotide reductase (p53R2/RRM5XXXXX2B) is a DNA hypomethylation-independent decitabine gene target that correlates with clinical response in myelodysplastic syndrome/acute myelogenous leukaemia. *Cancer Res.* **68**, 9358–9366 (2008).
167. Palii, S. S., Van Emburgh, B. O., Sankpal, U. T., Brown, K. D. & Robertson, K. D. DNA methylation inhibitor 5-Aza-2′-deoxycytidine induces reversible genome-wide DNA damage that is distinctly influenced by DNA methyltransferases 1 and 3B. *Mol. Cell. Biol.* **28**, 752–771 (2008).
168. Cameron, E. E., Bachman, K. E., Myohanen, S., Herman, J. G. & Baylin, S. B. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nature Genet.* **21**, 103–107 (1999).
This paper shows that in the preclinical setting, the synergistic interaction of demethylation followed by HDAC inhibition results in the reactivation of epigenetically silenced tumour suppressor genes in cancer cells.
169. Tong, A. W. & Nemunaitis, J. Modulation of miRNA activity in human cancer: a new paradigm for cancer gene therapy? *Cancer Gene Ther.* **15**, 341–355 (2008).
170. Calin, G. A. *et al.* MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukaemias. *Proc. Natl Acad. Sci. USA* **101**, 11755–11760 (2004).
171. Volinia, S. *et al.* A microRNA expression signature of human solid tumours defines cancer gene targets. *Proc. Natl Acad. Sci. USA* **103**, 2257–2261 (2006).
172. Hossain, A., Kuo, M. T. & Saunders, G. F. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol. Cell. Biol.* **26**, 8191–8201 (2006).
173. Yu, Z. *et al.* A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. *J. Cell Biol.* **182**, 509–517 (2008).
174. Tan, J. *et al.* Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev.* **21**, 1050–1063 (2007).
175. Herman, J. G. & Baylin, S. B. Gene silencing in cancer in association with promoter hypermethylation. *N. Engl. J. Med.* **349**, 2042–2054 (2003).
176. Bhaumik, S. R., Smith, E. & Shilatifard, A. Covalent modifications of histones during development and disease pathogenesis. *Nature Struct. Mol. Biol.* **14**, 1008–1016 (2007).
177. Look, A. T. Oncogenic transcription factors in the human acute leukaemias. *Science* **278**, 1059–1064 (1997).
178. Pui, C. H. & Jeha, S. New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. *Nature Rev. Drug Discov.* **6**, 149–165 (2007).
179. Rowley, J. D. Chromosome translocations: dangerous liaisons revisited. *Nature Rev. Cancer* **1**, 245–250 (2001).
180. Haferlach, T., Bacher, U., Kern, W., Schnittger, S. & Haferlach, C. Diagnostic pathways in acute leukaemias: a proposal for a multimodal approach. *Ann. Haematol.* **86**, 311–327 (2007).
181. Deschler, B. & Lubbert, M. Acute myeloid leukaemia: epidemiology and aetiology. *Cancer* **107**, 2099–2107 (2006).
182. Armstrong, S. A. & Look, A. T. Molecular genetics of acute lymphoblastic leukaemia. *J. Clin. Oncol.* **23**, 6306–6315 (2005).
183. Pui, C. H., Relling, M. V. & Downing, J. R. Acute lymphoblastic leukaemia. *N. Engl. J. Med.* **350**, 1535–1548 (2004).
184. Viswanathan, S. R., Daley, G. Q. & Gregory, R. I. Selective blockade of microRNA processing by Lin28. *Science* **320**, 97–100 (2008).
185. Choong, M. L., Yang, H. H. & McNiece, I. MicroRNA expression profiling during human cord blood-derived CD34 cell erythropoiesis. *Exp. Haematol.* **35**, 551–564 (2007).
186. Rodriguez, A. *et al.* Requirement of bic/microRNA-155 for normal immune function. *Science* **316**, 608–611 (2007).
187. Kohlhaas, S. *et al.* Cutting edge: the Foxp3 target miR-155 contributes to the development of regulatory T cells. *J. Immunol.* **182**, 2578–2582 (2009).
188. Ceppi, M. *et al.* MicroRNA-155 modulates the interleukin-1 signalling pathway in activated human monocyte-derived dendritic cells. *Proc. Natl Acad. Sci. USA* **106**, 2735–2740 (2009).
189. Costinean, S. *et al.* Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice. *Blood* **114**, 1374–1382 (2009).
190. Gore, S. D. *et al.* Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukaemia. *Clin. Cancer Res.* **8**, 963–970 (2002).
191. Gore, S. D.e.a. Impact of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndrome and acute myeloid leukaemia. *Clin. Cancer Res.* **7**, 2330–2339 (2001).
192. Giles, F. *et al.* A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory haematologic malignancies. *Clin. Cancer Res.* **12**, 4628–4635 (2006).
193. Garcia-Manero, G. *et al.* Phase 1 study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukaemia. *Blood* **112**, 981–989 (2008).
194. Silverman, L. R. *et al.* A Phase I Trial of the Epigenetic Modulators Vorinostat, in Combination with Azacitidine (azaC) in Patients with the Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukaemia (AML): A Study of the New York Cancer Consortium. *Blood Abstr.***112**, 1252–1252 (2008).
195. Odenike, O. M. *et al.* Phase I Study of belinostat (PXD101) plus azacitidine (AZC) in patients with advanced myeloid neoplasms. *J. Clin. Oncol. Abstr.* **26** (2008).
196. Fiskus, W. *et al.* Histone deacetylase inhibitors deplete enhancer of zeste 2 and associated polycomb repressive complex 2 proteins in human acute leukaemia cells. *Mol. Cancer Ther.* **5**, 3096–3104 (2006).

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Competing interests
The authors declare competing financial interests. See web version for details.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
ABL1 | *AML1* | *CEBPA* | *CEBPB* | *EBF1* | *ELL* | *ETO* | *FLT3* | *HOXA9* | *IKZF1* | *KIT* | *KRAS* | *LEF1* | *MEN1* | *MLL* | *MYC* | *NF1* | *NPM1* | *NRAS* | *PAX5* | *PML* | *RARA* | *SPI1* | *TCF3*
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CORRIGENDUM

Leukaemogenesis: more than mutant genes

Jianjun Chen, Olatoyosi Odenike and Janet D. Rowley

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In table 2 on page 30 of the above article, the reference for miR-196a and miR-196b was mistakenly indicated as reference 103. Accordingly, this reference has been replaced with references 89, 104 and 108–110.