



Molecular genetics in acute leukemia

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Improved techniques in identifying the chromosome changes and the affected genes that are involved in acute leukemias have led to improved treatments for these diseases. Identification of consistent chromosomal changes has allowed us to target the location of particular genes and has enabled us to focus our treatments more specifically to certain subtypes of leukemia. Translocations, in particular, are common cytogenetic abnormalities in human leukemia, and the prevalence of certain types of translocations varies with age. Cancers, lymphomas and leukemias are now known to be genetic diseases and it is recognized that genotype-specific therapies should be used that take into account the genetic alterations of the particular leukemia. *Leukemia* (2000) 14, 513–517.

Keywords: chromosome translocations in leukemia; *MLL*; fusion genes; genotype-specific therapy; acute leukemia

Introduction

Human acute leukemias are genetically very diverse. The consistent chromosome changes we identify in tumors pinpoint the location of genes whose functions are critical in the growth potential of that particular cell type. Identification of these genes that are located at the breakpoints in dozens of translocations, many of which were previously unknown, will provide unique insights into the function of these genes in normal cells, as well as their altered function in malignant cells. Moreover, we now know that some cytogenetic abnormalities have prognostic significance.

We have to move from the current therapies which often consist of essentially the same treatment for all types of lymphoid or myeloid leukemia, varying only in intensity and with or without the use of bone marrow transplant. Over the next decade, we must develop genotype-specific therapy; we must be prepared to identify the genotype of the leukemic cells of all patients. DNA assays, PCR, and new genotyping techniques that are coming, are providing new tools to define these subtypes of acute leukemia and this in turn will allow us to develop more specific therapies for patients.

One of the newest tools for cytogeneticists is spectral karyotyping, or SKY.^{1–3} Malcolm Ferguson-Smith⁴ isolated individual human chromosomes using flow sorting and then developed DNA probes unique to each human chromosome. The probes from all of the chromosomes are labeled with different fluorochromes and applied to metaphase cells. Then, with false coloring or spectral karyotyping, we can distinguish each chromosome. As we study patients with many different complex abnormalities, SKY gives us another tool to look at both the chromosome changes and the genotype-specific changes in these patients. In this era of cost-containment, one might consider karyotyping patients or doing PCR as an avoidable expense. However, in order to move into the next decade and into the next millennium, it will be necessary to persuade

the healthcare finance organizations that this is a critical part of the appropriate care for patients.

Cytogenetic abnormalities

Consistent chromosome translocations that we identify in tumors pinpoint the location of genes whose functions are critical in the growth potential of that particular cell type. Since 1973, we have progressed from defining translocations, beginning with t(8;21) and t(9;22), to defining the specific morphology associated with these translocations, to understanding the genes involved, and to beginning to understand the genetic changes in these genes. My colleague, James Vardiman, first observed that acute promyelocytic leukemia (APL) had the specific 15;17 translocation.⁵ This unique association made it clear that chromosome translocations were playing an integral part in the development of leukemia. The challenge was to identify the genes involved in the changes.

The MRC report in Blood⁶ describes the correlation of chromosome abnormalities with the 5-year survival of patients with AML, both children and adults. Patients with a normal karyotype, and patients with good risk cytogenetics and recurring translocations such as t(8;21); t(15;17), are associated with a good prognosis. The poor risk patients are those with a deletion of the long arm of chromosome 7 and/or chromosome 5, as well as other complex karyotypic anomalies.⁶

The 11q23 translocation is a frequent cytogenetic abnormality found in hematologic malignancies, occurring in 5–6% of patients with acute myelogenous leukemia (AML), 7–10% of acute lymphoblastic leukemia (ALL), 60–70% of all acute leukemias in infants, and in most patients with t-AML/t-ALL secondary to therapy that is targeting topoisomerase II.⁷ Initially, the question was whether all these translocations involved different genes? A chromosome band has 10–15 million nucleotides and therefore the myeloid or lymphoid leukemias could involve different genes.

What was not really emphasized in these studies was the association of these particular abnormalities with the age of the patient. Data from the Fourth International Workshop on Chromosomes and Leukemia in 1984 show the frequency of various abnormalities in *de novo* AML by age⁸ (Figure 1). Translocations predominate in those <1 year of age and decline over time, while prevalence of 5q/7q abnormalities increases with age. There is a high frequency of 11q abnormalities, mostly 11q23, that were subsequently shown to involve the *MLL* gene in infants.⁸ 11q abnormalities decrease substantially with age, as does t(8;21) so that overall translocations account for <10% of all the abnormalities within the >60 age group whereas they are very important in younger patients. This is the general pattern for translocations except for t(15;17), which shows some differences in frequency along with age. In terms of 5q and 7q abnormalities and age, approximately 17% of the *de novo* patients >60 years of age have a combined 5q or 7q deletion. There is a great increase in these abnormalities in patients >60 years of age relative to all other age groups. Moreover, these patients carry a poor prognosis and are difficult to treat.

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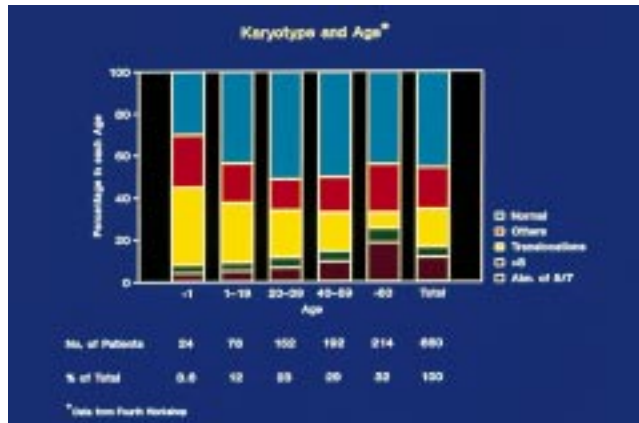


Figure 1 Approximately 17% of the *de novo* patients >60 years of age have either a combined 5q or 7q translocation. This is a significant increase compared to all other age groups, and represents a poor prognosis for these patients.

The consequences of the recurring translocations can be of two types. In Burkitt lymphoma and in the t(14;18) translocation involving the immunoglobulin gene (*IGH*) and *BCL2*, the immunoglobulin gene (gene A) is juxtaposed to the other gene, *MYC* in Burkitt's or to *BCL2* in follicular lymphoma (gene B) (Figure 2, upper panel).^{9,10} However, the immunoglobulin gene causes an abnormal or altered expression of the normal protein produced by *BCL2* or *MYC*; it is the abnormal expression, timing, and level that are associated with the malignant phenotype.

MLL translocations

In most of the translocations seen in myeloid leukemias, and in a substantial portion of the acute lymphoblastic leukemias and sarcomas, there is a break in both gene A and gene B; a fusion of these genes leads to the expression of altered fusion proteins (Figure 2, lower panel). One or both of the fusion genes can be expressed in the leukemia cells.¹¹ When the *MLL*

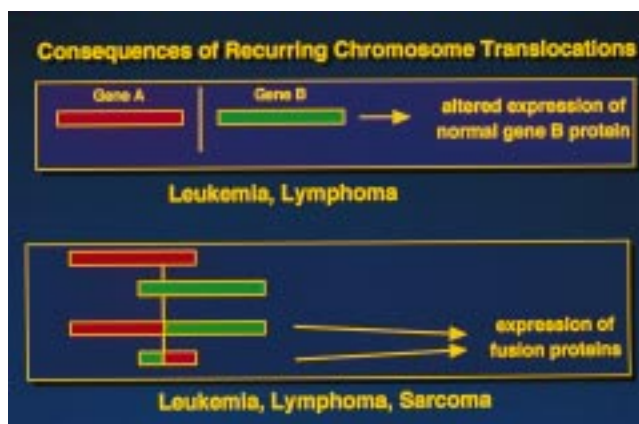


Figure 2 Upper panel: The consequences of recurring chromosome translocations in some lymphomas and leukemias are an altered expression of the normal gene B protein. Lower panel: In contrast, translocations seen in many of the myeloid and acute leukemias, as well as sarcomas, leads to an expression of a unique fusion protein transcribed from parts of gene A and gene B; one or both fusion proteins can be produced in the malignant cells.

gene was cloned, it was called *MLL* because it was present in both myeloid and lymphoid leukemias, as well as in a high proportion of mixed lineage leukemia.⁶ *ALL-1*, *Htrx*, and *HRX* are all other names for the *MLL* gene. *MLL* is a large gene, around 100 kb, with a large 11.7 kb cDNA and variably sized transcripts. There is a significant, but limited homology to *Drosophila trithorax*, from which the *HRX* name is derived.

Fluorescence *in situ* hybridization (FISH) is one of the important cytogenetic techniques that we can now use to define the genetic alterations in leukemic cells.¹² We can use DNA probes to study material from patients who have nothing available for RNA or DNA analysis. FISH can also be used for interphase cells, so even if you do not have metaphase cells available from a patient you can use these probes.

John Anastasi and his colleagues were able to use FISH in a sample that we obtained from the University of South Carolina.¹³ We obtained a bone marrow sample from a patient with a t(11;16); he first took videos of cells and then hybridized them with the appropriate probes for *MLL* (11q23) and *CBP* (16p13). We found that 100% of the monoblasts had fusions in *MLL/CBP*, 20% of granulocytes had fusions, and 10% of erythroblasts had fusions, while none of the lymphocytes had a fusion signal. This finding allowed us to determine the lineages involved with the 11;16 translocation.¹³

Michael Thirman and colleagues¹⁴ cloned a cDNA probe from the break point region of *MLL*. Virtually all translocations involving *MLL* occur within an 8.3 kb genomic region (Figure 3). By using this cDNA probe as a single probe in *Bam*HI-digested DNA, they could show two rearranged bands in lanes 1, 2, and 3; lane 4 is germ line, and there are single rearranged bands in lanes 5 and 6. By cutting this probe in half and using just the 5' part, rearranged bands are present in all five lanes; but using the 3' part in panel c, only those patients that had rearrangements of both bands still have a rearranged band, ie those in lanes 1, 2, and 3. In lanes 5 and 6, there were no rearrangements and we interpret that as a deletion of the 3' part of *MLL* in association with the translocation. We also use molecular Southern blot analysis¹⁴ for genotyping patients, as well as reverse transcription polymerase chain reaction (RT-PCR). In our hands, using RT-PCR with an *MLL* top primer and an unknown random bottom primer does not give very good results, so we need to clone all of these partner genes to have robust probes for diagnosis.

Consequences of translocations

There are at least 38 different translocations involving the *MLL* gene, which is more translocations than any other gene that has been identified (Figure 4). Chromosome bands with a name indicates genes have been cloned. To date, 19 of 38 translocated genes have been cloned (Figure 4). One of the problems in trying to clone these genes, is that some occur only in a few patients. Obtaining enough material to clone these breakpoints is extremely difficult.¹³ If researchers, cooperative groups, and large programs for leukemia collaborate, we could collect sufficient material to allow the cloning of all these genes.

An example of how we have progressed from identifying a translocation to cloning the involved genes, and to beginning to understand the alterations that occur in these genes is illustrated by the 8;21 translocation. The t(8;21) involves the *AML1* (*CBFA2*) gene on chromosome 21 and a gene called *ETO* on chromosome 8. *AML1* is a protein which is very critically involved in binding DNA and in regulating the transcrip-

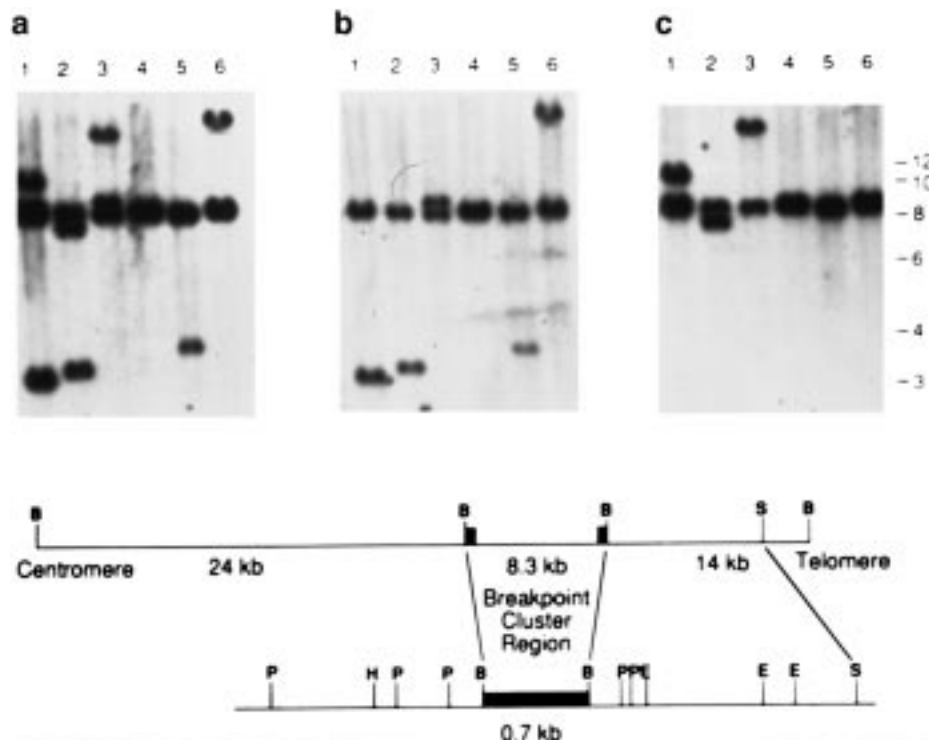


Figure 3 Genotyping patients has shown that virtually all translocations involving *MLL* occur within an 8.3 kb genomic region. By using this cDNA probe on *Bam*HI-digested DNA, Southern blot analysis shows two rearranged bands in lanes 1, 2 and 3; lane 4 is germ line and there are single rearranged bands in lanes 5 and 6.

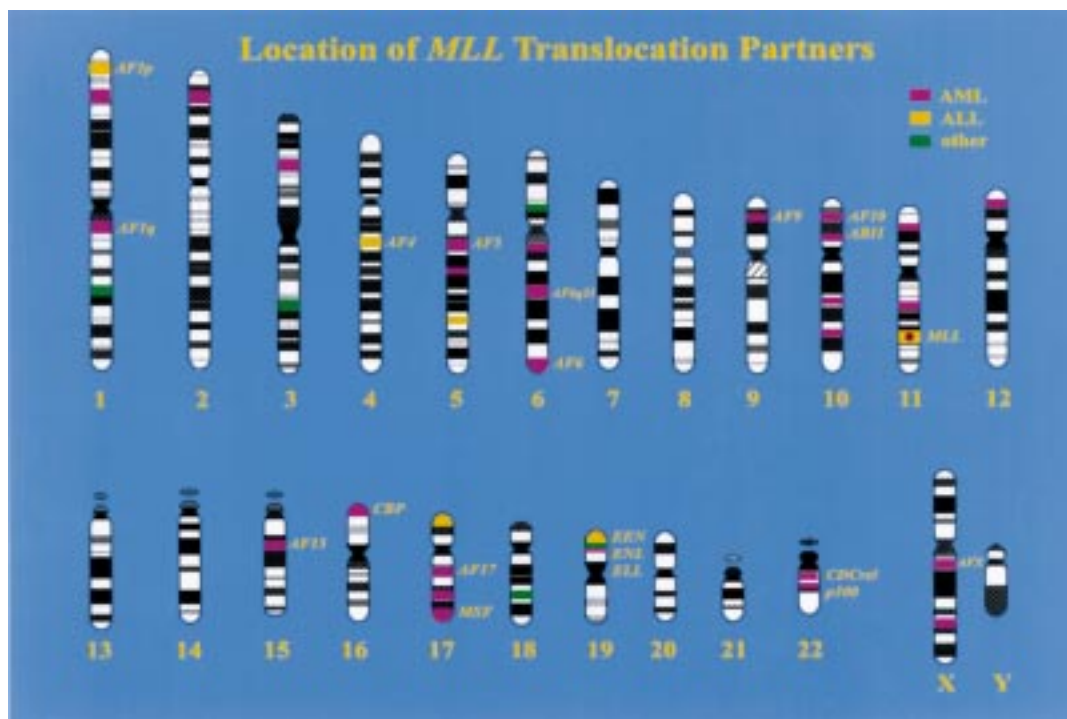


Figure 4 At least 38 different translocations involving the *MLL* gene have been identified so far, and half of these genes have been cloned. The major challenge in trying to clone these genes is finding enough patient material for analysis. The location of translocation breakpoints that involve *MLL* is indicated by colored bands. The involvement of *MLL* was determined by FISH, by Southern blot analysis or by RT-PCR. Symbols to the right of the band indicate that the translocation has been cloned and the partner gene has been identified and named. The phenotype of the malignant disease is indicated by the color of the band; the key is shown above.

tion of a number of myeloid-specific genes. The promoters of the target genes have a DNA core binding factor (CBF) domain that the AML1 protein binds to. AML1 is part of a heterodimer and the other component is CBF beta. CBF beta apparently stabilizes the binding of CBF alpha (AML1) to this DNA target site. The t(8;21) translocation breaks *AML1* in the middle of the gene, removing the transactivation domain and replacing it with part of the *ETO* gene on chromosome 8.

The t(8;21) translocation accounts for 15% of AML. Other translocations also involving *AML1* are fairly rare, but the CBF beta-myosin heavy chain gene fusion seen in the inv(16) also accounts for an important component of AML.¹⁵ Further, in the last few years it has become apparent that the t(12;21) involves *AML1* with *TEL*; this translocation accounts for about 30% of B-progenitor cell pediatric ALL. Thus, this single heterodimer accounts for a substantial part of both AML and ALL.⁷

TEL, the partner of *AML1* in the t(12;21), is another relatively promiscuous gene. There are about 32 translocations that have been identified with *TEL*, virtually all of them using FISH.^{12,13} We have had a major project in the laboratory involving analyzing all of our samples with 12p13 translocations looking for *TEL* rearrangements. We have found many of them, but relatively few have been cloned; therefore, we only know a few of the *TEL* partner genes. Again, this is an important area for all of us to work together to try to provide material so that we can clone these genes and have much better probes for diagnostic analysis.

As is well known, histone acetylation and deacetylation play an important role in the regulation of gene transcription.¹⁶ DNA is wound around the nucleosome complex and the histones have tails that are acetylated and deacetylated. The level of acetylation or deacetylation is very directly related to the function of these genes. For instance, the thyroid hormone and a heterodimer with a retinoic acid receptor X, binds to the thyroid response element on the DNA. The hormone complex is associated with a series of transcriptional corepressor proteins, such as Sin3 and N-COR, which are histone deacetylases. When these are bound to the protein that is bound to the gene, the gene is repressed and there is no transcription. The histone 'tails' are all closely wound around the DNA itself and the gene does not function. When these repressors are removed, a series of other proteins bind to the thyroid hormone that are transcriptional activators. The histone 'tails' then become accessible to the factors regulating gene transcription, and the gene can be expressed at high levels.

The consequences of the 15;17 translocation are related to the fusion of the *PML* gene on chromosome 15 with the retinoic acid receptor alpha (*RARA*) gene on chromosome 17. The *RARA* binds to its normal target response element. However, the *PML* gene is associated with histone deacetylators, and the *RARA* cannot function. The target genes that *RARA* would normally activate and which therefore would lead to cell differentiation are unresponsive to the normal physiologic levels of retinoic acid.¹⁷ On the other hand, when you treat a patient with pharmacologic, not physiologic, levels of all-trans retinoic acid (ATRA), you dissociate this histone deacetylation complex from the fusion protein and you get cells to mature.¹⁷ This is a response that you have seen as you have been treating your patients with t(15;17) translocations.

There is also a rare variant of this translocation, the t(11;17) translocation, where patients have what appears to be a variant form of APL. Instead of *PML* as the partner gene, it is the *PLZF* gene.¹⁷ When the deacetylase complex is removed from *RARA*, there is still a strong binding site on *PLZF* for the

histone deacetylase complex, which is not responsive to ATRA. Therefore, this fusion gene remains in a repressed state. If you treat patients with an 11;17 translocation with ATRA, you do not get a response. This point emphasizes the need to consider genotype-specific therapy; one must know the correct genotype of the patients in order to give them appropriate therapy. How we translate this information into clinical treatment of these patients remains to be determined.

In the t(8;21) translocation, the portion of the fusion protein provided by chromosome 8, namely *ETO*, is associated with the histone deacetylase complex.¹⁸ Therefore, the target genes of *AML1*, such as the IL-3 receptor and GM-CSF, are not activated because this is an inactive fusion protein. *ETO* is a gene that is normally functioning in the nervous system, but not normally functioning in most hematopoietic cells. The challenge is to discover what drugs to give to patients that will disassociate the histone deacetylase complex from *ETO* which will allow the *AML1-ETO* fusion protein to function.

Summary

We now have an additional tool, SKY, which allows us to see what we have been missing in patients with complex karyotypes.¹⁻³ One would hope that as we look at enough of these complex karyotypes we may well find patterns emerging, such as various rearrangements that lead to the recurring juxtaposition of two genes. SKY is also very important in helping to define the region of deletions more precisely. I believe that, for those of us in cytogenetics, our goal should be to improve the genotypic classification of these leukemias and to identify the involved genes. It has become very clear that this information will lead to unique insights into the function of these genes in normal cells, and their altered function in malignant cells. We have evolved from the study of straight cytogenetics to molecular genetics. It is the combination of cytogenetics and molecular genetics that will provide the tools and the insights that we will be using in the next millennium to treat our patients.

References

- 1 Veldman T, Vignon C, Schrock E, Rowley JD, Ried T. Hidden chromosome abnormalities in haematological malignancies detected by multicenter spectral karyotyping. *Nat Genet* 1997; **15**: 406-410.
- 2 Rowley JD, Reshmi S, Carlson K, Roulston D. Spectral karyotype analysis of T-cell acute leukemia. *Blood* 1999; **93**: 2038-2042.
- 3 Fleischman EW, Reshmi S, Sokova OI *et al*. Increased karyotype precision using fluorescence *in situ* hybridization and spectral karyotyping in patients with myeloid malignancies. *Cancer Genet Cytogenet* 1999; **108**: 166-170.
- 4 Carter NP, Ferguson-Smith ME, Affara NA, Briggs H, Ferguson-Smith MA. Study of X chromosome abnormality in XX males using bivariate flow karyotype analysis and flow sorted dot blots. *Cytometry* 1990; **11**: 202-207.
- 5 Rowley JD, Golomb HM, Vardiman J, Fukuhara S, Dougherty C, Potter D. Further evidence for a non-random chromosomal abnormality in acute promyelocytic leukemia. *Int J Cancer* 1977; **20**: 869-872.
- 6 Grimwade D, Walker H, Oliver F *et al*. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* 1998; **92**: 2322-2333.
- 7 Rowley JD. The critical role of chromosome translocations in human leukemias. *Annu Rev Genet* 1998; **32**: 495-519.

- 8 Sakurai M, Swansbury GJ. Fourth International Workshop on Chromosomes in Leukemia 1982: overview of association between chromosome pattern and cell morphology, age, sex, and race. *Cancer Genet Cytogenet* 1984; **11**: 265–274.
- 9 Rabbitts TH. Chromosomal translocations in human cancer. *Nature* 1994; **372**: 143–149.
- 10 Look TA. Oncogenic transcription factors in human acute leukemia. *Science* 1997; **278**: 1059–1064.
- 11 Grimwade D, Howe K, Langabeer S *et al*. Establishing the presence of the t(15;17) in suspected acute promyelocytic leukaemia: cytogenetic, molecular and PML immunofluorescence assessment of patients entered into the MRC ATRA trial. MRC Adult Leukaemia Working Party. *Br J Haematol* 1996; **94**: 557–573.
- 12 Sato Y, Bohlander SK, Kobayashi H *et al*. Heterogeneity in the breakpoints in balanced rearrangements involving bank 12p13 in hematologic malignancies identified by fluorescence *in situ* hybridization: *TEL* (ETV6) is involved in only one half. *Blood* 1997; **90**: 4886–4893.
- 13 Rowley JD, Reshmi S, Sobulo O *et al*. All patients with the t(11;16)(q23;p13.3) that involves *MLL* and *CBP* have treatment-related hematologic disorders. *Blood* 1997; **90**: 535–541.
- 14 Thirman MJ, Gill HJ, Burnett RC *et al*. Rearrangement of the *MLL* gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *New Engl J Med* 1993; **329**: 909–914.
- 15 Nucifora G, Dickstein JI, Torbenson V, Roulston D, Rowley JD, Vardiman JW. Correlation between cell morphology and expression of the *AML1/ETO* chimeric transcript in patients with acute myeloid leukemia without the t(8;21). *Leukemia* 1994; **8**: 1533–1538.
- 16 Warrell RP Jr, He LZ, Richon V, Calleja E, Pandolfi PP. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J Natl Cancer Inst* 1998; **90**: 1621–1625.
- 17 He LZ, Guidez F, Tribioli C *et al*. Distinct interactions of PML-RARalpha and PLZF-RARalpha with corepressors determine differential responses to RA in APL. *Nat Genet* 1998; **18**: 126–135.
- 18 Wang J, Hoshino T, Redner RL, Kajigaya S, Liu JM. ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. *Proc Natl Acad Sci USA* 1998; **95**: 10860–10865.